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Neurodevelopmental Consequences of Early Life Stress in Primates

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Neurodevelopmental Consequences of Early Life Stress in Primates

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B.S. Neuroscience, and Cell and Molecular Biology, Tulane University, 2006
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An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in

Graduate Division of Biological and Biomedical Science
Neuroscience
2013

Abstract

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Early life stress (ELS), particularly social stress, comes in many forms and has been implicated in the etiology of several psychopathologies. It is thought that the repeated activation of the stress response, and subsequent exposure to elevated levels of stress hormones (specifically the glucocorticoid cortisol in primates) during development, when the brain is especially vulnerable to insult, leads to alterations in the brain that result in alterations in behavior. Evidence suggests that white matter (WM) is sensitive to stress, and alterations in WM microstructure have been reported in several stress-related psychopathologies. Thus, it is the goal of this dissertation to investigate the development of WM in two nonhuman primate models of early social stress, social subordination stress and infant maltreatment, and how alterations in WM are related to behaviors related to those that are altered in human psychopathology. Diffusion tensor imaging (DTI) was employed to measure the microstructural integrity of WM tracts in these models at different ages from birth through adolescence. Findings in subordinate animals during prepuberty include increased FA in prefrontal and frontal regions. FA in these regions positively correlated with submissive behaviors in the social group. To investigate the effects of inherited factors on these measures serotonin transporter (5HTT) genotype polymorphism was included in the analysis, and showed interaction effects with dominance rank in several regions of WM, where subordinate animals with

the s-allele had decreased FA. Anxious and aggressive behaviors measured during laboratory tasks negatively correlated with FA in these regions, suggesting a role for 5HTT polymorphism in these behaviors via effects on WM microstructure. Animals maltreated as infants showed alterations in FA throughout infancy and in adolescence. Both increases and decreases in FA were detected, with decreases predominating at the older ages. Affected regions included the internal capsule, cerebellar, frontal, parietal, temporal, and prefrontal WM. Maltreated animals showed increased emotional reactivity in the social group, but had blunted responses to threat during a laboratory task. These results provide evidence that WM is sensitive to ELS, that alterations in WM microstructure are related to behavior, and effects in these two domains appear to be experience specific.

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Acknowledgements

The author would like to thank her committee for their willingness to share their expertise and wisdom during the studies presented. Special thanks are owed to Dr. Mark Wilson for sharing data used in this dissertation. Infinite thanks are owed to Anne Glenn, Christine Marsteller, Dora Guzman, Jennifer Miles, Jennifer Whitley, Marta Checchi, Shannon Bounar, Erin Olmoguez, Vasiliki Michopoulos, Jodi Godfrey and the entire staff of the Yerkes National Primate Research Center Field Station for their invaluable help in collecting the data presented, to Dr. Martin Styner and Yundi Shi for DTI training and processing, to Dr. Kai McCormack and Dr. Alison Grand for behavioral data, to Dr. Jessica Raper for her help developing and implementing the Human Intruder ethogram, and to the Yerkes Imaging Core and the Biomedical Imaging Technology Center for development of the imaging sequences used. Lastly, but most certainly not least, are the endless thanks due to Dr. Mar Sanchez for her unmatched willingness to provide support and guidance in any way possible throughout these studies.

Table of Contents

[1] Modified slightly from Howell BR, Godfrey J, Gutman DA, Michopoulos V, Zhang X, Nair G, Hu X, Wilson ME, Sanchez MM. (2013) Social subordination stress and serotonin transporter polymorphisms: associations with brain white matter tract integrity and behavior in juvenile female macaques. *Cerebral Cortex*. In press.

[2] Modified slightly from Howell BR, McCormack KM, Grand AP, Sawyer NT, Zhang X, Maestripieri D, Hu X, Sanchez MM. Brain white matter microstructure alterations in adolescent rhesus monkeys exposed to early life stress: associations with high cortisol during infancy. *Biology of Mood & Anxiety Disorders*. Submitted.

List of Tables and Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 1 : Introduction

1.1 Stress, early adversity and human health

A stressor is “any unpredictable or uncontrollable stimulus that could - potentially- pose a threat to the organism” (Levine and Ursin 1991), and can be broadly classified as either systemic (e.g. hypoxia) and/or psychogenic (e.g. social). Animals have evolved complex physiological and behavioral responses to acute stressors/threats that work to maintain a dynamic equilibrium, or homeostasis, and minimize cost to the animal (Chrousos and Gold 1992; Chrousos 2009).These responses are very effective at maintaining homeostasis in response to acute stressors, but under conditions of chronic stress, such as the social pressure of modern society, sustained activation of these stress response systems can sometimes do more damage than they prevent (McEwen and Stellar 1993; McEwen 2004). Chronic stress has been linked to poor health outcomes for decades (Selye 1955; Juster, McEwen et al. 2010) and is associated with both poor psychological and physical outcomes including exacerbation of risk factors for cardiovascular disease (Brunner 1997), obesity and diabetes (Dallman, Pecoraro et al. 2003), alterations in immune response (Segerstrom and Miller 2004), infertility (Wilson and Kopitzke 2002), addiction (Sinha 2008) and psychopathology (Juster, McEwen et al. 2010). Growing evidence from studies in humans and other animals suggests that these outcomes are also more severe when chronic stress is experienced early in life and the psychopathological and pathophysiological trajectories may depend on the developmental timing of exposure to stress (Kaplow and Widom 2007; Lupien, McEwen et al. 2009).

Early life stress (ELS) is associated with poor behavioral and health outcomes in adulthood including several psychopathologies (McEwen 2003; Lupien, McEwen et al. 2009; Schafer and Ferraro 2013). Children and adolescents exposed to community-wide natural disasters are at greater risk to develop post traumatic stress disorder (PTSD),

depression, and anxiety, but most victims don't show long-term maintenance of symptoms (Wang, Chan et al. 2013). However, children exposed to more chronic social stress early in life such as abuse, neglect, or poverty, are at increased risk to develop persistent psychopathologies that remain into adulthood (Graham, Heim et al. 1999; MacMillan, Fleming et al. 2001; Herrenkohl, Klika et al. 2012). These environmental insults combine with inherent factors (i.e. gene polymorphisms (Nugent, Tyrka et al. 2011)) to contribute to the development of psychopathologies (Klengel and Binder 2013). ELS has been associated with increased risk to develop depression, anxiety disorders (including PTSD), substance abuse, attention-deficit hyperactivity disorder, conduct disorders, difficulties regulating emotion, difficulties maintaining social relationships (Toth, Manly et al. 1992; Teicher, Andersen et al. 2002; Teicher, Andersen et al. 2003; Nemeroff 2004), inflammation and other immune issues (Danese, Pariante et al. 2007; Shirtcliff, Coe et al. 2009) and even cancer (Jacobs and Bovasso 2000). Many of the brain regions involved in regulation of the stress response are affected by ELS, including the hypothalamic-pituitary-adrenal (HPA) axis itself (Tarullo and Gunnar 2006). These alterations are thought to underlie the increased risk for psychopathology. The main goal of this dissertation is to examine the neurobehavioral impact of the most significant early experiences for primates: maternal care and social stress.

This chapter will begin with a review of the behavioral, endocrine, and neural characteristics of the stress response and its dysregulation in psychiatric disorders. Given that social stress has a critical impact on brain development, the second section will briefly highlight the phases of postnatal brain development in primates and the deleterious impact of early social stress on brain development. Sex differences in these developmental processes will be discussed, as they have potentially important implications on interpreting the effects of chronic social stress and ELS. The third and fourth sections will describe the impact of different early social stressors on the stress

response and brain development in humans and monkeys, respectively. As the topic of this thesis will focus on the effects of early social stressors on white matter (WM) development, another section will be devoted to the description of the neuroimaging techniques used to measure WM integrity in vivo, their strengths and limitations, as well as evidence demonstrating the impact of social stress on WM integrity. The final section will expose the rationale and specific hypotheses for each study described.

1.2 The stress response

1.2.1 Behavioral, autonomic and endocrine responses to stress

As stated above, the function of the stress response is to maintain the body's systems within a dynamic equilibrium, or homeostasis, while minimizing the cost to the animal, a feat accomplished by mobilizing energy for systems necessary for immediate survival and inhibiting systems that are not necessary for immediate survival (e.g. the reproductive system). The stress response does this very well when stressors are acute, but chronic exposure to stress can lead to pathology (McEwen 1998). There are multiple systems involved in the stress response which work in a coordinated manner to respond to threats to the organism. These include behavioral, autonomic, and endocrine stress responses. The behavioral responses to threat include freezing (or fight or flight), increased arousal and alertness, increased vigilance, focus and attention, and in the long-term, suppression of feeding and reproductive behavior (Chrousos and Gold 1992). The autonomic arm responds quickly to stressors and involves activation of the sympathetic nervous system and often inhibition of the parasympathetic nervous system via brainstem structures and the hypothalamus (Ulrich-Lai and Herman 2009). This autonomic response is purely neural and acts via direct innervation of the sympathetic nervous system to affect sympathetic target organs including the heart, gastrointestinal

(GI) tract, and lungs, among others, where it acts to increase heart rate and respiration to support the increased metabolic needs associated with the “fight or flight” response (Molina 2005). In addition to these local actions, activation of the sympathetic nervous system in response to stress also leads to systemic release of catecholamines from the adrenals (referred to as the sympathetic-adrenomedullary [SAM] system), part of the neuroendocrine stress response that also supports the “fight or flight” response (Ulrich-Lai and Herman 2009).

The HPA axis is another major neuroendocrine system that responds to stress in parallel to SAM activation. Briefly, when a stressor is detected specific pathways are activated, resulting in parvocellular neurons of the paraventricular nucleus of the hypothalamus (PVN) being activated and releasing corticotropin-releasing hormone (CRH), and other neuropeptides, such as arginine vasopressin (AVP), into the portal vasculature of the median eminence, which then act on the anterior pituitary to cause release of adrenocorticotropic hormone (ACTH) into the general circulation. ACTH acts upon cells in the adrenal cortex to cause the synthesis and release of glucocorticoids (GCs), such as corticosterone in rodents and cortisol in primates (Charmandari, Tsigos et al. 2005). These are highly catabolic steroid hormone whose main function is to mobilize energy substrates to provide fuel to organs such as the heart and skeletal musculature to respond to the threat. The HPA axis has a negative feedback mechanism to shut down its activity via GCs, so that cortisol, for example, turns off axis activation by inhibiting ACTH synthesis at the level of the pituitary, and CRH at the level of the hypothalamus, and also by acting on extrahypothalamic structures, including limbic regulatory regions such as the hippocampus and prefrontal cortex (PFC), that inhibit the axis (Herman and Cullinan 1997; Charmandari, Tsigos et al. 2005; Ulrich-Lai and Herman 2009).

Stress-induced increases in circulating cortisol levels are superimposed on circadian rhythms of cortisol, with the highest levels occurring in the morning around

waking, and the lowest occurring at night around the onset of sleep, a result of changes in pulsatile release of CRH and AVP from the hypothalamus (Charmandari, Tsigos et al. 2005). Although GCs can have fast, nongenomic effects (Dallman 2005), they are mainly slow-acting and act via two types of intracellular receptors: (1) glucocorticoid receptors (GR), which have low affinity for GCs and are primarily responsible for negative feedback of the HPA axis (i.e. to shut down the stress response) and (2) mineralocorticoid receptors (MR), which have high affinity for GCs and are primarily responsible for maintaining basal HPA axis activity (Sanchez 2006). Both of these receptors translocate to the nuclei of cells once cortisol is bound, affecting gene transcription by acting as transcription factors (Charmandari, Tsigos et al. 2005; Ulrich-Lai and Herman 2009). In primates GRs are expressed in the PVN of the hypothalamus and cortical regions, but strongly in the PFC, where MRs are also highly expressed (Sanchez, Young et al. 2000; Sanchez 2006). It is through these GC receptors that stress-induced elevations of GCs can affect the PFC, particularly during development. Several disorders are associated with either sustained activation or dysregulation of the stress response and include depression, chronic fatigue syndrome, and anxiety disorders such as PTSD and panic disorder, severe chronic disease, and obsessive-compulsive disorder (Chrousos and Gold 1992; McEwen 2007).

1.2.2 Central control of the stress response

Stress responses are regulated by several central nervous system structures and evidence suggests that this control is specific to the type of stressor (Herman and Cullinan 1997; Herman, Ostrander et al. 2005; Ulrich-Lai and Herman 2009). Systemic stressors (e.g. hemorrhage) activate the stress response via direct catecholaminergic innervation from the brainstem to the CRH producing neurons of the PVN (Silverman, Hoffman et al. 1981). It has also been proposed that social stressors, which involve

higher order sensory processing, act via a complex network of limbic structures, including the bed nucleus of the stria terminalis (BNST), amygdala, hippocampus, and PFC to modulate the stress response (Herman and Cullinan 1997; McEwen and Gianaros 2010). The BNST regulates the stress response (activate or inhibit) and plays an important role in mediating the communication between limbic structures (amygdala and hippocampus) and hypothalamic and brainstem structures involved in the regulation of homeostatic function (Walker, Toufexis et al. 2003). The amygdala activates the stress response, mainly through projections of the central nucleus (CeA) (Herman and Figueiredo et al. 2003). The CeA plays an important role in both the autonomic stress response via direct connections with brainstem nuclei, and the HPA axis response via indirect projections to the PVN through the BNST (Herman, Figueiredo et al. 2003; Jankord and Herman 2008). The medial amygdala (MeA) also regulates HPA axis stress responses via indirect projections to the PVN through the BNST and preoptic area (Goldstein, Rasmusson et al. 1996; Jankord and Herman 2008). Basolateral (BLA) nuclei also play an important role, mostly through projections to CeA, but also through projections to MeA and BNST (Goldstein, Rasmusson et al. 1996; Jankord and Herman 2008). Ascending aminergic inputs from the raphé nucleus (serotonin, 5HT) and the locus coeruleus (norepinephrine) also activate the HPA axis (Ziegler, Cass et al. 1999; Herman, Figueiredo et al. 2003). The HPA axis is inhibited by the hippocampus, which plays a prominent role in the cortisol negative feedback described above (Herman and Cullinan 1997; Ulrich-Lai and Herman 2009). PFC also plays a critical role in the stress response by integrating and relaying information about the stressor from primary sensory and association cortices to subcortical structures, thus modulating the behavioral and endocrine response in a stressor-specific manner (López, Akil et al. 1999; Cerqueira, Almeida et al. 2008; Dedovic, Duchesne et al. 2009). In particular, orbital frontal cortex (oPFC) and medial PFC (mPFC) play important roles via

indirect connections to the PVN and regions of the brain stem that mediate the stress response, and also through reciprocal connections with the amygdala (Öngür, An et al. 1998; Ghashghaei and Barbas 2002). The posterior oPFC sends inhibitory projections to the intercalated masses (IM) of the amygdala, thus inhibiting inhibition of the CeA by IM (i.e. disinhibition), subsequently activating the HPA axis (Barbas 2007). Both the oPFC (although these projections are light) and the mPFC project to the CeA, which inhibits the HPA axis, facilitating negative feedback inhibition of the system in parallel to the hippocampus (Diorio, Viau et al. 1993; Herman, Ostrander et al. 2005; Cerqueira, Almeida et al. 2008).

1.2.3 Effects of chronic stress on the HPA-axis and regulatory limbic regions

There is abundant evidence showing that chronic exposure to stressful stimuli alters the HPA axis, including regulatory neurocircuitry, impacting other physiological systems (e.g. alterations in metabolism, growth and tissue repair, immune/GI/reproductive function) and these effects are thought to be a result of allostatic load, or the "wear and tear" that results from chronic stress (McEwen and Gianaros 2010). Chronic stress can result in hypertrophy of the adrenals, stunted growth, infertility, heart disease, myopathy, hypertension, and impaired disease resistance (Chrousos 2009). The effects on the HPA axis itself appear to be dependent on several factors including the type of stressor, the stage of development of the organism during exposure, and the time since stressor onset (Miller, Chen et al. 2007). Many studies have reported hypocortisolemia related to chronic stress, particularly in response to ELS, and this is thought to be due to a down regulation of components of the HPA axis subsequent to an initial elevation in response activations to the stressor (Gunnar and Vazquez 2001; Sanchez, Noble et al. 2005). Heim and colleagues suggest several possible mechanisms for this hypocortisolemia including reduced synthesis or availability of

stress hormones (e.g. adrenal insufficiency), hypersecretion of CRH from both the PVN and extrahypothalamic sites (CeA and BNST) and subsequent downregulation of CRH receptors in the pituitary, increased negative feedback sensitivity, and morphological changes described in the following section (Heim, Ehlert et al. 2000).

Several animal studies have demonstrated atrophy of apical dendrites in the CA3 region and inhibition of neurogenesis in the dentate gyrus of the hippocampus (McEwen 1999; McEwen 2001). Human neuroimaging studies have found evidence of hippocampal atrophy related to chronic stress, which is thought to alter HPA axis negative feedback regulation by this region (Bremner 1999). In contrast to the hippocampus, chronic stress has been related to increased dendritic arborization in the amygdala and BNST (Vyas, Mitra et al. 2002; Vyas, Bernal et al. 2003), and increases in amygdala volume (McEwen and Gianaros 2010). Limbic neuron hyperexcitability (Joels, Karst et al. 2007) has been reported following chronic stress, suggesting morphological and electrophysiological changes that could support increased emotional reactivity.

Subregions of PFC also show opposite effects of chronic stress. For example, mPFC dendritic complexity is reduced and oPFC dendritic complexity is increased in response to chronic stress (McEwen 2007). The sum of all of these effects appears to be a hyperactive HPA axis (Jankord and Herman 2008), which is thought to contribute to the feed-forward effects of chronic stress, and lead to subsequent brain alterations (Charmandari, Kino et al. 2003) and risk for psychopathology (Lupien, McEwen et al. 2009).

1.2.4 Stress regulatory neurocircuitry, chronic stress, and psychopathology

Brain regions important for the regulation of the stress response are also important for regulating emotional behaviors affected in stress-related psychopathology. For example, the amygdala, in particular the CeA, is also important in the regulation of fear and anxiety responses and fear learning (LeDoux 2000) which are altered in stress, affective, and anxiety disorders (Heim and Nemeroff 1999). Studies have shown that stress and GCs can lead to long-lasting increases in fear and anxiety responses through increased CRH expression in CeA (Kalin, Takahashi et al. 1994; Shepard, Barron et al. 2000; Kolber, Roberts et al. 2008). Increased activity of these amygdala and BNST CRH efferent pathways to the hypothalamus and brain stem has been proposed to mediate the increased reactivity to potential threats (increased fear, anxiety, vigilance, sympathetic and HPA responses) reported in individuals exposed to ELS (Heim and Nemeroff 1999; Sanchez, Ladd et al. 2001). Hyperactivity of the amygdala has been reported in neuroimaging studies of anxiety disorders, such as PTSD (Rauch, Shin et al. 2003), effects thought to be partially mediated by stress-induced plasticity in this region (Shekhar, Truitt et al. 2005). In addition to the amygdala, the PFC is important for emotional regulation and is also affected by stress. In part due to strong reciprocal connections with the amygdala, the mPFC and oPFC regulate emotionality and stress responses and are altered in behavioral disorders such as depression and anxiety (Drevets, Price et al. 2008). Electrical stimulation of the white matter tracts connecting the frontal pole and medial temporal pole (e.g. PFC and amygdala) has successfully treated depression and other psychopathologies (Gutman, Holtzheimer et al. 2009; Haber and Brucker 2009). The oPFC is implicated in the representation of emotional information and the regulation of emotional processes (Dolan 2007), and thus oPFC damage causes socioemotional deficits, including emotional outbursts, impulsivity, difficulty with goal-directed behavior and failure to follow social norms (Tucker, Luu et

al. 1995; Bechara, Damasio et al. 2000; Hartikainen, Ogawa et al. 2000). The mPFC and hippocampus are involved in extinction of fear learning, a process thought to be altered in psychopathologies like anxiety disorders (Morgan, Grant et al. 2002; Ji and Maren 2007). Chronic stressor exposure not only results in a number of psychopathologies including increased fear, anxiety and reactivity to stress, but also in alterations in reward, motivation, and mood regulatory brain circuits (Southwick, Vythilingam et al. 2005; Nestler and Carlezon Jr 2006), leading to much of the symptomatology observed in anxiety and mood disorders, which are considered chronic-stress related disorders (Heim and Nemeroff 2001).

1.3 Neurobehavioral development in primates

The lasting effects of ELS are thought to be so detrimental because they occur at a time of rapid developmental changes in the brain (Fig. 1.1), creating windows of vulnerability in which adverse experience can be encoded (Rice and Barone Jr 2000; Andersen 2003; Knudsen 2004). In the following section brain development in humans and monkeys will be reviewed and compared to examine similarities in developmental processes that exist between these two species, and that make the rhesus monkey a valuable model organism for the study of ELS.

1.3.1 Brain development in primates

Prenatally in primates, cells proliferate, migrate, and start making connections through branching, arborization, and extending axons (Hayashi 1992)(Fig. 1.2). This process results in an overabundance of neuron dendrites, axons, and synapses at birth that are selectively refined (pruned) throughout development in response to

environmental stimuli (Hayashi 1992). The peak of cortical synaptic density, and interestingly neurotransmitter receptor density, occurs between postnatal month 2 and 4 in rhesus macaques, and then declines to adult levels around 3 years of age in most regions, but continues to decline through 20 years in PFC (Rakic, Bourgeois et al. 1986; Lidow, Goldman-Rakic et al. 1991; Bourgeois, Goldman-Rakic et al. 1994). In monkeys peak limbic synapse density occurs at postnatal month 2 (Rakic and Nowakowski 1981) and reaches full functional maturity at 4 months (Bachevalier and Mishkin 1984; Kalin and Shelton1989). In humans this process occurs over a longer time and in a region-specific manner, with auditory cortex reaching peak synaptic densities at 3 months, and PFC not reaching peak synaptic density beyond 3.5 years (Huttenlocher and Dabholkar 1997).

In both monkeys and humans these changes in synapse densities are paralleled by region specific decreases in gray matter (GM) and increases in WM (Lenroot and Giedd 2006; Malkova, Heuer et al. 2006). Although early reports in humans of GM volume changes with age suggested linear decreases (Reiss, Abrams et al. 1996), more recent studies of GM volumes describe a region-specific inverted “U” shape during development, with frontal and parietal GM peaking at 12 years of age, temporal GM peaking at 16 years, and occipital GM continuing to increase through 20 years (Giedd, Blumenthal et al. 1999; Giedd 2004). These developmental patterns suggest region specific sensitive periods, with some lasting for several years as a result of protracted developmental trajectories (Andersen 2003). More detailed analyses of longitudinal data suggest that cortical maturation (i.e. GM loss) occurs in “low-order” regions (e.g. regions that process visual or somatosensory stimuli) prior to the association cortices that integrate this sensory input such as portions of the temporal and frontal cortices (Gogtay, Giedd et al. 2004). Cortical development also seems to follow the concept of “ontogeny recapitulates phylogeny” with phylogenetically older regions such as the

piriform and entorhinal cortex maturing before evolutionarily newer regions such as the inferior temporal cortex and PFC (Giedd 2004; Gogtay, Giedd et al. 2004; Shaw, Kabani et al. 2008).

WM volumes, including the corpus callosum (CC), increase linearly during childhood and adolescence (Reiss, Abrams et al. 1996; Giedd, Blumenthal et al. 1999; Giedd 2004; Lenroot and Giedd 2006), and appear to be due at least in part to increases in myelination (Deoni, Mercure et al. 2011). The general pattern of postnatal myelination is similar in humans and monkeys, and begins in the cerebellum, pons, and internal capsule (IC), and then proceeds from caudal to rostral regions from the splenium of the CC and optic radiations, to the occipital and parietal lobes, and finally into the genu of CC, frontal, and prefrontal WM (Gibson 1991; Barnea-Goraly, Menon et al. 2005; Gao, Lin et al. 2009; Deoni, Mercure et al. 2011; Shi, Short et al. 2013). In monkeys the elimination of axons occurs both pre- and postnatally in a region specific manner with some regions such as the CC not reaching adult axon numbers until postnatal day 60 (LaMantia and Rakic 1990). In both monkeys and humans myelination begins prenatally in most regions and continues in association areas, namely temporal and prefrontal regions, into early adulthood (Gibson 1991). Cerebellar, temporal and prefrontal tract myelination occurs during childhood, with the cerebellum achieving adult levels of myelin between 3 and 6 months of age in monkeys and 1 to 2 years in humans, while WM of commissural and association tracts only reach adult levels of myelination at 3.5 years in monkeys and around 20 years in humans (Gibson 1991). This suggests that commissural and association tracts, such as the uncinate fasciculus (UF), WM tract connecting PFC with temporal regions and the last WM tract to myelinate in humans (Lebel, Walker et al. 2008), are particularly sensitive to ELS because of their protracted development.

Much of what we know about the rapid developmental changes that occur early in life in the amygdala comes from studies of rhesus monkeys. Amygdala development in rhesus monkeys follows nuclei-specific patterns. The medial nucleus is near adult size at birth, while the lateral and basal nuclei increase in volume from birth through 3 months of age (Chareyron, Lavenex, et al. 2012). The volume of the central nucleus is about half of the adult size at birth and continues to increase beyond one year of age (Chareyron, Lavenex, et al. 2012). These nuclei specific patterns are reflected in a postnatal increase in total amygdala volume, which occurs at the highest rates during the first 4 months of life (Payne, Machado et al. 2010). Given the focus of this dissertation on the development of WM, it is interesting that oligodendrocyte numbers in the amygdala increase in parallel with the increase in amygdala volume after 3 months of age, while neuronal size, astrocyte number, and neuron number do not change (Chareyron, Lavenex, et al. 2012). This suggests that the increase in amygdala volume is not only due to neuronal changes, but also in the glia that produce the myelin in WM. It is possible that this increase in oligodendrocytes in the amygdala is related to the maturation of amygdala circuits reflected during behavioral development. For example, in monkeys, amygdala afferents from portions of temporal cortex that relay visual information mature at week 3 when an animal first begins to respond appropriately to social cues, while efferents from these same temporal regions to oPFC do not become mature until 2 months when curiosity and frustration become apparent (Machado and Bachevalier 2003).

Maturational processes such as synapse and axon elimination and myelination occur at different times in different regions as discussed above, and occur in parallel with emergence of specific behaviors in much the same way as described above for amygdala circuits. For example, in humans the protracted development of the PFC and temporal cortex parallels development of attention and inhibitory control, a relationship

supported by several functional imaging studies in adults and children (Casey, Giedd et al. 2000). Casey and colleagues suggest that this maturation of cognitive control is related to a decrease in synapses and myelination. Cognitive and affective control has been postulated to depend on the development of frontostriatal and fronto-amygdalar circuits in humans (Hare and Casey 2005). These examples highlight the close connections between the development of prefrontal-amygdala circuits and emergence of behaviors they control, and lead to questions about what happens when these closely coupled processes are altered.

1.3.2 Development of the HPA axis: GCs

Human newborns are able to activate the HPA axis in response to stressors, but do not exhibit a mature circadian rhythm in cortisol; however they do show two peaks of cortisol about 12 hours apart that are independent of the time of day (Gunnar 1992). Some studies have reported an adult-like circadian cortisol rhythm as early as 2 weeks while others don't detect a mature rhythm until 3 months of age (Price, Close et al. 1983; Santiago, Jorge et al. 1996). At about 3 months of age, there is a decrease in the cortisol response to physical examinations, an effect that continues throughout the first year of life (Gunnar 1992), and is paralleled by decreases in basal cortisol (Tollenaar, Jansen et al. 2010). This period of apparent stress insensitivity is thought to mirror the "stress hyporesponsive period" (SHRP) described in rodents, and may be dependent on the quality of the caregiving provided (Gunnar and Quevedo 2007). In rodents, the SHRP is characterized by a quiescent HPA axis, evidenced by both an inability to mount a cortisol response to stressors that are effective in activating the axis at other ages, and by failure to suppress circulating cortisol levels in response to challenge with the synthetic GC dexamethasone, suggesting insensitivity to the inhibitory effects of high levels of GCs observed in adults (Sapolsky and Meaney 1986; Vazquez 1998). The purpose of this

hyporesponsive period is thought to be to maintain the low level of GCs necessary for normative brain growth and development (Sapolsky and Meaney 1986). There is evidence in humans that this period of relative stress hyporesponsivity is dependent on the quality of parental care, and may extend through childhood (Gunnar and Fisher 2006), which is consistent with the hypothesis that GC levels need to be tightly regulated during brain development. This is also supported by alterations in the stress response and brains of humans exposed to ELS. The role of quality caregiving in buffering the stress response during development also highlights the importance of social experience in the development of the HPA axis in humans, a trait that is shared by nonhuman primates.

Development of the HPA axis in nonhuman primates follows a similar pattern as that described for humans. Basal HPA circadian rhythms have been reported in infant rhesus monkeys, but still seem immature at 5 months of age, with high cortisol levels at sunrise, followed by a nonsignificant decline from sunrise to midday, and a steep decline from midday to sunset (Raper, Bachevalier et al. 2012). In juvenile monkeys cortisol sharply declines between morning and afternoon, and afternoon and night, demonstrating an adult-like diurnal pattern of cortisol secretion (Sanchez, Noble et al. 2005; Barrett, Noble et al. 2009). Although there is little evidence of a SHRP in rhesus monkeys, early social experience, specifically nurturing maternal behavior, does buffer the HPA axis response (Sanchez 2006). It is important to note that highly rejecting, abusive, and low protecting rhesus mothers fail to buffer the HPA axis in their infants (McCormack, Grand et al. 2003). This parallels the importance of social experience in the development of the HPA axis described in humans above.

1.3.3 Sex differences in neurobehavioral development

The brains of adult men and women are different (Cosgrove, Mazure et al. 2007). These differences appear to have a developmental basis, suggesting the possibility that stress may have sex-dependent effects that may interact with developmental timing. Female brains mature earlier than males. For example, total cerebral volume peaks at approximately 11 years old in females but not until 14.5 in males (Giedd, Blumenthal et al. 1999; Lenroot, Gogtay et al. 2007), and both subcortical and cortical GM volumes peak for females about 1-2 years earlier than males (Lenroot, Gogtay et al. 2007). Also, males have more prominent age-related decreases in GM volume and increases in WM volume than females during adolescence (De Bellis, Keshavan et al. 2001). The linear increase in WM is present regardless of sex, but is smaller in females than males, both during childhood and adolescence (Giedd, Blumenthal et al. 1999; Lenroot, Gogtay et al. 2007). In adolescence, girls show mature levels of myelination (indirectly measured using DTI) earlier than boys (Wang, Adamson et al. 2012), and more robust age-related increases in CC area have been reported in males during childhood (De Bellis, Keshavan et al. 2001), supporting the idea of earlier WM development in females. Evidence for earlier maturation in females in subcortical limbic regions is not consistent. Increased amygdala volume in males and decreased hippocampal volumes in females in childhood are reported in some studies (Caviness, Kennedy et al. 1996), but not in others (Lange, Giedd et al. 1997). Larger left amygdala volumes in males, and larger bilateral hippocampus volumes in females have been reported in adolescence (Neufang, Specht et al. 2009). Still others report no sex differences in volumes in subcortical structures, but do report differences in maturational changes with the left amygdala only significantly increasing in volume in boys and right hippocampus only increasing in volume in girls (Giedd, Vaituzis et al. 1996). Taken together these sex differences in brain development suggest that vulnerability to stress in these regions may also differ as a function of both

sex and developmental timing, with girls being more vulnerable to earlier insults due to earlier brain development discussed above.

Sex differences in emotional reactivity have also been described in children, with girls being more reactive than boys (McManis, Bradley et al. 2001). This relationship continues into adolescence when girls are more reactive to social stress (Shih, Eberhart et al. 2006). Women show increased cortisol reactivity to interpersonal stress, but not to achievement-based stressors (Stroud, Salovey et al. 2002; Kelly, Tyrka et al. 2008), supporting previous findings that females are more behaviorally reactive to social stress, although studies of stress reactivity are not consistent and often report greater stress responses in men (Kudielka and Kirschbaum 2005). In adults, this increase in emotional reactivity has been related to enhanced activity in the amygdala and PFC in women (Domes, Schulze et al. 2010), suggesting that sex dependent differences in PFC-amygdala circuits could be related to increased emotional reactivity. These differences in stress and emotional reactivity are thought to underlie the increased risk for depression and anxiety disorders observed in women (Seeman 1997; Olff, Langeland et al. 2007), and suggest that social stress is especially salient to women. The sex differences in brain development and responses to social stress described above provide evidence that sex is an important factor to consider when investigating the different trajectories of ELS and chronic social stress induced developmental psychopathology.

1.4 Early social stress in humans

Social condition is thought to play a major role in disease (Link and Phelan 1995). In particular, social stress during childhood is associated with behavioral and cognitive impairments and increased risk for psychopathology and health problems later in life,

including anxiety and mood disorders (Teicher, Andersen et al. 2002; Teicher, Andersen et al. 2003; Gunnar and Quevedo 2007; Weber, Rockstroh et al. 2008). These outcomes are thought to be a consequence of the strong influence early social experiences has on brain development, one of the many adaptations of primate brains to complex social environments (Dunbar and Shultz 2007). Many forms of ELS involve adverse social environments, and include early social deprivation as seen in children adopted from orphanages (sometimes referred to as post-institutionalized children), low socioeconomic status (SES) during childhood, and childhood maltreatment (including both abuse and neglect).

1.4.1 Early social deprivation

Early social deprivation, such as that experienced by post-institutionalized children, is a pervasive form of early social stress and has adverse effects on several behavioral and physiological systems. Spitz first described the alterations associated with this early deprivation in infants as *anaclitic depression*, which included withdrawal, cognitive deficits, weight loss, and overall developmental decline (Spitz 1945; Spitz and Wolf 1946). Deficits in growth and alterations in the HPA axis have also been reported in post-institutionalized children and are thought to be due to the chronic stress associated with lack of social contact (Loman and Gunnar 2010; Johnson, Bruce et al. 2011). Many of the alterations in behaviors and neuroendocrine systems observed in post-institutionalized children could be regulated by the amygdala, thus neuroimaging studies have often focused on this limbic structure. Increases in amygdala volume and reactivity, with some evidence for premature engagement, have been reported in post-institutionalized children (Tottenham and Sheridan 2009; Tottenham 2012). Results of diffusion tensor imaging (DTI) studies of the effects of early social deprivation on WM tract integrity have found decreased structural integrity in the UF, one of the major WM

tracts connecting the PFC with subcortical limbic structures including the amygdala (Eluvathingal, Chugani et al. 2006). Diffuse fronto-striatal connectivity, suggesting incomplete pruning during development, have also been reported (Behen, Muzik et al. 2009). These findings are supported by findings of reduced structural integrity in frontal, temporal, and parietal WM that also include components of the UF in this population (Govindan, Behen et al. 2010). These studies of post-institutionalized children underscore the importance of early social experience on normative brain development, particularly in frontal-amygdala circuits, providing further support for the role of alterations in these regions in other forms of ELS.

1.4.2 Low SES during childhood

An example of cumulative adverse social experiences is low SES, which represents a complex form of chronic social stress associated with socioemotional and cognitive impairments in children, also involving poor access to material and social resources, and exposure to increased violence, parental absence, and drug abuse (Adler, Boyce et al. 1994; Bradley and Corwyn 2002; Pearlin, Schieman et al. 2005; Hackman and Farah 2009). Low SES during childhood has also been shown to increase risk for depression in women (Gilman et al. 2002). These effects are thought to be mediated by the chronic, cumulative stress experienced by low SES children (Baum, Garofalo et al. 1999; McEwen and Gianaros 2010). Low SES children and adolescents show poor cognitive and academic performance, and socioemotional development (Bradley and Corwyn 2002). Low SES during childhood is associated with increased risk factors for cardiovascular disease (Blane, Hart et al. 1996) and obesity (Shrewsbury and Wardle 2008), and has even been linked to alterations in physical growth (Malina, Little et al. 1985; Block and Krebs 2005). In addition to these physical health outcomes children in

low SES environments are also at higher risk to develop psychopathologies (Johnson, Cohen et al. 1999; Muntaner, Eaton et al. 2004), including depression, a risk that is higher in women than men (Gilman, Kawachi et al. 2002; Lorant, Deliège et al. 2003).

The neural substrates underlying the association of behavioral alterations and increased risk for psychopathology within low SES populations are not well understood. In part, this is due to the fact that this at risk population has been understudied because of the complexity and varied forms of early adversity they experience (e.g. violence in their neighborhoods, parental drug abuse, paternal absence, physical, sexual and emotional trauma, etc.). However, recent studies report high levels of basal salivary cortisol in both low SES adults (Cohen, Grieve et al. 2006) and children (Lupien, King et al. 2000), suggesting experience-related alterations (activation) in the HPA axis. The effects of SES on the brain are understudied (Hackman and Farah 2009; Tomalski and Johnson 2010), although there is some recent evidence of low SES effects on brain GM (Noble, Norman et al. 2005; Hackman and Farah 2009; Jednoróg, Altarelli et al. 2012). There is also evidence of altered PFC function in low SES children (Kishiyama, Boyce et al. 2009), as suggested by findings of decreased executive control in this population (Noble, Norman et al. 2005). Another study reported that SES predicted specialization of the left inferior frontal gyrus, and was positively correlated with gray and white matter volumes in this region (Raizada, Richards et al. 2008). Another study showed a relationship between phonological awareness and activation in the left fusiform gyrus and cerebellum of children with low SES when none was detected in those with high SES (Noble, Wolmetz et al. 2006). Despite the paucity of studies examining the neurobiological underpinnings of behavioral alterations and psychopathology associated with low SES, there is evidence that this is a form of cumulative social chronic stress and that there are alterations in brain systems previously shown to be sensitive to stress involved in the etiology of psychopathology.

1.4.3 Childhood maltreatment

Childhood maltreatment is a form of ELS that contrasts low SES in that it typically occurs during a much shorter developmental window than low SES, although these conditions are often comorbid (Garbarino and Kostelny 1992). Child maltreatment includes both physical and emotional abuse and neglect, and is most often perpetrated by a child's primary caregiver(s) (U.S. Dept. of Health and Human Services, 2012). This is interesting given that the adverse effects of low SES are thought to be mediated at least in part by harsh parenting (McLoyd 1998). Child maltreatment has also been implicated in poor cognitive and socioemotional development, and physical health (Cicchetti and Toth 2005). Child maltreatment is linked to: several of the leading causes of death in adults (Felitti, Anda et al. 1998), obesity (Danese and Tan 2013), alterations in physical growth (Elmer and Gregg 1967), and increased risk to develop psychopathology, including anxiety and mood disorders (Heim and Nemeroff 1999). Maltreated children also show increased incidence of attention-deficit-hyperactivity disorder (ADHD) (Famularo, Kinscherff et al. 1992), as well as difficulty with inhibitory control of behavior and emotions (Shields and Cicchetti 1998). This causes them to be more impulsive and aggressive and puts them at higher risk for substance abuse (Sinha 2008; Andersen and Teicher 2009).

Childhood maltreatment also results in long-term structural and functional brain alterations (Hart and Rubia 2012). Regional volume loss in specific cortico-limbic structures (hippocampus, amygdala, PFC) has been reported in adults with histories of childhood maltreatment (Bremner, Randall et al. 1997; Stein, Koverola et al. 1997; Bremner 2003). These reductions in limbic structure volume are paralleled by decreased structural integrity of WM measured using DTI in WM tracts important for emotion regulation, including the cingulum bundle, arcuate fasciculus, and fornix in adults

exposed to parental emotional abuse (Choi, Jeong et al. 2009). Reported brain structural changes in children, measured using magnetic resonance imaging (MRI), include more diffuse alterations than in adults, such as reductions in cortical WM volume, CC area (cross hemispheric fibers) and intracranial volume (De Bellis, Baum et al. 1999; De Bellis, Keshavan et al. 1999; Bremner 2002; Teicher, Andersen et al. 2002; Teicher, Andersen et al. 2003). Alterations in WM integrity also parallel these volumetric alterations in children and adolescents and have been reported in several tracts including the CC (suggesting alterations in the integration of information between the hemispheres), the superior longitudinal fasciculus (large association tract involved in both cognitive, e.g. language, and emotion regulation), cingulum bundle (these findings were specifically in the portion of the tract that projects to the hippocampus), and the fronto-occipital fasciculus (involved in emotional visual function)(Jackowski, Douglas-Palumberi et al. 2008; Huang, Gundapuneedi et al. 2012). These differences between adults and children exposed to maltreatment are not completely understood, but could be due to brain circuitry reorganization processes that occur during adolescence (Lebel, Rasmussen et al. 2008; Giedd and Rapoport 2010; Knickmeyer, Styner et al. 2010), heterogeneity of the populations studied, the variety of comorbid psychopathologies present in studies of structural alterations of these limbic regions, and continued, or concurrent, exposure to maltreatment in children (De Bellis, Keshavan et al. 1999; Kaufman and Charney 1999). Although all this evidence suggests that ELS can impact the development of cortico-limbic and cortical association tracts involved in emotional, behavioral and cognitive regulation affected in these populations, the relation between structural and functional changes, or the emergence of these alterations during development is not clearly understood.

1.5 Nonhuman primate models of social stress early in life

Nonhuman primate models are crucial when studying the neural mechanisms of developmental psychopathology because they are more closely related to humans phylogenetically than rodents while still allowing for experimental manipulations not possible in studies investigating humans directly (Gibbs, Rogers et al. 2007). Rhesus monkeys are a nonhuman primate species widely used to study the neurobehavioral effects of social environment due to several advantages. They live in large troops made up of several multi-generational female-headed families and several immigrant adult males, resulting in a complex social environment. They also have well-developed brains that closely resemble humans' in respect to organization and neurochemistry (Reep 1984; Barbas 2000; Croxson, Johansen-Berg et al. 2005; Thiebaut de Schotten, Dell'Acqua et al. 2012), particularly regarding the PFC, which is very different in primates versus rodents (Preuss and Goldman-Rakic 1991; Preuss and Goldman-Rakic 1991; Preuss 1995; Öngür and Price 2000). Maturational processes in the brain occur in similar region specific patterns during development, with similar temporal and anatomical patterns, in humans and monkeys, although over a condensed period of time in monkeys (approximately 1:4 years, monkey to human time) (Diamond 1991; Gibson 1991; Huttenlocher and Dabholkar 1997). GC and CRH receptor distributions are comparable in rhesus monkey and human brains, particularly regarding their high expression in PFC (Seckl, Dickson et al. 1991; Sanchez, Young et al. 1999; Sanchez, Young et al. 2000; Pryce 2008), suggesting that some effects of ELS-induced elevations in stress hormones and neuropeptides (e.g. GCs and CRH) could be similar in these species. Several nonhuman primate models of ELS and social stress have been used to study the effects of these experiences on the brain and behavior (Sanchez, Ladd et al. 2001), which will be reviewed in the next section.

1.5.1 Social isolation and maternal deprivation

Experimental manipulations of the early rearing environment to model ELS include rearing in partial social isolation, as done by Dr. Harry Harlow in the 1960's and 70's. This results in severe behavioral and social deficits including stereotypies, self injurious behavior, inability to read social cues or develop normal social relationships, and hyperaggressiveness (Suomi, Harlow et al. 1971). Rearing in complete social isolation led to similar outcomes, but with increased severity, and also included increased fear and anxiety (Seay and Gottfried 1975). Alterations in the HPA axis have also been reported in this model (Sanchez, Ladd et al. 2001), and recently several studies provided evidence for gene-by-environment interactions and epigenetic mechanisms for the effects of ELS (Barr, Newman et al. 2004; Provençal, Suderman et al. 2012). These manipulations of the early social environment have been invaluable in understanding the importance of social stimuli on socioemotional and physiological development, but these are all somewhat artificial paradigms with questionable ethological validity, except perhaps for extreme cases of social deprivation in children, such as the orphanage-rearing described in previous sections.

Maternal separation in nonhuman primates has also been used to model ELS. Separation from the mother is a potent stressor for infant monkeys that leads to increased activity, vocalizations, and HPA axis activations (Harlow, Harlow et al. 1971; Bayart, Hayashi et al. 1990). There is evidence of alterations in PFC (Rilling, Winslow et al. 2001) and HPA axis (Sanchez, Noble et al. 2005) of maternally separated monkeys, effects thought to be mediated by exposure to elevated levels of stress hormones (Sanchez, Ladd et al. 2001). Cameron and colleagues have gone one step further and looked at the long-term effects of complete maternal deprivation on infant behavior as a function of when the mother and infant were separated. The primary findings of these

studies are consistent with those reported in children. That is, rhesus infants separated from their mothers at earlier ages (beginning at 1 week of life) showed the most detrimental effects (O'Connor and Cameron 2004). These effects included social disturbances and alterations in fear behaviors, as well as alterations in both PFC and amygdala (O'Connor and Cameron 2004). Although these NHP models of maternal separation have provided critical information on the importance of the mother-infant bond in normative neurobehavioral development, they are still artificial manipulations/experiences that would not typically occur in this species.

The variable foraging demand model contrasts the other models described briefly above in that it was specifically developed as an ethologically valid model of ELS that exploits species typical behavior, i.e. foraging for food. This model consists of variable, and most importantly, unpredictable, availability of food (i.e. periods of intense foraging alternated with periods of free access to food), and has been used to study disruption of the early social environment in bonnet macaques (Sanchez, Ladd et al. 2001). This manipulation alters maternal behavior by reducing the amount of time spent responding to infant solicitations for care. This results in long-term behavioral alterations in the infant that include social incompetence, increased fearfulness, and both behavioral and physiological hyperresponsiveness to stressful stimuli (Coplan, Andrews et al. 1996). Reductions in WM integrity in the anterior limb of the internal capsule are found in adult monkeys exposed to this form of ELS, providing evidence that ELS has long-term effects on the brain (Coplan and Abdallah et al. 2010). Although the variable foraging demand paradigm arguably has more ethological validity than social deprivation or maternal separation, it still involves experimental manipulations that may affect behavioral outcomes in unexpected and difficult to interpret ways. All of these models have been crucial to underscore the critical role of early social environment (including the mother) on primate development, but they are based on artificial manipulations that

this species may not otherwise be exposed to, thus the need for studies using nonhuman primate models with stronger ethological validity. I chose the two rhesus monkey models used in this dissertation, social subordination stress and infant maltreatment, in part because they both have high ethological validity.

1.5.2 Social subordination in rhesus monkeys

SES in human society has parallels with the social structure of other primate societies. Rhesus monkeys, for example, live in societies organized by a strict dominance hierarchy that functions to maintain group stability (Bernstein and Gordon 1974; Bernstein 1976). An animal's place within the hierarchy determines your access to resources, such as food and mates (Sade 1967). These matrilineal hierarchies are maintained through contact and non-contact aggression and threats of aggression from dominant to subordinate animals (Bernstein and Gordon 1974; Bernstein, Gordon et al. 1974; Bernstein 1976). The defining feature of social subordination is the presentation of submissive behaviors from subordinate animals in response to agonistic interactions with dominant animals (Bernstein and Gordon 1974; Bernstein, Gordon et al. 1974; Bernstein 1976). These agonistic encounters are unpredictable, and often unprovoked, increasing uncertainty and stress in subordinates (Silk 2002). This presumably reduces a subordinate animal's sense of control over the social and physical environment. Consequences of this unpredictable, continual harassment in adult females include stress-related phenotypes, such as HPA dysregulation, evidenced by hypercortisolemia and impaired GC negative feedback (Shively, Laber-Laird et al. 1997; Jarrell, Hoffman et al. 2008; Kaplan, Chen et al. 2010; Michopoulos, Higgins et al. 2012). Subordinate animals receive higher rates of aggression and lower rates of affiliation (Michopoulos, Higgins et al. 2012). It is interest then to note that these two behaviors were the best predictors of increased cortisol in subordinates found in a meta-analysis of several

primate species (Abbott, Keverne et al. 2003). Thus, social subordination in rhesus monkeys is a well-established nonhuman primate model of chronic psychosocial stressor exposure, with high ethological validity. This model has been used to study the adverse effects of chronic social stress on a broad range of adult behavioral, neuroendocrine and health outcomes (Gust, Gordon et al. 1991; Kaplan, Adams et al. 1996; Morgan, Grant et al. 2002; Michopoulos, Berga et al. 2009; Paiardini, Hoffman et al. 2009; Kaplan, Chen et al. 2010; Tung, Barreiro et al. 2012). Given the success of this model in studying effects of chronic social stress in adults, recent investigations, including the one presented in Chapter 2 of this dissertation, are beginning to focus on the effects of this chronic stressor on developing monkeys.

Infant rhesus monkeys acquire the relative rank of their mothers within their social group (Sade 1967), a process thought to be attributable to latent observational learning as well as harassment from higher ranking females (Holekamp and Smale 1991). Thus infants of low ranking mothers are subject to the pressures of their rank beginning very early in development. While we know social subordination affects pubertal timing (Wilson, Gordon et al. 1986; Zehr, Van Meter et al. 2005; Wilson, Bounar et al. 2013), an effect that is associated with increased emotional reactivity (Wilson, Bounar et al. 2013) , the subordinate phenotype is less well understood in developing monkeys, which is one of the goals of this dissertation. It is important to recognize that this form of social stress extends throughout the life of the animal, not just during early life, which is one of the main differences between this and the infant maltreatment model utilized in this dissertation. The social stress experienced by subordinate animals does begin early in the months following birth, but most likely occurs after weaning and continues throughout life, which makes it a form of chronic stress that does not only occur during a short developmental window. This allows for the contrast of its effects with those of infant

maltreatment, which occurs very early in life, but during a relatively short developmental window.

1.5.3 Infant maltreatment in rhesus monkeys

Child maltreatment is not a uniquely human phenomenon, but are also reported in both wild and captive populations of nonhuman primates, including macaques, baboons, and marmosets (Troisi, D'Amato et al. 1982; Troisi and D'AMATO 1984; Johnson, Kamilaris et al. 1996; Maestripieri, Wallen et al. 1997; Maestripieri 1998; Maestripieri and Carroll 1998; Brent, Koban et al. 2002). The labs of Dr. Dario Maestripieri and Dr. Mar Sanchez have studied spontaneous macaque infant maltreatment as a translational animal model of human childhood maltreatment. Infant maltreatment in this model is comprised of (1) physical abuse in which the mother exhibits aberrant violent behaviors towards the infant (drags, crushes, sits on, or roughly grooms the infant) during the first 2-3 months of life, which triggers overt signs of distress in the infant (vocalizations, tantrums, etc.), and (2) high rates of infant rejection early in life, which is a physically undamaging component consisting of pushing the infant away when it solicits contact with the mother, also resulting in infant distress (i.e. tantrums, screams) (Maestripieri 1998). Studies using this model have identified socioemotional alterations and activation of the stress response comparable to those seen in maltreated children (McCormack, Sanchez et al. 2006; Sanchez 2006; McCormack, Newman et al. 2009; Sanchez and Pollak 2009; Sanchez, McCormack et al. 2010; Koch, McCormack et al. 2013), some of them associated with decrease in brain 5-HT function (Maestripieri, Higley et al. 2006; Sanchez, Alagbe et al. 2007). Delays in social development including delayed independence from the mother and less exploration and play have also been reported in this model (Maestripieri and Carroll

1998; Maestripieri, Jovanovic et al. 2000). These findings parallel long-term consequences found in maltreated humans, supporting the construct validity of this model to gain understanding of the mechanisms underlying the poor developmental outcomes associated with this adverse early experience in humans.

1.6 ELS effects on brain WM using DTI

Brain WM is important for rapid axonal conductance between distant regions (Fields 2008). Alterations in WM integrity have been reported in several psychiatric disorders (Taylor, Hsu et al. 2004; White, Nelson et al. 2008; Thomason and Thompson 2011), a link possibly due in part to WM's sensitivity to early experiences (Eluvathingal, Chugani et al. 2006; Katz, Liu et al. 2009; Coplan, Abdallah et al. 2010; Govindan, Behen et al. 2010; Frodl, Carballedo et al. 2012). Brain WM undergoes massive developmental increases in volume during early life, due in part to increases in myelination, making this developmental process vulnerable to environmental influence (Davison and Dobbing 1966; Andersen 2003). There is also evidence that myelination is sensitive to GCs as discussed below, suggesting that effects of ELS on WM are a consequence of GC exposure early in life. Brain WM is beginning to be recognized as an important factor underlying behavioral control and neural plasticity (Fields 2008; Thomason and Thompson 2011), and thus alterations in brain WM could underlie some of the behavioral alterations observed in response to chronic social stress and ELS. It is for these reasons that the focus of the current dissertation is on investigating the effects of these types of stressors on brain WM integrity.

1.6.1 DTI as a measure of WM integrity

DTI is a powerful imaging modality for *in vivo* characterization of WM in the brain (Le Bihan 2003). DTI is able to capture the spatiotemporal patterns of water diffusion in the brain on a microscopic scale using a variation of a typical MRI magnetization sequence (Le Bihan, Poupon et al. 2006; Jones and Leemans 2011). DTI capitalizes on a couple of key physiological properties of the brain: (1) the brain is mostly water (Kreis, Ernst et al. 1993) and (2) the myelin sheath surrounding myelinated axons is hydrophobic. WM can be mapped because myelinated axons restrict what would otherwise be non-directional diffusion (i.e. isotropic) of water, resulting in anisotropy (Le Bihan, Mangin et al. 2001). It is important to note that the scale at which this diffusion is measured is at the level of entire WM tracts, and not at the level of the individual axon (Jellison, Field et al. 2004); however the anisotropy measured using DTI can provide valuable information about the underlying neuronal properties (e.g. myelination, axonal diameter, axonal packing density in the tract, and subcellular composition, etc.) of the axons comprising the tracts. These diffusion properties include fractional anisotropy (FA), radial diffusivity (RD), and axial diffusivity (AD) (note that this is not an exhaustive list). These metrics can be used to characterize the local microorganization of brain WM. FA is calculated as the ratio of diffusion parallel to the fibers, to diffusion perpendicular to the fibers, and thus can be affected by either changes in diffusion perpendicular to the tract (measured by RD, which decreases with increased axonal myelination (Keller and Just 2009; Zhang, Jones et al. 2009; Bennett, Madden et al. 2010)), or to changes in diffusion parallel to the tract (measured by AD, which increases with axonal density, caliber, and microtubular packing and organization (Kumar, Macey et al. 2010; Kumar, Nguyen et al. 2012)). This is not only based on neuroimaging evidence, but also supported by combined DTI and histological studies performed in rodent and nonhuman primate brains (Song, Sun et al. 2002; Song, Sun et

al. 2003; Choe, Stepniewska et al. 2012). Thus, higher FA values in the presence of decreased RD, but no AD changes, could be interpreted as increased WM tract integrity due to increased myelin, whereas increased FA in parallel to increased AD, without RD changes, would indicate increased fiber tract organization. DTI studies describe a global increase in FA and, where effects of age on RD and AD were reported, decrease in RD and AD with age (Klingberg, Vaidya et al. 1999; Barnea-Goraly, Menon et al. 2005; Gao, Lin et al. 2009; Deoni, Mercure et al. 2011; Shi, Short et al. 2013). These reports are consistent with previous histological studies of WM maturation (Gibson 1991).

1.6.2 Strengths and limitations of DTI

Although DTI is a powerful imaging modality in the study of brain WM, as with any methodology, it has its strengths and limitations. DTI is susceptible to certain image artifacts, particularly motion. Methods have been developed to minimize these effects during image acquisition, and computational methods are used to address them during data processing (Le Bihan, Poupon et al. 2006). As described above, DTI measures microstructural properties of WM *indirectly*, and although it is an invaluable tool for *in vivo* WM tract identification it cannot provide the same detailed information that post-mortem tracer and histological studies offer (e.g. tract directionality). DTI is one of the best imaging modalities available to study WM connectivity and microstructure *in vivo*, a necessity when studying subjects longitudinally, making it especially useful in studies of brain development (Cascio, Gerig et al. 2007). Thus, it should not be treated as a substitute for these methods (Stone and Kötter 2002), but rather as an option for *in vivo* studies of longitudinal brain development needed to generate hypotheses to guide more detailed, although more time intensive, histological investigations at specific ages.

1.6.3 Stress effects on brain WM: role of GCs

The mechanisms by which stress could affect brain WM appear to involve primarily, although not exclusively, effects of GCs on axon myelination by oligodendrocytes. Although it is generally thought that stress results in decreased brain myelination, recent studies provide evidence for more complex regional and age-dependent effects of GCs, resulting in both increases or decreases in myelination (Jauregui-Huerta, Ruvalcaba-Delgadillo et al. 2010). In rodents, oligodendrocytes that form the myelin sheath express both GR and MR intracellular receptors (Bohn, Howard et al. 1991). Recent *in vitro* evidence suggests that GCs suppress proliferation of oligodendrocyte precursor cells in GM and WM (Alonso 2000). Developmental studies in rodents have provided evidence that GCs modulate oligodendrocyte differentiation and myelogenesis via regulation of key oligodendroglial proteins such as myelin basic protein (MBP) (Kumar, Cole et al. 1989). The effects of synthetic GCs differ as a function of gestational age, with GC-related decreases in MBP immunoreactivity and numbers of oligodendrocytes associated with younger ages (Antonow-Schlorke, Helgert et al. 2009). These findings in molecular studies of oligodendrocytes are supported by studies in sheep that report delays in myelination resulting from prenatal GC administration (Dunlop, Archer et al. 1997; Huang, Harper et al. 2001). Early postnatal exposure to a single dose of the synthetic GC methylprednisone in rodents resulted in decreased myelination (Gumbinas, Oda et al. 1973); however, more chronic manipulations of postnatal cortisol increased myelination (Casper, Vernadakis et al. 1967). Paradoxically, early adrenalectomy also results in increased myelination (Meyer and Fairman 1985). The synthetic GC dexamethasone has also been shown to signal the initiation, and enhance the rate of, myelination in *in vitro* studies (Chan, Phillips et al. 1998). GCs also upregulate genes involved in myelination (Zhu, Wiggins et al. 1994). Interestingly, there is evidence that GCs selectively inhibit cell death in oligodendrocytes but not in neurons

(Lee, Yan et al. 2008). Taken together, these studies provide evidence that brain WM is very sensitive to GCs, and thus, potentially social stress via exposure to elevated cortisol levels. Due to the strong role of brain WM on behavioral control (e.g. Nagy, Westerberg et al. 2004), GC-induced alterations in brain WM development could likely underlie the alterations reported in stress-related psychopathology. Although all this may be the result of stress-induced exposure to elevated GC levels during development, the relationship between age of exposure, type, and chronicity of the stressor is unclear and likely very complex.

1.7 Overall rationale and hypotheses

Based on the literature reviewed above, ELS, via repeated activations of the stress response and prolonged exposure to elevated levels of stress hormones, can interact with genetic factors (e.g. serotonin transporter polymorphisms) leading to increased risk for psychopathology in humans. However, different types, timing, and chronicity of social stress and ELS can result in different neurobehavioral alterations. Sex differences in vulnerability, trajectory and manifestations of stress-related effects, with females in general being at higher risk for stress-related psychopathologies, also exist. Brain alterations associated with ELS, including those reported in WM microstructure, are thought to contribute to this risk; however, how different types and durations of early experience lead to different alterations in brain development, how these brain alterations unfold during development, and how they are related to sex are not well understood in children. This is partially due to limitations of doing prospective, longitudinal studies in at risk pediatric populations, where confounding/comorbid conditions further compromise developmental analysis. Thus, the overarching goal of the studies presented

in this dissertation is to use two ethologically valid nonhuman primate models of social stress to: (1) Compare alterations in brain WM at different stages of development in order to address the hypothesis that WM microstructure in prefrontal-amygdala circuits will be altered in both of these models based on previous evidence of alterations in behaviors and HPA function regulated by these circuits in both models; (2) Address the hypothesis that alterations in WM microstructure in other regions, such as tracts connecting association cortices will also be present, however the regions affected and even the directionality of the effects will differ as a function of the specific experience due to differences in age of exposure to, type and duration of the stressor (i.e. lifetime for social subordination versus the first 3-6 postnatal months for infant maltreatment); (3) Examine how WM microstructure relates to socioemotional behavior in these two models.

These goals will be achieved with the following specific aims:

Aim 1: Investigate how social subordination stress in rhesus monkeys and genetic factors (5HTT gene polymorphisms) interact to affect brain WM tracts in prepubertal female macaques using DTI, and associations with socioemotional behavior: Given the literature reviewed above, I hypothesized that prefrontal tracts, specifically the UF, would be particularly affected in prepubertal female subordinate monkeys. In order to assess the functional correlates of dominance rank related brain differences, I also examined the associations between brain WM tract integrity, emotional behavior and measures of stress physiology in these juveniles. I chose to study females for this aim because they have been shown to be more vulnerable to 5HTT mediated effects of ELS (Barr, Newman et al. 2004), and the matrilineal social dominance hierarchies present in rhesus monkey groups are more ethologically salient

to females because they remain with their natal groups for life, in contrast to males, which emigrate at puberty.

Aim 2: Investigate how maltreatment experienced during the first 3-6 months of life affects brain WM in adolescent rhesus monkeys using DTI, and its associations with alterations in socioemotional behavior and neurochemistry: I investigated the relationship between WM integrity and plasma cortisol measured during the first month of life, when maltreated animals were experiencing high rates of abuse and rejection, as well as associations with aggression observed in adolescence. Based on the literature reviewed above I hypothesized that there would be microstructural differences as shown using FA, RD, and AD in WM tracts associated with the regulation of the HPA axis and aggressive behavior (cortico-limbic tracts, association cortices) in maltreated monkeys as compared to control animals. I also hypothesized that these alterations would have significant correlations with aggression, providing evidence that these microstructural WM changes are involved in mediating the long-term behavioral effects of ELS.

Aim 3: Investigate how the experience of infant maltreatment during the first 3-6 months of life, independent of potential confounding effects of heritable factors, affects microstructural integrity and emotional reactivity during infancy in rhesus monkeys. Using a cross-fostering paradigm to control for the confounding effects of heritable factors, I studied the effects of infant maltreatment longitudinally on WM and emotional reactivity. I also collected hair samples at birth and during infancy to measure accumulated cortisol to confirm that infant maltreatment is, indeed, a stressful experience. I hypothesized that cortisol accumulated in hair would be

greater in maltreated infants than controls, and that this increase in cortisol would be accompanied by decreases in WM integrity (i.e. decreased FA) in prefrontal-amygdala circuits during the same infant developmental period. In the longitudinal analysis, I hypothesized that the alterations in WM microstructure (i.e. decreases in FA) would occur in regions undergoing active myelination as part of normative development, thus alterations in prefrontal-amygdala circuits would not be present at the earliest ages studied, but would become evident at older ages. I also hypothesized that maltreated infants would exhibit increased emotional reactivity, confirming that the behavioral alterations previously reported were related to the experience of maltreatment. An additional hypothesis is that all these brain, behavioral and stress physiology alterations will be the product of the adverse experience, and not due to heritable factors. I also hypothesized that females would show the most drastic effects based on previous reports of increased vulnerability to ELS in female monkeys on the HPA axis (Clarke 1993; Barr, Newman et al. 2004) and the brain (Spinelli, Chefer et al. 2010).

Figure 1.1

Schematic diagram of pre- and post- natal brain development in humans, highlighting the developmental processes occurring during early life stress (reproduced with permission from Lenroot and Giedd 2006).

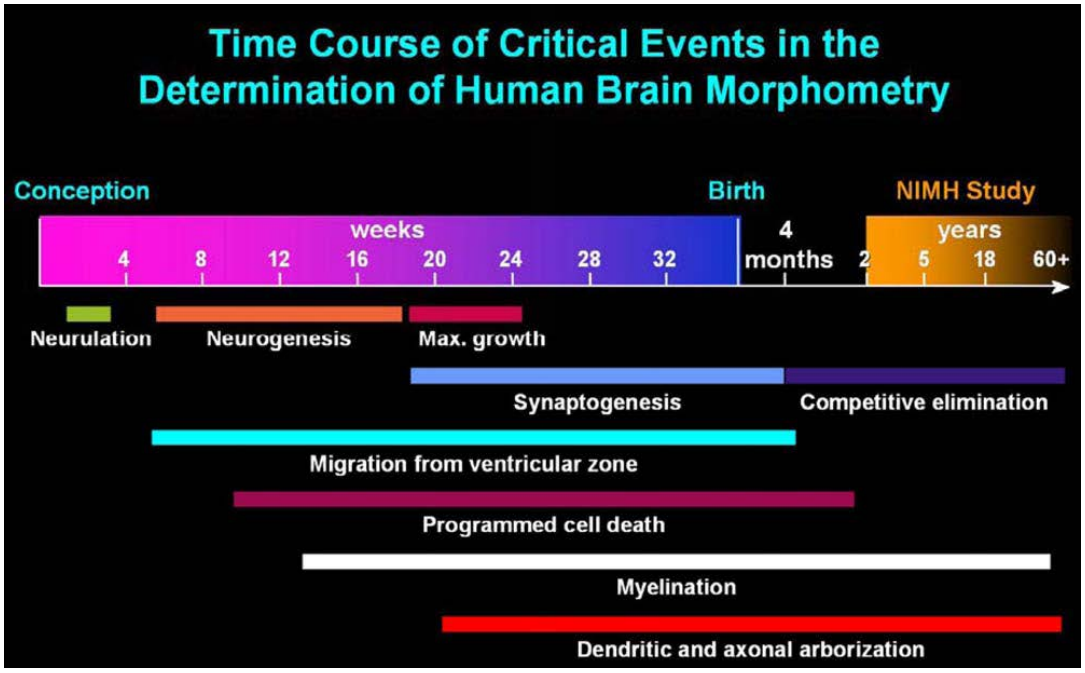
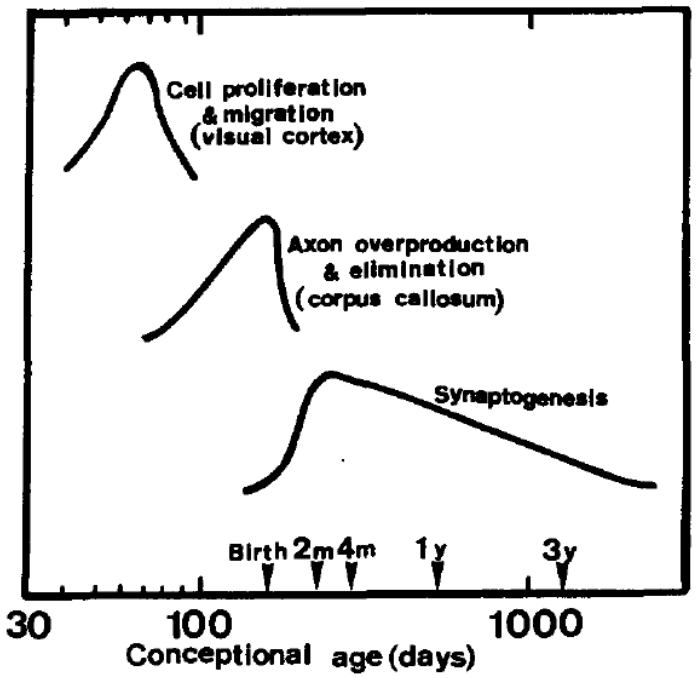


Figure 1.2

Schematic diagram of pre- and post- natal cerebral development in rhesus monkeys, highlighting the developmental processes occurring during early life stress (reproduced with permission from Hayashi 1992).



Chapter 2 : Long-term effects of early life stress on brain white matter and behavior: juvenile period in a model of social subordination stress[3]

[3] Modified slightly from Howell BR, Godfrey J, Gutman DA, Michopoulos V, Zhang X, Nair G, Hu X, Wilson ME, Sanchez MM. (2013) Social subordination stress and serotonin transporter polymorphisms: associations with brain white matter tract integrity and behavior in juvenile female macaques. *Cerebral Cortex*. In press.

2.1 Abstract

We examined the relationship between social rank and brain white matter (WM) microstructure, and socioemotional behavior, and its modulation by serotonin transporter (5HTT) polymorphisms in pre-pubertal female macaques. Using diffusion tensor imaging (Ardekani, Figarsky et al.) and tract-based spatial statistics (TBSS), social status differences were found in medial prefrontal (mPFC) WM and cortico-thalamic tracts, with subordinates showing higher WM structural integrity (measured as fractional anisotropy, FA) than dominant animals. 5HTT genotype-related differences were detected in the posterior limb of the internal capsule, where s-variants had higher FA than l/l animals. Status by 5HTT interaction effects were found in (1) external capsule (middle longitudinal fasciculus), (2) parietal WM and (3) short-range PFC tracts, with opposite effects in dominant and subordinate animals. In most regions showing FA differences, opposite differences were detected in radial diffusivity (RD), but none in axial diffusivity (AD), suggesting differences in tract integrity likely involve differences in myelin. These findings highlight that differences in social rank are associated with differences in WM structural integrity in juveniles, particularly in tracts connecting prefrontal, sensory processing, motor and association regions, sometimes modulated by 5HTT genotype. Differences in these tracts were associated with increased emotional reactivity in subordinates, particularly with higher submissive and fear behaviors.

2.2 Introduction

The primate brain has evolved to adapt to complex social environments (Dunbar and Shultz 2007) and, as a consequence, early social experiences have a strong influence on its development. In particular, social stress during childhood is associated with behavioral and cognitive impairments and increased risk for psychopathology and health problems later in life, including anxiety and mood disorders (Teicher, Andersen et al. 2002; Teicher, Andersen et al. 2003; Gunnar and Quevedo 2007; Weber, Rockstroh et al. 2008). These long-term adverse effects of stress are more prevalent in females than in males (Becker et al. 2007) and often emerge during puberty (Kessler et al. 2001; Reardon et al. 2009; Seeman 1997). However, it is likely that adverse social experiences prior to puberty are also critical for neurobehavioral changes.

In addition, genetic factors can affect vulnerability to these stressful experiences. For example, allelic variants in the promoter region of the gene encoding the serotonin (5HT) transporter (5HTT) affect emotional and neuroendocrine stress responses and modulate the vulnerability to the deleterious effects of early life stress, both in humans (Caspi et al. 2003; Caspi et al. 2010; Stein et al. 2008; Zalsman et al. 2006) and nonhuman primates (Barr et al. 2004; Bennett et al. 2002; Champoux et al. 2002; McCormack et al. 2009). In both species, the short (s) allele carriers -with less functional 5HTTs- seem more vulnerable to stress (Holmes and Hariri 2003; Suomi 2003). There is also evidence that these gene by environment interactions are more salient in females (Barr et al. 2004; Eley et al. 2004; Sjoberg et al. 2006). Despite the reported role of social stress and genetic variation in the etiology of psychopathology in females, particularly during the peripubertal period, the underlying neurobiological mechanisms are still not clearly understood.

One potential mechanism could be through effects on brain white matter (WM), which is sensitive to adverse experiences (Choi et al. 2009; Coplan et al. 2010; Eluvathingal et al. 2006; Govindan et al. 2010) and 5HT (Whitaker-Azmitia et al. 1996), particularly during development. The important functional role of brain WM has been underscored by the development of neuroimaging methods that visualize fiber tracts and measure their structural integrity *in vivo*, such as diffusion tensor imaging (Ardekani, Figarsky et al.) (Thomason and Thompson 2011). DTI measures water diffusion in the brain and provides measures of WM fiber tract properties, including fractional anisotropy (FA), a measure of structural integrity of WM tracts. Because increased tract microstructural integrity (via, for example, increased myelin) can affect information transfer by increasing conduction speed along the axon (Lang and Rosenbluth 2003; Paus 2010), brain WM tract integrity is recognized as an important mechanism underlying behavioral control (Fields 2008; Thomason and Thomson 2011). In addition, brain WM integrity is vulnerable to early stress/adversity (Coplan et al. 2010; Eluvathingal et al. 2006; Frodl et al. 2012; Govindan et al. 2010; Katz et al.2009) and is affected by polymorphisms in the 5HTT gene (Pacheco et al. 2009).

Prospective studies assessing the impact of persistent exposure to social stressors on brain WM in children are difficult. An example of cumulative adverse social experiences is low socioeconomic status (SES), which represents a complex form of chronic early social stress associated with socioemotional and cognitive impairments in children, also involving poor access to material and social resources (Bradley and Corwyn 2002; Hackman and Farah 2009). Low SES during childhood has also been shown to increase risk for depression in women (Gilman et al. 2002). Although there is some recent evidence of low SES effects on brain grey matter (GM) (Hackman and Farah 2009; Jednorog et al. 2012; Noble et al. 2012) the effects of continual adverse early social

experiences such as low SES on brain WM and associated effects on socioemotional deficits in girls are not understood.

To address some of these questions, the present study used a nonhuman primate model of chronic social stress, notably social subordination in rhesus monkeys, to investigate how this adverse experience and 5HTT polymorphisms interact to affect brain WM tracts in prepubertal female macaques using DTI. Subordinate dominance status in rhesus monkeys is enforced through unpredictable and recurring contact and non-contact aggression from more dominant animals in the group. Both subordinate adult (Bernstein et al. 1974; Bernstein and Gordon 1974; Bernstein 1976; Shively and Kaplan 1984) and juvenile females (Bernstein and Ehardt 1985) receive more aggression from higher-ranking group mates and terminate these interactions by emitting submissive behavior, a defining feature of subordination. Because offspring of group living macaques assume the relative rank of their mother (Sade 1967), offspring of subordinate mothers are exposed to high rates of aggression from birth. Consequences of this unpredictable, continual harassment in adult females include stress-related phenotypes, including hypothalamic- pituitary-adrenal axis (HPA) dysregulation, evidenced by hypercortisolemia and reduced glucocorticoid (Price, Close et al.) negative feedback (Jarrell et al. 2008; Kaplan et al. 2010; Shively et al. 1997). Thus, social subordination is a well-established nonhuman primate model of chronic psychosocial stress used to study its adverse effects on a broad range of adult behavioral, neuroendocrine and health outcomes (Gust et al. 1991; Kaplan et al. 1996; Kaplan et al. 2010; Michopoulos et al. 2009; Morgan et al. 2002; Paiardini et al. 2009; Tung et al. 2012). While the subordinate phenotype is less well understood in juveniles, they undergo delayed puberty (Wilson et al. 1986; Wilson et al, 2013; Zehr et al. 2005), an effect exacerbated in 5HTT s-allele carriers (Wilson and Kinkead 2008) and associated with increased emotional reactivity (Wilson et al, 2013). However, the neurobiological

effects of social subordination on these juvenile females are unknown. In this study, we used DTI and tract-based spatial statistics (TBSS) to examine differences in brain WM tract integrity between dominant and subordinate females prior to puberty, and its modulation by 5HTT polymorphisms. WM tract integrity was measured by FA, in parallel with radial diffusivity (RD) and axial diffusivity (AD) measures to aid with the interpretation of the local microstructural mechanisms involved (Bennett et al. 2010; Burzynska et al. 2010; Hu et al. 2011; Keller and Just 2009; Metwalli et al. 2010; Shamy et al. 2010; Shi et al. 2012; Tang et al. 2012; Taubert et al. 2012; Wheeler-Kingshott and Cercignani 2009). Given recent evidence that social subordination is associated with reduced GM density in prefrontal cortex of adult male macaques as measured by MRI (Sallet et al. 2011), we hypothesized that prefrontal tracts would be particularly affected in prepubertal female subordinate monkeys, as well. In order to assess the functional correlates of social rank-related brain differences, we also examined the associations between brain WM tract integrity, emotional behavior and measures of stress physiology in these juveniles.

2.3 Methods

2.3.1 Subjects

Subjects were thirty-five prepubescent female rhesus monkeys (*Macaca mulatta*) living in four social groups consisting of 60-100 adult females and their juvenile offspring, and 4-6 adult males. Animals were housed in outdoor enclosures (three quarters of an acre area) with access to climate-controlled indoor facilities at the Yerkes National Primate Research Center (YNPRC) Field Station in Lawrenceville, GA. Free access to a standard low-fat, high-fiber diet (Purina Mills Int., Lab Diets, St. Louis, MO)

and water was provided. Subjects were studied between 14-22 mo, prior to menarche, which was reached by these females at 29.92±0.62 mo (Wilson et al. 2013) consistent with other reports for outdoor-housed female rhesus monkeys around 26 mo of age (Wilson et al. 1986). All procedures were approved by the Emory University Institutional Animal Care and Use Committee in accordance with the Animal Welfare Act and the U.S. Department of Health and Human Services "Guide for Care and Use of Laboratory Animals".

2.3.2 Determination of social rank

Each subject's relative dominance rank within her natal group was determined based on outcomes of dyadic agonistic interactions in which a subordinate female is one who unequivocally emits a submissive behavior in response to an approach or to an actual aggressive act from a more dominant animal (Bernstein 1976). Thus, dominant rank was not determined by who aggresses whom but rather who submits to whom. Each animal's relative rank was calculated as the ratio of her rank to the total number of animals in her group, exclusive of animals <12 months old. Thus, a subject ranking 25 out of a group of 100 animals received a relative rank of 0.25. For the present analysis, we compared the most subordinate females in the cohort (animals with a relative rank of >0.60, representing the 40% lowest ranking animals; n = 13) to more dominant females (n = 22). This relative rank value was also used in regression analyses to determine its relation to other behavioral and physiological phenotypes (see below).

2.3.3 5HTT genotyping

All subjects were genotyped for polymorphisms in the 5HTT promoter gene (SLC6A4), as described previously (Hoffman et al. 2007). Of the 35 females, 17 had both alleles of the long promoter length variant (l/l: 10 dominant, 7 subordinate) and 18 had at least one short allele (s-variant: 12 dominant -9 l/s, 3 s/s-, 6 subordinate -4 l/s, 2 s/s-

). Subjects with l/s or s/s genotype were combined (s-variant or */s) based on the low occurrence of the s/s genotype and previous reports that both genotypes result in similar phenotypes in rhesus monkeys (Champoux et al. 2002).

2.3.4 Coefficients of relatedness

Because this colony is pedigreed, kinship coefficients were determined (Hamilton 1964) per rank and 5HTT genotype. Average coefficients for dominant-l/l, dominant-s-variant, subordinate-l/l and subordinate-/s-variant groups were 0.040, 0.045, 0.062, and 0.082, respectively, indicating that any shared genetic factors within each status-genotype category would not explain the phenotypic differences between the groups.

2.3.5 DTI data

2.3.5.1 DTI acquisition

Subjects were transported from their social group to the YNPRC Imaging Center the day before the scans. The scanning age was not different between dominant and subordinate subjects (20.68±0.29 vs. 20.89±0.33 mo) nor between l/l and s-allelic variant females (20.68±0.36 vs. 20.90±0.28), as described in Results, and corresponds to a juvenile period before puberty onset (menarche) for these females (29.92±0.62 mo) (Wilson et al. 2013). A DTI and a T1-weighted MRI scan were acquired during the same session using a 3T Siemens Magnetom TRIO system (Siemens Med. Sol., Malvern, PA) and an 8-channel phase array coil. All animals were scanned supine in the same orientation, achieved by placement and immobilization of the head in a custom-made head holder via ear bars and a mouth piece. A vitamin E capsule was taped on the right temple to mark the right side of the brain. Scans were acquired under isoflurane anesthesia (1-1.2% to effect, inhalation), following initial induction with telazol (5mg/kg, i.m.). Animals were fitted with an oximeter, ECG, rectal thermistor and blood pressure

monitor for physiological monitoring, an i.v catheter to administer dextrose/NaCl (0.45%) to maintain normal hydration, and an MRI-compatible heating pad. Upon completion of the scans and full recovery from anesthesia, each female was returned to her social group.

DTI scans were acquired following published protocols by our group for rhesus monkeys (Hecht et al. 2013), using a single-shot dual spin-echo EPI sequence with GRAPPA (R=3), voxel size=1.3x1.3x1.3mm with zero gap, 60 directions, TR/TE=5000/86ms, FOV= 83mm, b:0, 1000s/mm^2, and 12 averages. T1-MRI scans were acquired using a 3D magnetization prepared rapid gradient echo (3D-MPRAGE) parallel imaging sequence with GRAPPA (R=2), voxel size=0.5x0.5x0.5mm^3, TI/TR/TE=950/3000/3.49ms, FOV=96mm, 8 averages.

2.3.5.2 DTI image analysis

Preprocessing

The FMRIB Software Library FSL, FMRIB, Oxford UK, (Smith et al. 2004; Woolrich et al. 2009) FDT tool was used to fit a tensor model at each voxel in native diffusion space to calculate the diffusion properties selected for this study (FA and AD; RD was calculated at each voxel by averaging the second and third eigenvalues for each voxel using the fslmaths command in FSL) after correcting for B0 inhomogeneity-induced distortion and eddy current effects, and skull-stripping using the BET FSL tool (Smith 2002).

These FSL diffusion analysis tools have been previously applied with success to rhesus brain DTI data by our group (Hecht et al. 2013) and others (Bendlin et al. 2011; Makris et al. 2007; Willette et al. 2010; Willette et al. 2012). The diffusion properties examined (FA, RD, and AD) characterize the local microorganization of brain WM and have been previously used in combination in DTI studies describing developmental changes of brain tracts (Shi et al. 2012) and effects of lesions (Shamy et al. 2010) in this

species, as well as effects of experience in humans e.g. (Tang et al. 2012). This is because changes in FA (calculated as the ratio of diffusion parallel to the fibers to the diffusion perpendicular to the fibers) can be due to either changes in perpendicular diffusion along the tract measured by RD, which decreases with increased axonal myelination (Bennett et al. 2010; Keller and Just 2009; Zhang et al. 2009) or to changes in parallel diffusivity measured by AD, which increases with axonal density, caliber, and microtubular packing and organization (Kumar et al. 2010; Kumar et al. 2012), evidence also supported by combined DTI and histological studies performed in rodent and nonhuman primate brains (Choe et al. 2012; Song et al. 2002; Song et al. 2003). Thus, higher FA values in the presence of decreased RD, but no AD changes, is generally interpreted as increased WM tract integrity due to increased myelination, whereas increased FA in parallel to increased AD, without RD changes, indicates increased fiber tract organization.

Tract-based Spatial Statistics

The FSL TBSS tool (Smith et al. 2006) was used to identify the centers of all major WM tracts present in all subjects. This method uses nonlinear registrations (Rueckert et al. 1999) to align all subjects' FA data to a predetermined common space or template image, from which a group mean FA image is calculated. In our study, the data was registered to the Wisconsin 112RM-SL monkey atlas (McLaren et al. 2009; McLaren et al. 2010), resulting in a final resolution of 0.5mmx0.5mmx0.5mm. The mean FA image was then skeletonized and thresholded (only voxels with FA>0.2 were included) so that only the centers of major tracts are included in the analysis, excluding small peripheral tracts that may confound findings due to anatomic individual variability and partial volume effects. Each subjects' individual FA values are then projected onto the mean FA skeleton (Smith et al. 2006). Skeletonized-FA data significantly minimizes the number of voxels included in the voxel-wise statistical analysis (described below),

cutting down multiple comparisons, and is less dependent on the accuracy of the initial registrations. A version of the tbss_non_FA script modified to use the Wisconsin 112RM-SL monkey atlas space was applied to generate each subject's skeletonized RD and MD maps.

Statistical analysis of DTI measures

A voxel-wise two-way analysis of variance (Neuner, Kupriyanova et al.) was run to examine the effects of social status (high vs. low-ranking) and 5HTT genotype (l/l vs. s-variant) on skeletonized FA data using Analysis of Functional NeuroImages (Papaioannou, Dafni et al.) (Cox 1996). Results were considered significant at $p<0.05$, after cluster-correction for clusters larger than 150 significant contiguous voxels (18.75 mm^3), a more stringent criteria than previously used for similar rhesus monkey DTI studies (Willette et al. 2010). Results (significant clusters >150 voxels) were displayed in the Wisconsin 112RM-SL rhesus atlas (McLaren et al. 2009; McLaren et al. 2010), which is in the brain coordinate space of the Saleem-Logothetis rhesus atlas (Saleem and Logothetis 2012).

Binary masks were created for the regions showing FA differences (i.e. voxel clusters of significant FA differences detected in the voxel-wise ANOVA, status and/or 5HTT genotype effects). The mean RD and AD were calculated within these regions, following previously published approaches (Smith et al. 2004; Tang et al. 2012). A two-way ANOVA was run in SPSS to examine the effects of status and 5HTT genotype on RD and AD in those clusters with significant FA differences, with significance level set at $p<0.05$. The mean FA was also calculated for each cluster to examine its correlations with behavioral and cortisol data using Pearson correlation (see details below). Finally, a test-retest analysis of stability and replicability of DTI FA, RD and AD measures was

performed in clusters with significant effects in a small group of animals (n=5) that were scanned twice, a few weeks apart, using a paired t-test.

DTI probabilistic tractography

To identify the most likely fiber tract(s) passing through the WM regions with significant status and/or 5HTT genotype effects, we performed probabilistic tractography in each individual macaque brain using the FSL imaging suite and methods published by our group for nonhuman primates (Hecht et al. 2013), rodents (Gutman et al. 2012a; Gutman et al. 2012b) and humans (Gutman et al. 2009). A pipeline was constructed using the NiPype framework (Gorgolewski et al. 2011) to perform image registration and to generate tractography maps for each subject. Briefly, the reference volume from the DTI data set (the "nodif" or b0 volume) was registered to the template Wisconsin 112RM-SL rhesus atlas (McLaren et al. 2009; McLaren et al. 2010) using a 12-DOF linear registration as implemented in the FSL FLIRT module. Regions of interest (ROIs) for probabilistic tractography were derived from the significant cluster masks previously identified in the TBSS analysis. For each ROI, probabilistic tractography was computed in template space for each voxel within the seed mask using the default parameters in FSL using distance correction, resulting in a probabilistic tractography map for each ROI (based on the 7 significant clusters identified) for each subject. All tractography used a multi-fiber reconstruction algorithm implemented in FSL, bedpostX, which permits the reconstruction of geometrically complex pathways, including crossing fibers (Behrens et al. 2007). After tractography, each individual raw tract map was thresholded at 1% of the robust mean intensity and binarized, and combined to produce a group probability map; in this composite image (group probability map) the intensity of each voxel represents the number of individual subjects that showed connectivity with that voxel after thresholding. For visualization, the

composite images were then thresholded at a group level to highlight only voxels that were common to at least 40% of subjects; of note we evaluated the effects of both relaxed and more stringent subject and group level thresholds which produced qualitatively similar results. Resulting fiber-tracts were identified using available rhesus brain and fiber pathways atlases (Saleem and Logothetis 2012; Schmahmann and Pandya 2006; Schmahmann et al. 2007a).

2.3.6 Behavioral data

2.3.6.1 Group social observations

Focal behavioral observations (2 x 30 min) were collected monthly from 14.3±0.24 until 22.5±0.24 mo for each subject using an established rhesus ethogram (Altmann 1962) with modifications (Maestripieri et al. 2006). Behavioral categories included affiliative (proximity, grooming, contact), agonistic (aggression, submission), anxiety-like yawn, body shake, scratch (Schino et al. 1996; Troisi et al. 1991), and play behaviors. Data were recorded using netbooks and a custom-designed program that captures initiator, behavior, recipient and time (Graves and Wallen 2006). Inter-observer reliability, calculated as inter-observer % agreement on the frequencies and durations of behaviors emitted by a female during formalized observations, exceeded 92%. Durations and frequencies of behaviors were averaged across observation sessions for analysis.

2.3.6.2 Emotionality testing

The Human Intruder (HI) paradigm (Kalin and Shelton 1989) and the Approach-Avoidance (AA) task (Meunier et al. 1999) were performed before the scans (at 18.37±0.11 mo) to examine associations of the subjects' emotional behavior with the

neuroimaging data. These tasks evoke strong and distinct behavioral responses to novel, threatening, stimuli (Kalin and Shelton 1989; Machado et al. 2009; Meunier et al. 1999), and have been used to examine the effects of early stress (Grand et al. 2005) and 5HTT genotypes (Bethea et al. 2004) in rhesus monkeys. The Human Intruder (HI) paradigm (Kalin and Shelton 1989) is comprised of three consecutive ten-minute conditions: (1) an alone (AL) condition that elicits distress vocalizations and locomotion; (2) a profile (PR) condition where an unfamiliar experimenter enters the testing room and presents his/her facial profile towards the monkey, who typically stops locomoting/vocalizing and freezes while scanning the intruder; and a stare (ST) condition, during which the experimenter faces the animal and makes continuous eye contact with it, a threatening behavior for rhesus macaques that induces aggressive and submissive behaviors towards the intruder. Sessions were videotaped and scored for frequencies and durations of behaviors following a previously published ethogram and procedures (Machado and Bachevalier 2006), with an inter-rater reliability >92%.

The Approach Avoidance (AA) task (Meunier et al. 1999) was done a week apart from the HI and consisted of six five-minute sessions, during which a different novel object (three neutral, three fear-evoking) was presented in a tray per session, in parallel to a food reward (jellybean) to drive approach behaviors. For the present analysis, we only report responses to the most fear-evoking object (battery operated toy pig that moved and emitted sounds). AA sessions were also videotaped and scored for frequencies and durations of behaviors (exploration and locomotion; aggressive, submissive, anxiety-like and fearful behaviors; latency to touch/inspect objects and to eat the jellybean) using a published ethogram (Machado and Bachevalier 2006). Inter-observer reliability was greater than 92%.

2.3.6.3 Statistical analysis: behavioral associations with rank and neuroimaging data

A principal component analysis (PCA) using a rotation method of Oblimin with Kaiser normalization was performed using SPSS 19.0 software separately for the AA and HI data to group behaviors into similar patterns of responses that represent the most prominent behavioral phenotypes, as previously done in rhesus monkeys (Williamson et al. 2003). The AA PCA included behavioral responses to the motorized pig and the HI PCA included behavioral responses to the most threatening conditions, the PR and ST (Tables 2.1 and 2.2). Behaviors with loading scores ≤0.4 were excluded from the analysis. Composite scores were calculated for each PCA component identified for correlational analysis.

Linear regression assessed the relation between relative social rank and levels of aggression, submission and affiliation exhibited in the social group. In addition, Pearson product moment correlations were used to examine the associations between neuroimaging measures (mean FA in clusters with significant status and/or 5HTT genotype effects) and behavioral data (behaviors in the social group and factors derived from the HI and AA PCAs). A Sidak correction for multiple correlations was used to set the significance level for the regression analyses. In addition to the magnitude of the correlation coefficient, Cohen d values were also reported to provide the effect size of the associations. Using established conventions (Cohen 1992), effect sizes >0.3 and <0.7 were considered moderate and those >0.7 large.

2.3.7 Cortisol data

Serum cortisol levels were measured to examine HPA axis basal activity and stress reactivity a week before the HI and AA tasks. For this, a baseline blood sample was

collected from the animal's saphenous vein in the awake state, within 10 minutes from initial access of the group, following previously published protocols (Arce et al. 2010; McCormack et al. 2009). Immediately after, the subject was transported to a novel behavioral testing room and placed in a testing cage for 30 minutes, when another blood sample (post-stress) was obtained to measure stress-induced increase in cortisol secretion. This brief social separation is a potent stressor in monkeys that activates the HPA axis (Arce et al. 2010). Cortisol assays were performed at the YNPRC Biomarkers Core Lab using a commercially available RIA kit (Beckman-Coulter/DSL, Webster TX), with a range from 0.50-60 µg/dl, and intra- and inter-assay CVs of 4.9% and 8.7%, respectively.

2.3.7.1 Statistical analysis: associations with rank and neuroimaging data

Linear regression models assessed the relation between relative social rank and cortisol levels (baseline and stress-induced increases [the latter measured as the delta from baseline to post-stress levels]). Pearson product moment correlations were used to examine the associations between cortisol (baseline and stress increases) and FA measures. Statistical significance level was set at $p<0.05$.

2.4 Results

2.4.1 DTI data

2.4.1.1 Social status and 5HTT genotype effects on FA measures

No differences in scanning age were detected by two-way ANOVA for social status (dominants=21.05±0.30, subordinates=20.69±0.33 mo; $F_{1,31}$=0.476, p=0.495) or 5HTT genotype (l/l=20.76±0.37, */s=21.06±0.26 mo; $F_{1,31}$=0.476, p=0.495).

Two-way ANOVA of the TBSS results identified several regions of the FA skeleton that exceeded the criteria for significance ($p<0.05$, cluster-corrected for clusters >150 significant, contiguous, voxels).

A main effect of status was found in three separate clusters (Figures 2.1-2.2): one in left medial prefrontal cortex (mPFC) (cluster 1, 204 voxels), and two in the WM along the left dorsal medial wall (cluster 2, 202 voxels; cluster 3, 196 voxels). In all these clusters low-ranking animals had higher FA than dominants regardless of 5HTT genotype. Probabilistic tractography identified cluster 1 as involving local, short range, intrahemispheric mPFC fibers, some of them cross-hemispheric (crossing through the genu of the corpus callosum -CC- in 40-60% of the animals; Fig. 2.1C). Clusters 2 and 3 both involved short-range cortico-cortical fibers (intra- and inter-hemispheric) in the dorsomedial wall interconnecting frontal regions corresponding to the supplementary motor, premotor and primary motor cortex in most animals, and also connecting these frontal motor areas with somatosensory cortex in 25-50% of subjects (Fig. 2.2C). In addition, cluster 3 also contained cortico-thalamic tracts connecting somatosensory and primary motor cortices with thalamic regions (Fig. 2.2D). Based on proximity and overlap in the tractography results, clusters 2 and 3 appear to be part of the same network.

A main 5HTT genotype effect was detected in a single cluster (cluster 4, 153 voxels), in the left posterior limb of the internal capsule (PLIC, Fig. 2.3), with s-variants showing higher FA than l/l animals regardless of social status. Probabilistic tractography identified this cluster as involving mostly cortical descending pathways, including corticorubral and corticospinal tracts with bilateral connectivity (via the corpus callosum) in about 50% of the animals (Fig. 2.3A).

A significant status x 5HTT genotype interaction effect was detected in three different clusters: one in the left external capsule (EC; cluster 5, 242 voxels; Fig. 2.4), one in the right parietal cortex WM (cluster 6, 167 voxels; Fig. 2.5) and a third in left prefrontal cortex (PFC) WM (cluster 7, 157 voxels; Fig. 2.6). In all three of these clusters dominant l/l animals had lower FA than s-variant animals, while the opposite was true for low-ranking animals (l/l had higher FA than s-variants). Probabilistic tractography identified cluster 5 (EC) as involving the left middle longitudinal fasciculus (MLF; Fig. 2.4C), a long association parieto-temporal tract that reciprocally connects the inferoparietal cortex with cortical areas in the superior temporal sulcus (STS) and gyrus (STG) as identified in macaques by tract-tracing (Schmahmann and Pandya 2006) and diffusion spectrum imaging (Schmahmann et al. 2007a). Cluster 6 (parietal cortex WM) involved short range parietal “U” fibers (Fig. 2.5C), short cortico-cortical association fiber tracts. Cluster 7 involved short-range prefrontal tracts connecting dorsolateral, ventrolateral and dorsomedial PFC in the left hemisphere and across the two hemispheres in 40-60% of subjects (Fig. 2.6C).

2.4.1.2 Status and 5HTT genotype effects on RD and AD measures

We used a two-way ANOVA to analyze status and 5HTT genotype effects on RD and AD in those clusters with significant FA differences and found 2 different patterns of results:

(1) In most clusters the significant FA effects (status, 5HTT genotype or status x genotype) were accompanied by opposite effects on RD, but not on AD; thus, in clusters 1, 2 and 3, where a main status effect of FA was detected (with subordinates showing increased FA), an opposite effect was found on RD, so that subordinate animals showed lower RD than dominants (cluster 1, $F_{1,31}$=4.445, p=0.043; cluster 2, $F_{1,31}$=10.025, p=0.003; cluster 3, $F_{1,31}$=8.993, p=0.005), without differences in AD; similarly, in cluster 4, where a main 5HTT genotype effect was detected on FA (with s-variants showing higher FA than l/l animals), the opposite genotype effect was detected on RD, so that s-allele carriers showed lower RD than l/l animals ($F_{1,31}$=18.272, p=0.0000169), without differences in AD; furthermore, in clusters 6 and 7, which showed status x genotype effects on FA (with dominant l/l animals showing lower FA than s-variant animals, while the opposite was true for low-ranking animals), an opposite interaction effect was detected on RD, so that dominant l/l subjects showed higher RD than s/* and the opposite was true for low-ranking animals (cluster 6: $F_{1,31}$=8.369, p=0.007; cluster 7: $F_{1,31}$=4.535, p=0.041), with no effects detected on AD; (2) Only cluster 5 showed a different pattern of effects, where the status x genotype interaction effect on FA (with dominant l/l animals showing lower FA than s-variant animals, while the opposite was true for low-ranking animals) was accompanied by similar effects on AD, but no effects at all on RD, so that high-ranking, l/l animals had lower AD than high s/*, and the opposite was true for low-ranking animals ($F_{1,31}$=8.305, p=0.007).

2.4.1.3 Test-retest analysis of stability and replicability of DTI measures

No significant test-retest differences were detected for DTI measures in any of the seven identified clusters described above. This analysis suggests the measures are stable and reproducible and that the experimental group differences reported are not spurious.

2.4.2 Behavioral data

2.4.2.1 Group social observations: status and 5HTT genotype associations

Regression analyses demonstrated that higher-ranking juveniles, indeed, direct higher rates of aggressive behaviors towards others ($r=-0.46$, $p<0.01$), whereas rates of aggression received ($r=-0.35$, $p<0.03$) and submissive behaviors emitted increased significantly with more subordinate status ($r=0.34$, $p=0.04$). The degree by which social rank predicted these behaviors was not affected by adding 5HTT genotype to the regression model.

2.4.2.2 Emotionality testing: PCA

Four components were identified in the HI paradigm PCA (Table 2.1): fear, submission, anxiety and distress. The first component accounted for 22.1% of the variance and was labeled “fear” based on the positive loading of freezing during the PR condition, behavior reflecting fearfulness/anxiety in this task (Kalin and Shelton 1989). In addition, turn away during the ST condition loaded negatively. The second component accounted for 19.1% of the variance and included lipsmack and gaze avert during the ST condition, both of which loaded positively and indicate “submissive” or pacificatory behaviors used to neutralize a potentially threatening interaction (Altmann 1962). The third component (labeled “anxiety”) accounted for 17.5% of the variance and included anxiety-like behaviors yawn, scratch (Schino et al. 1996) and toothgrinding also reflecting distress and anxiety (Machado and Bachevalier 2006; Williamson et al. 2003) during the ST condition, all with positive loadings. The fourth component (labeled “distress”) accounted for 13.6% of the variance and included coo vocalizations and threats during ST (both with negative loadings), a positive score in this component

indicating low frequencies of these behaviors and thus lower reactivity and distress based on previous reports of PCA loadings for rhesus monkeys (Williamson et al. 2003).

Three components were identified in the AA task PCA for behaviors exhibited in response to the most fear-evoking object (motorized pig) (Table 2.2): anxious aggression, vigilance/anxiety and impulsivity. The first component accounted for 30.2% of the variance and included threat object (positive loading), look away from object (functioning to avoid interaction with the object; positive loading) and bite object (negative loading). While threat is considered an aggressive behavior for this species (Altmann 1962), in the context of the AA and the other behaviors it may reflect more defensive aggression (Meunier et al. 1999) and we considered positive scores on this component as indicating "anxious aggression". The second component accounted for 20.5% of the variance and included locomotion (positive loading) and visually inspecting the object (negative loading), thus a high score on this component indicates high exploration and low "vigilance/anxiety". The third component accounted for 15.3% of the variance and included latency to take the jellybean and latency to explore the testing box, both with negative loadings, thus a high score on this component indicates shorter latencies indicating increased "impulsivity".

2.4.3 Behavioral correlations with FA measures

Mean FA in significant clusters was associated with specific behaviors from the social group as well as from component scores of the HI and AA PCAs, all showing moderate to large effect sizes (Table 2.3). Mean FA in cluster 1 (left mPFC tracts) positively correlated with HI-component-1 (fear) and with submissive behavior in the social group, so that animals with higher FA were more fearful and submissive. Mean FA in cluster 2 (left cortico-thalamic tracts) was also positively correlated with social submission. Mean FA in cluster 4 (left PLIC) was positively correlated with group

observations of submissive behavior and negatively with play behavior. FA in cluster 5 (left MLF) positively correlated with HI-component-2 (submission towards HI). FA in cluster 6 (right parietal "U" fibers) negatively correlated with HI-component-3 (anxiety) and AA-component-1 (anxious aggression), so that animals with higher FA showed less anxiety-related behaviors. FA in cluster 7 (left short range prefrontal fibers) negatively correlated with AA-component-1 (anxious aggression), and positively with HI-component-2 (submissive behaviors towards HI), with animals with higher FA exhibiting less anxious aggression and more submission towards HI.

Additional correlational analyses were performed to better understand how behaviors in different contexts related to each other as well as to relative dominance ranks within the social groups. HI-component-1 (fear) was negatively correlated with social aggression ($r=-0.445$, $p=0.014$, Cohen's $d=-0.994$), suggesting that animals that were more fearful during the HI displayed less social aggression in their groups (i.e. subordinate animals). Indeed, more subordinate status predicted higher scores on this fear-related component ($r=0.343$, $p=0.059$, Cohen's $d=0.730$). In addition, HI-component-3 (anxiety) correlated positively with AA-component-1 (anxious aggression; $r=0.613$, $p=0.0003$, Cohen's $d=1.552$) but negatively with submissive behaviors in the social group ($r=-0.400$, $p=0.029$, Cohen's $d=0.873$), so that less anxiety during the HI task was associated with less anxious aggression during the AA, and with more submission in the group. Finally, more subordinate status predicted higher scores on the HI-submissive component ($r=0.390$, $p=0.030$, Cohen's $d=0.847$), consistent with higher rates of lip-smacking during the stare condition of the HI ($r=0.459$, $p=0.005$, Cohen's $d=1.033$). However, subordinate status also predicted a higher frequency of threats ($r=0.326$, $p=0.053$, Cohen's $d=0.690$) whereas more dominant status predicted longer duration of freezing during the stare condition of the HI ($r=-0.358$, $p=0.032$, Cohen's

d=-0.767), reflecting two different strategies for dealing with a threatening situation, depending on rank.

2.4.4 Cortisol data: associations with status, 5HTT genotype and FA measures

Relative rank was also included in regression analyses to determine its relation to HPA axis activity (baseline and stress-induced cortisol). More subordinate status significantly predicted higher baseline cortisol concentrations ($r=0.37$, $p=0.04$, Cohen's $d=0.797$) but a more blunted cortisol stress response to the social separation test ($r=-0.44$, $p<0.01$, Cohen's $d=-0.980$). The degree by which social rank predicted these phenotypes was not affected by adding 5HTT genotype to the regression model.

When examining the associations between mean FA in each significant cluster and cortisol measures (baseline and stress-induced increases), no significant correlations were found in any brain regions.

2.5 Discussion

To the best of our knowledge, this is the first DTI study to examine the relationship between social subordination and brain WM tract integrity in association with socioemotional behavior, and its modulation by 5HTT polymorphisms in a prepubertal female macaque model. At this juvenile stage of development social rank-related differences were found in tracts connecting prefrontal, motor, association and sensory processing brain regions, with subordinate females showing higher WM integrity than dominant animals, sometimes modulated by 5HTT genotype. Microstructural differences in these tracts could affect emotional and sensory processing, leading to

increased emotional reactivity in subordinates, as supported by the associations detected between FA and submissive and fear behaviors, and consistent with reports in human conditions (Kim and Whalen 2009; Noriuchi et al. 2010). In most brain regions showing differences in FA, a measure of WM tract integrity, opposite differences were detected in RD, but not AD, suggesting that differences in tract integrity likely involve differences in myelin (Bennett et al. 2010; Choe et al. 2012; Hu et al. 2011; Keller and Just 2009; Song et al. 2002; Song et al. 2003; Zhang et al. 2009). The one exception was the EC (MLF), where FA differences were accompanied by differences in AD, but not RD, indicating different mechanisms involved e.g. differences in axon density/caliber, cytoskeletal organization (Choe et al. 2012; Kumar et al. 2010; Kumar et al. 2012; Song et al. 2002). While our study design cannot disentangle genetic or prenatal effects from the postnatal experience of social subordination, these findings provide important evidence that differences in social status up to the prepubertal period are associated with differences in brain WM structure linked to behavioral function.

At the behavioral level, subordinate juveniles received more aggression from higher-ranking animals and exhibited more submission than dominants, consistent with the behavioral phenotype reported in the literature for pre-adult low-ranking rhesus females (Bernstein and Ehardt 1985). In adults, the unpredictable, continual harassment experienced by subordinate females results in stress-related phenotypes, including hypercortisolemia and HPA dysregulation (Jarrell et al. 2008; Kaplan et al. 2010; Michopoulos et al. 2012; Shively et al. 1997). Consistent with those reports, the juvenile subordinates in this study also showed elevated basal cortisol suggesting that subordination during the prepubertal period is, indeed, a psychosocial stress that results in neuroendocrine signs of chronic stress already at this young age.

Neuroimaging analyses showed that subordinate females had increased WM tract structural integrity (increased FA) compared with higher-ranking animals in 3 regions.

These included mPFC WM involving local, intra- and interhemispheric tracts, as well as WM along the dorsomedial wall which involved: (1) fibers interconnecting frontal regions in the supplementary motor, premotor and primary motor cortex, and these frontal motor areas with somatosensory cortex and (2) cortico-thalamic tracts connecting somatosensory and primary motor cortices with thalamus, which transform sensory information into action and are also potentially part of the mirror neuron or motor representation systems (Rizzolatti and Fadiga 1998). The fact that the effects of social subordination were limited to limbic prefrontal and frontal tracts suggests specificity of rank-related differences, i.e. these were not widespread effects. This observation is important given that PFC and frontal regions are particularly vulnerable to early life stress in primates (De Bellis et al. 2002; Kaufman and Charney 2001; Sanchez et al. 1998; Sanchez et al. 2001; Spinelli et al. 2009; Teicher et al. 2003) and WM alterations have been reported in these regions in children and nonhuman primates exposed to other forms of chronic stress during development (Govindan et al, 2010; Paul, 2008; Eluvathingal et al., 2006; Coplan et al, 2010). A caveat of our study design, though, is that it cannot separate the contribution of postnatal social stress experience from potential genetic or maternal experiential prenatal effects on the structural differences observed in subordinates. However, consistent with the possibility that subordination effects on brain white matter could be a consequence of chronic stress (as supported by the elevated cortisol levels in subordinates), region-specific effects of chronic stress have been reported specifically on mPFC neuronal structure, including retraction of dendritic fields and synaptic loss, in primates and rodents (Arnsten 2009; Joels et al. 2007). These smaller dendritic fields with fewer synapses can actually result in electrically more compact neurons with enhanced neuronal output due to reduced integration of input (Joels et al. 2007; Thomason and Thompson 2011), and consequently in less flexible/versatile functional and behavioral responses. Our findings

of increased FA in subordinates' local mPFC tracts potentially involved in emotional regulation (Arnsten 2009; Drevets 2001), as well as in frontal and cortico-thalamic tracts that transform sensory information into action (Rizzolatti and Fadiga 1998), and the association of this increased tract integrity with higher fear and submission –behaviors associated with lower rank- would be consistent with this notion of decreased behavioral flexibility. That is, these brain structural differences in subordinates (increased tract integrity potentially due to increased myelin) could lead to increased conduction speed along mPFC and frontal tracts (Paus 2010), which would support prepotent behavioral responses potentially adaptive for survival of subordinates in their social groups (i.e. higher submission and fear behaviors). Although these interpretations are speculative because our design does not allow us to establish causality between brain and behavioral differences, it is possible that the brain structural phenotypes in subordinates function to facilitate attending and reacting to cues to successfully navigate the social environment and minimize the risk of aggression from more dominant animals (Silk 2002). These speculations are consistent with reports of increased FA in ventromedial PFC of squirrel monkeys exposed to early life stress, an observation that was interpreted as "adaptive", preparing the individual to cope with challenges in their environment (Katz et al. 2009). Altogether, these findings suggest that different positions in the social hierarchy likely lead to different female strategies to deal with threatening or uncertain situations, potentially related to brain structural differences.

Structural effects of social subordination have been recently reported in prefrontal GM of adult male rhesus monkeys by MRI, as well as in the STS and amygdala (Sallet et al. 2011), and there is evidence that low SES, a complex mix of chronic social stress and limited access to resources, results in reduced prefrontal and frontal volumes and gyrification, associated with socioemotional and cognitive deficits in children (Hackman and Farah 2009; Jednorog et al. 2012). Our findings provide further evidence

that structural differences associated with social status in primates extend to brain WM as well, specifically affecting integrity of medial prefrontal and frontal tracts in developing females. Consistent with our findings of a positive correlation between FA in these tracts and fear, increased FA in prefrontal regions involved in emotional control has also been linked to increased emotional reactivity, in particular anxiety, in human studies (Phan et al. 2009; Han et al. 2008; Zhang et al. 2012). Thus, dispelling the common misconception that higher FA is better functionally, altogether this evidence supports the recent view e.g. (Thomason and Thompson 2011) that higher FA in developing individuals may reflect less cognitive flexibility and could potentially impact neurocircuit organization during learning, resulting in prepotent responses potentially adaptive to that specific environment, as suggested for subordinates in our study.

In contrast to the correlations detected between status-related differences in tract integrity and behavior, no associations were found with the levels of the stress hormone cortisol, despite being elevated in subordinate juveniles. This suggests that rank-related FA differences are not mediated by the concurrently high basal cortisol concentrations. Future studies will need to elucidate whether subordination-related brain structural effects are a consequence of chronic exposure to high cortisol prior to this prepubertal period, or whether they are mediated by other biological or genetic factors.

We also examined how 5HTT polymorphisms modulated social rank effects. The effects of 5HTT allelic variation on emotional reactivity are thought to reflect brain structural differences caused by exposure to different levels of 5HT during development (Whitaker-Azmitia et al. 1996), since adult levels of brain transporters are similar (Jedema et al. 2010; Murthy et al. 2010; Shioe et al. 2003; Willeit et al. 2001). In fact, there is evidence that s-allele carriers have reduced GM volume in mPFC, frontal, temporal and parietal cortices and amygdala (Canli et al. 2005; Jedema et al. 2010; Pezawas et al. 2005), and reduced FA in the uncinate fasciculus (UF, connecting

ventromedial PFC with temporal regions) (Pacheco et al. 2009), which could underlie activity differences in these limbic circuits (Hariri et al. 2002; Rao et al. 2007). In this study we did not detect genotype effects on UF, but rather on the PLIC, with s-variant animals showing higher FA than l/l subjects, and higher FA being associated with increased social submission and less play, suggesting a potential effect on social interactions. Tractography identified the tracts affected as cortical descending pathways, including the corticospinal and corticorubral tracts, involved in the voluntary motor control of upper and lower limbs for locomotion and manipulation of objects, as well as of posture and muscle tone control (Humphrey et al. 1984). Reduced FA in the PLIC has been associated with motor impairments (Puig et al. 2011; Sach et al. 2004) and hypotonia (Yamada et al. 2006) in clinical conditions. Based on this evidence, the higher FA in s-variants could result in higher muscle tone and readiness to locomote associated with their higher submission, but the association with reduced play is difficult to explain.

We also found social status by 5HTT genotype interaction effects in several regions, with opposite genotype effects in subordinates and higher-ranking animals. These included the left external capsule, identified by tractography as the left MLF, a long association fiber pathway connecting the inferoparietal area with cortical regions in the STS and STG in macaques (Schmahmann and Pandya 2006; Schmahmann et al. 2007a). In this cluster, higher FA was associated with higher gaze-averting/submission rates towards the human intruder. The MLF is involved in the processing of auditory spatial information in macaques (Schmahmann and Pandya 2006; Schmahmann et al. 2007a), with left STG dominance reported for processing of conspecific vocalizations in fMRI studies (Joly et al. 2012). Increased FA in left parieto-temporal tracts including the MLF, involved in auditory processing, has been associated with increased language comprehension in humans (Wong et al. 2011). Based on all this evidence, the higher MLF

FA shown by s-variant than l/l females, at least in higher ranking animals, could potentially result in faster, more efficient, cortical auditory processing.

Additional interaction effects were found in "U" parietal tracts (cortico-cortical association fibers connecting adjacent gyri and involved in sensory information integration) and short-range tracts connecting PFC regions within and between hemispheres. Although the opposite effects of genotype on subordinate and dominant animals are difficult to interpret, the negative correlation between FA in these tracts and anxiety during the HI task is interesting given that similar associations have been reported between microstructural WM abnormalities (low FA) in frontolimbic and parietal regions and psychopathology in some clinical populations, also modulated by 5HTT genotype (Alexopoulos et al. 2009).

It is important to note the limitations of this study. First of all, our research design does not allow to demonstrate causality between social rank, brain WM microstructure and behavioral differences, because it cannot disentangle whether the WM structural differences in subordinates are the result of postnatal social experience, maternal prenatal experience e.g. prenatal stress (Wadhwa 2005), or transgenerational transmission of brain and behavioral phenotypes via genetic or epigenetic mechanisms (Nelson and Nadeau 2010). Because rhesus monkey offspring assume their mother's social rank, effects of postnatal social experience are potentially confounded by their mother's prenatal experience. Thus, if the mothers experienced social stress during gestation, this could alter brain maturation and lead to behavioral deficits later in life (Harris and Seckl 2011; Seckl 2008). Furthermore, our recent report that social status differences in adult females' peripheral gene expression are associated with differences in DNA methylation patterns (Tung et al. 2012) supports the possibility that some of the social rank effects on brain and behavioral/neuroendocrine phenotypes are mediated via epigenetic modifications, which could also be transmitted from mothers to daughters. An

additional limitation is that, because we only studied the animals at one age point, we can't determine, either, when the social rank- or 5HTTgenotype-related differences emerged or whether they reflect differences in developmental trajectories. Given all these caveats, the findings of this study should be interpreted with caution. While future studies need to determine the unique contributions of genetic/epigenetic factors versus pre- and postnatal experience on social rank-related brain differences, evidence from our group and others support that at least some of the systems studied are very plastic and sensitive to social experience, including social rank changes during adulthood. For example, rank rearrangement results in distinct behavioral and physiological phenotypes (Shively et al. 1997) and genome-wide expression patterns (Tung et al. 2012) that match a female's current, but not former, rank. The central question for future studies is whether brain structure exhibits similar social experience-induced plasticity.

In addition to the study design limitations described above, technical limitations of the DTI neuroimaging procedures also need to be noted. We performed a combined analysis of FA, RD and AD, because these DTI properties provide good overall indices of changes in tract structural integrity and the underlying mechanisms due to their strong relationships with histological measures of WM microstructure (i.e. myelin thickness, axon density/caliber/diameter, fiber spread, etc). However, some of those relationships remain controversial (Wheeler-Kingshott and Cercignani 2009) and our level of analysis can't truly demonstrate the microstructural/cellular processes involved in the social status and/or 5HTT genotype differences detected, or their actual biological/physiological meaning besides the behavioral correlations reported here. These questions are particularly complex in developing primates, where it is not clearly understood whether ongoing WM maturation processes such as myelination and axon pruning may contribute to DTI properties in a different way than in adults (Paus 2010). Finally, the FA thresholding used for the TBSS method excluded small fiber tracts from

the analysis that could also be affected, which is a methodological limitation due to generation of potential false negatives.

Taking into account all these potential mitigating factors, our data nonetheless suggest the existence of differences in brain structural connectivity between subordinate and higher-ranking prepubertal females, in part modulated by 5HTT polymorphisms. Structural differences in the main tracts affected, which connect prefrontal, motor, association and sensory processing brain regions, were associated with specific behavioral phenotypes, particularly submissive, fear and anxious behaviors that vary as a function of social status. Ongoing longitudinal analyses will determine if these rank-related differences persist into adolescence a period of important brain circuitry reorganization (Giedd and Rapoport 2010; Knickmeyer et al. 2010; Lebel et al. 2008; Shi et al. 2012) and adulthood, while planned studies will begin to examine whether prenatal and genetic/epigenetic factors synergize with postnatal social experiences to influence neurobehavioral development.

Table 2.1

Factor loadings of Principal Components Analysis for the human intruder test, no eye contact (NEC) condition.

Behaviors	**Factor 1** Fear	**Factor 2** Submission	**Factor 3** Anxiety	**Factor 4** Distress
Freeze duration- NEC	0.85			
Turn away frequency- Stare	-0.79			
Avert gaze frequency- Stare		0.88		
Lipsmack frequency- Stare		0.67		
Anxiety-like behaviors- Stare			0.83	
Toothgrind- Stare			0.72	
Coo- Stare				-0.79
Threat- Stare				-0.76

Table 2.2

Factor loadings of Principal Components Analysis for the Approach-Avoidance test.

Behaviors	**Factor 1** Anxious-aggression	**Factor 2** Anxious vigilance	**Factor 3** Impulsivity
Threaten object frequency	0.94		
Look away from object frequency	0.76		
Bite object frequency	-0.43		
Locomote frequency		0.82	
Visually inspect object frequency		-0.80	
Latency to take reward			-0.76
Latency to explore box			-0.66

Table 2.3

Pearson correlations of mean FA in regions where significant effects of status and/or 5HTT genotype with behavioral data collected in the social group and factors derived from the PCA analysis of emotionality tests (HI and AA). Shown are p-values associated with these correlations. Applying a Sidak correction yields a critical p-value of 0.005. However, all effects sizes, reflected in the magnitude of the correlation coefficient as well as the associated Cohen's d value, indicate medium to large effects.

CLUSTER OF SIGNIFICANT FA VOXELS	**BEHAVIOR/COMPONENTS**	**r df = 33**	**p-value**	**Cohen's d**
Cluster 1, main status effect	HI component 1, fear Submissive behaviors in group	0.36 0.38	0.04 0.02	0.78 0.85
Cluster 2, main status effect	Submissive behaviors in group	0.35	0.05	0.74
Cluster 4, Main 5HTT genotype effect	Submissive behaviors in group Play in the group	0.44 -0.35	0.01 0.04	0.97 -0.75
Cluster 5, status by genotype effect	HI component 2, submissive	0.42	0.02	0.93
Cluster 6, status by genotype effect	AA component 1, anxious aggression Hi component 3, anxiety	-0.53 -0.62	0.002 0.0002	-1.23 -1.56
Cluster 7, status by genotype effect	AA component 1, anxious aggression HI component 2, submissive	-0.41 0.44	0.02 0.01	-0.9 0.98

Figure 2.1

Results of the DTI analysis: main effect of status.

(A) TBSS 2x2 ANOVA results showing main effect of status (high/dominant vs. low/subordinate), cluster 1: left medial prefrontal cortex (mPFC) white matter. (B) Results from the two-way ANOVA showing that low ranking animals have significantly higher FA than high ranking ones. (C) Representation of the affected tracts using probabilistic tractography: group probability map (subject level threshold at 1% of the robust mean intensity with distance correction applied) showing that the cluster of significant voxels in Fig. 2.1A includes local, short range, intra-hemispheric mPFC fibers, with some cross-hemispheric fibers (crossing through the genu of the corpus callosum – CC-), in 40-60% of the animals (yellow). Left to right images represent sagittal, coronal, and axial planes. The composite Images (group probability maps) show voxels that were common to at least 15 (40%) animals. Colors represent percentage of subjects that showed connectivity with that voxel in the single subject analysis: 40-60% animals in yellow, 60-80% animals in orange and 80-100% animals in red.

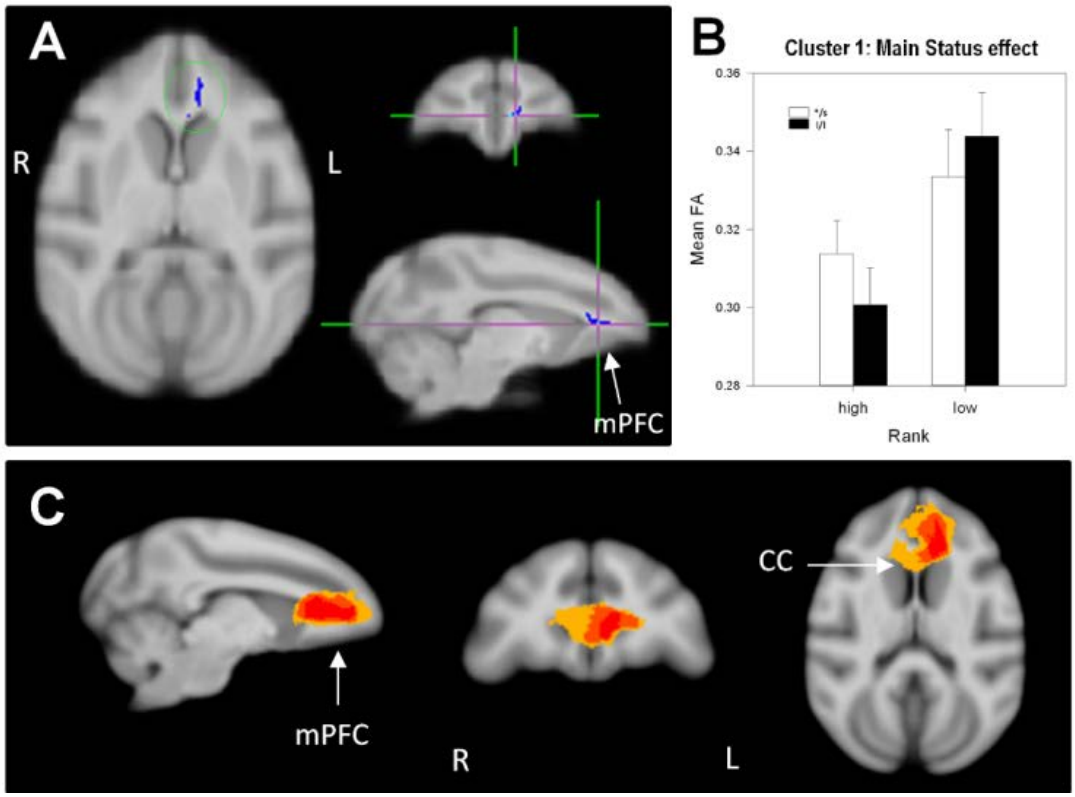


Figure 2.2

Results of the DTI analysis: main effect of status.

(**A**) TBSS 2x2 ANOVA results showing main effect of status in two clusters, 2 and 3: white matter along the left dorsal medial wall. (**B**) Results from the two-way ANOVA showing that low ranking animals have significantly higher FA than high ranking ones. (**C & D**) Probabilistic tractography: group probability map (subject level threshold at 1% of the robust mean intensity with distance correction applied) showing that (**C**) both clusters of significant voxels in Fig. 2.2A involved short range cortico-cortical fibers (intra- and inter-hemispheric) in the dorsomedial wall, interconnecting frontal regions corresponding to the primary and supplementary motor area (SMA) and premotor cortex in most animals, as well as connections of these frontal motor areas with somatosensory cortex (SSC) in about 25-50% of subjects. Left to right images represent coronal and axial planes. The composite Images (group probability maps) show voxels that were common to at least 15 (40%) animals. Colors represent percentage of subjects that showed connectivity with that voxel in the single subject analysis: 40-60% animals in yellow, 60-80% animals in orange and 80-100% animals in red. PMD: dorsal premotor cortex (**D**) Represents the group probability map of additional caudal tractography in cluster 3, suggesting the involvement of cortico-thalamic tracts connecting somatosensory and primary motor cortices with thalamic regions. Left to right images represent sagittal and coronal planes. Thresholds and color codes as in 2C.

Figure 2.2 (continued)

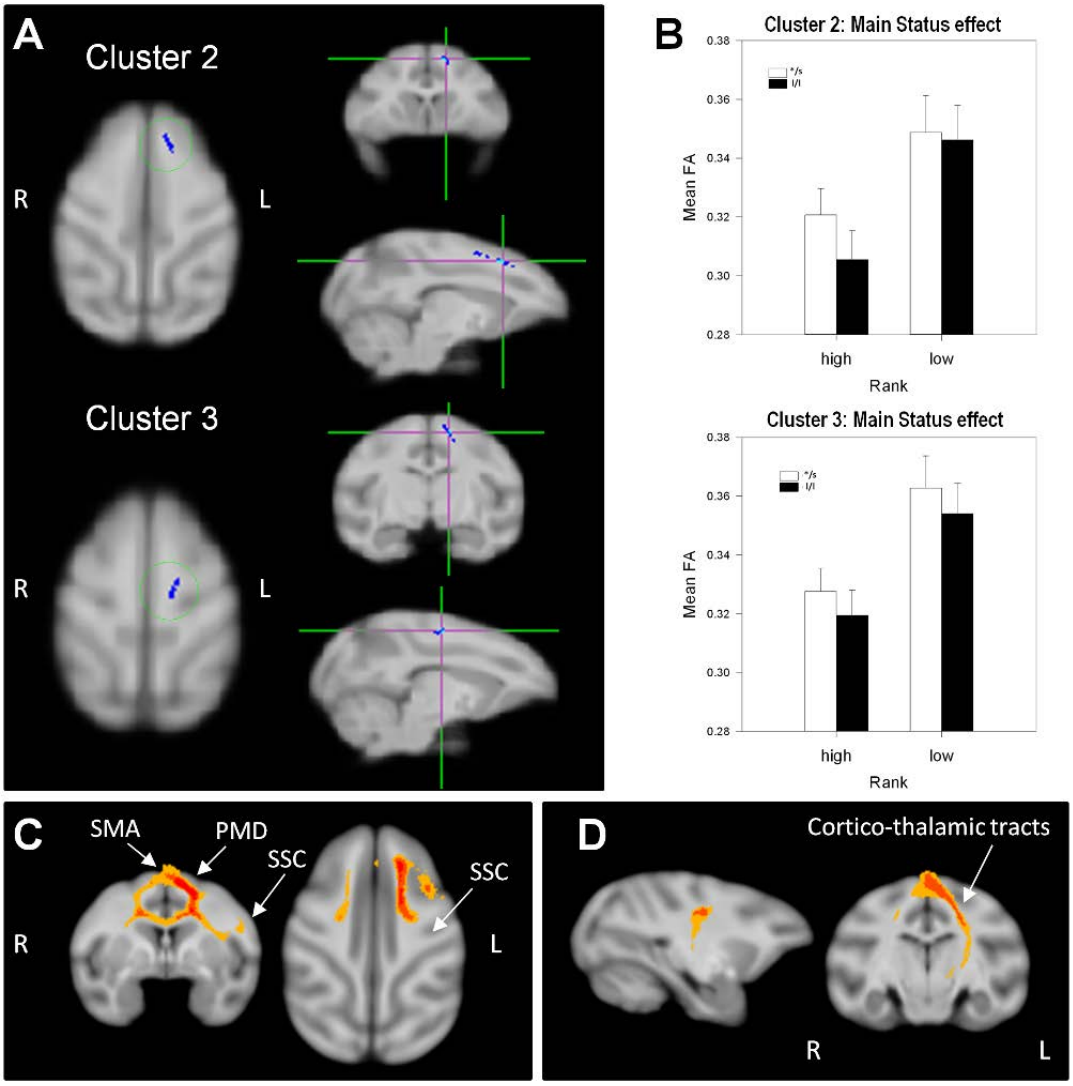


Figure 2.3

Results of the DTI analysis: main effect of 5HTT genotype.

(**A**) TBSS 2x2 ANOVA results showing main effect of 5HTT genotype (l/l vs */s –or short-variant-) in a cluster (cluster 4) in the left posterior limb of the internal capsule. (**B**) Results from the two-way ANOVA showing that l/l animals have significantly lower FA than */s ones. (**C**) Probabilistic tractography: group probability map (subject level threshold at 1% of the robust mean intensity with distance correction applied) showing that the cluster of significant voxels in Fig. 2.3A involves mostly cortical descending pathways, including corticorubral and corticospinal tracts with bilateral connectivity via the corpus callosum in about 50% of the animals. Left to right images represent sagittal, coronal and axial planes. The composite Images (group probability maps) show voxels that were common to at least 9 (25%) animals. Colors represent percentage of subjects that showed connectivity with that voxel in the single subject analysis: 25-50% animals in yellow, 50-75% animals in orange and 75-100% animals in red. The slightly different group threshold applied to the composite image of this cluster was chosen for display purposes, but more stringent group level threshold results showed qualitatively similar results (just slightly narrower bands) Abbreviations: SSC=somatosensory cortex; STS=superior temporal sulcus.

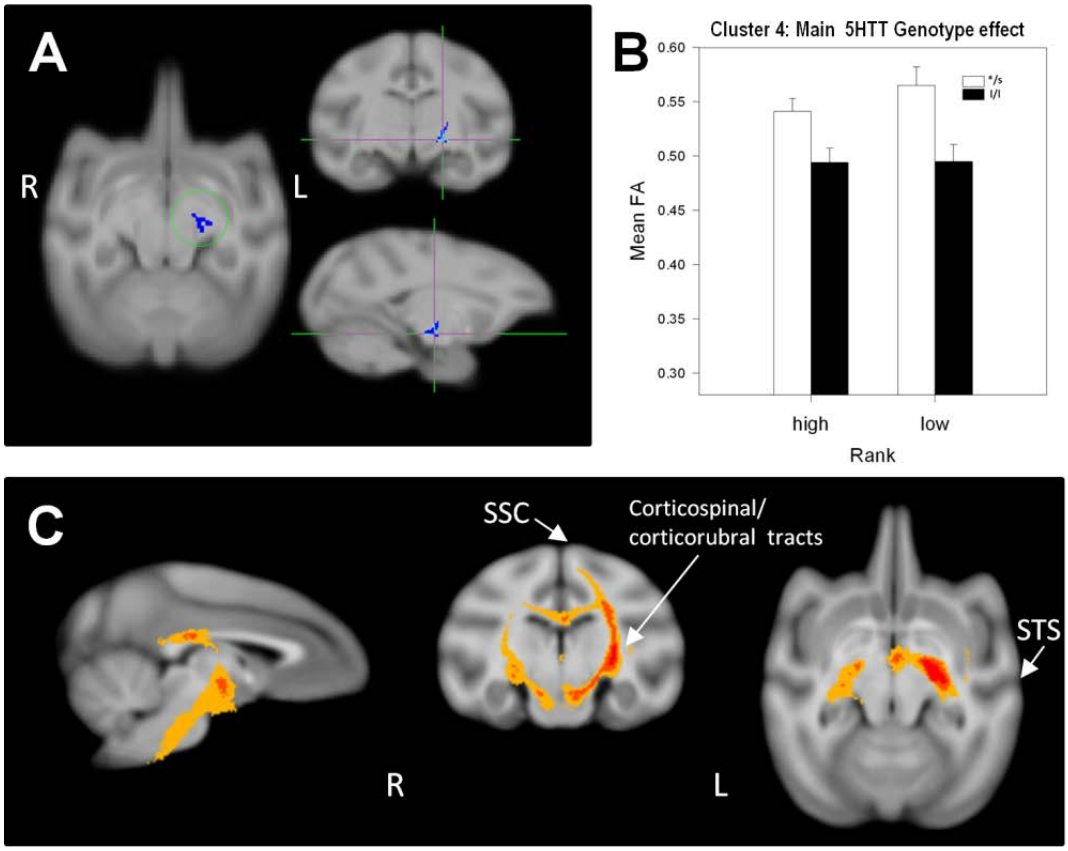


Figure 2.4

Results of the DTI analysis: status x 5HTT genotype interaction effect.

(**A**) TBSS 2x2 ANOVA results showing significant status x 5HTT genotype interactions in a cluster (cluster 5) in the left external capsule. (**B**) Results from the two-way ANOVA showing that dominant l/l animals had lower FA than s-variant ones, whereas l/l subordinates had higher FA than */s. (**C**) Probabilistic tractography: group probability map (subject level threshold at 1% of the robust mean intensity with distance correction applied) showing that the cluster of significant voxels in Fig. 2.4A corresponds to the left middle longitudinal fasciculus (MLF), which reciprocally connects the inferoparietal area with cortical regions in the superior temporal sulcus (STS) and gyrus (STG). Left to right images represent sagittal, two coronal (rostral versus caudal) and axial planes. The composite Images (group probability maps) show voxels that were common to at least 15 (40%) animals. Colors represent percentage of subjects that showed connectivity with that voxel in the single subject analysis: 40-60% animals in yellow, 60-80% animals in orange and 80-100% animals in red. STG: superior temporal gyrus; STS: superior temporal sulcus.

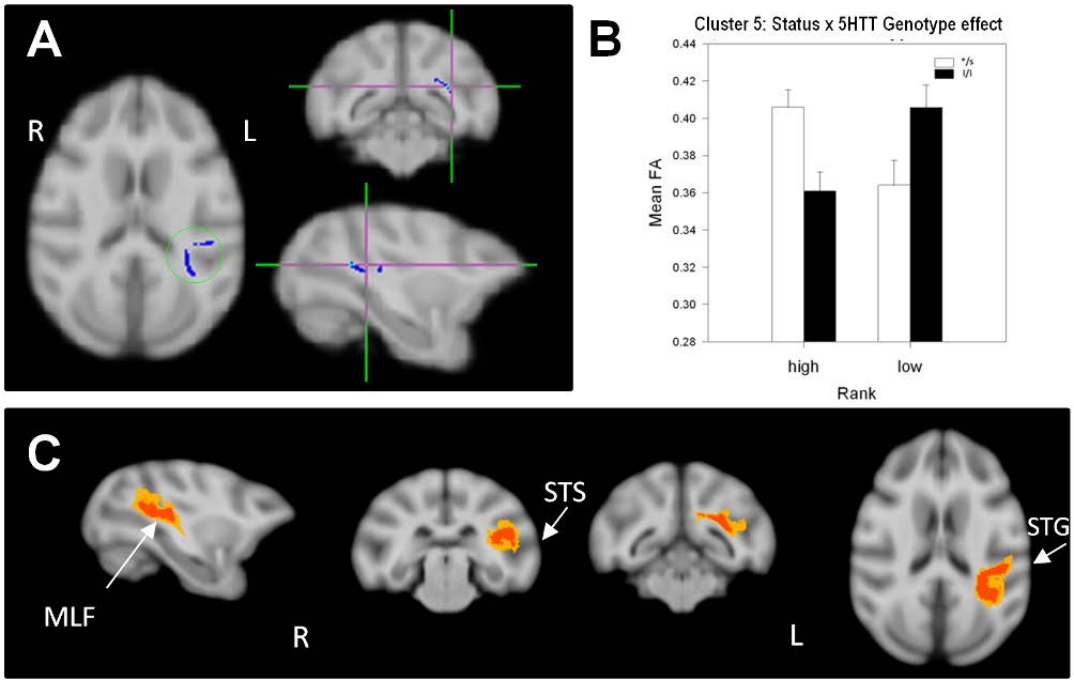


Figure 2.5

Results of the DTI analysis: status x 5HTT genotype interaction effect.

(**A**) TBSS 2x2 ANOVA results showing a status x genotype interaction in a cluster (cluster 6) in the right parietal white matter. (**B**) Results from the two-way ANOVA showing that dominant l/l animals had lower FA than s-variant ones, and l/l subordinates had higher FA than low */s. (**C**) Probabilistic tractography: group probability map (subject level threshold at 1% of the robust mean intensity with distance correction applied) showing that the cluster of significant voxels in Fig. 2.5A corresponds to short range parietal cortico-cortical "U" fibers in the right hemisphere. Left to right images represent sagittal, coronal and axial planes. The composite Images (group probability maps) show voxels that were common to at least 15 (40%) animals. Colors represent percentage of subjects that showed connectivity with that voxel in the single subject analysis: 40-60% animals in yellow, 60-80% animals in orange and 80-100% animals in red. Abbreviations: ips=intraparietal sulcus.

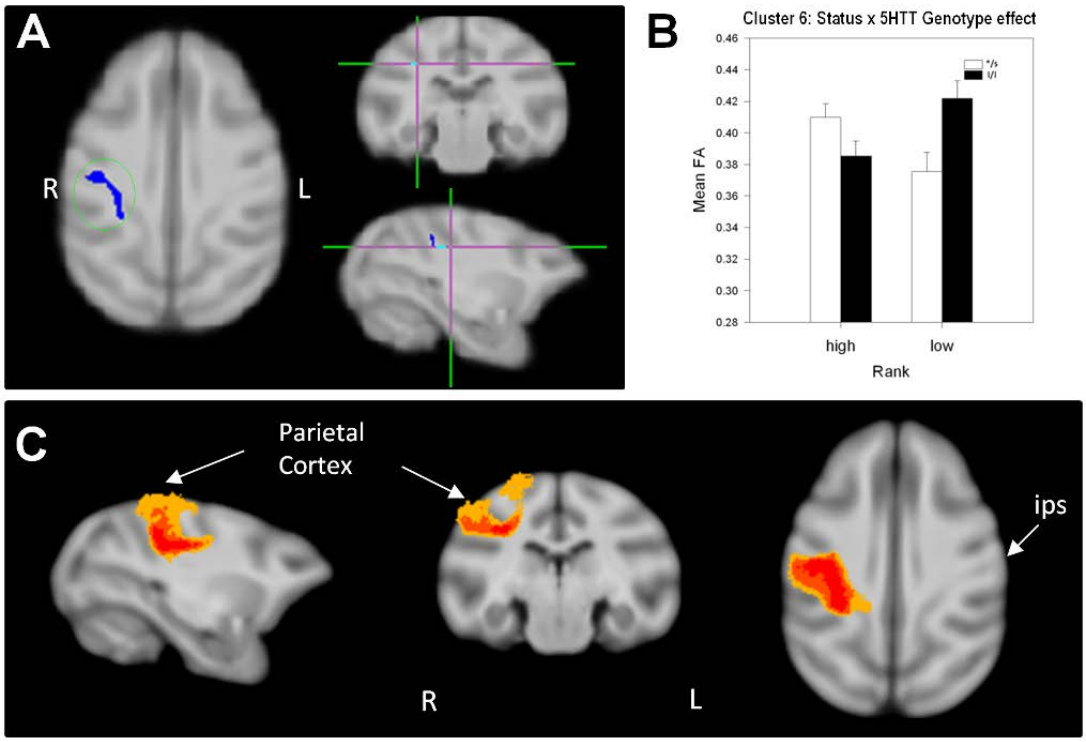
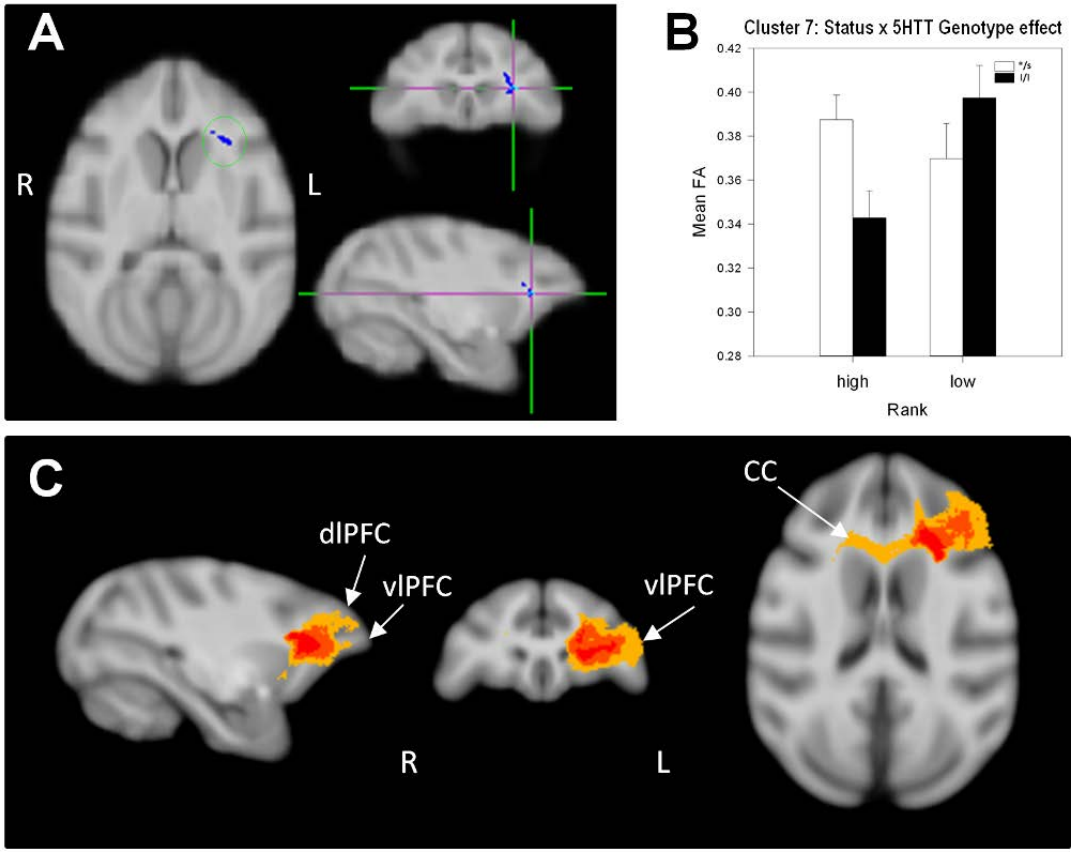


Figure 2.6

Results of the DTI analysis: status x 5HTT genotype interaction effect.

(**A**) TBSS 2x2 ANOVA results showing a status x genotype interaction effect in a cluster (cluster 7) in the left prefrontal white matter. (**B**) Results from the two-way ANOVA showing that dominant l/l animals had lower FA than s-variant ones, and l/l subordinates had higher FA than low */s. (**C**) Probabilistic tractography: group probability map (subject level threshold at 1% of the robust mean intensity with distance correction applied) showing that the cluster of significant voxels in Fig. 2.6A corresponds to the left, short range, prefrontal white matter tracts connecting dorsolateral,ventrolateral and dorsomedial PFC in the left hemisphere and bilaterally via the genu of the CC in 40-60% of subjects. Left to right images represent sagittal, coronal and axial planes. The composite Images (group probability maps) show voxels that were common to at least 15 (40%) animals. Colors represent percentage of subjects that showed connectivity with that voxel in the single subject analysis: 40-60% animals in yellow, 60-80% animals in orange and 80-100% animals in red. Abbreviations: CC=corpus callosum; dlPFC=dorsolateral PFC; vlPFC=ventrolateral PFC.



Chapter 3 : Brain white matter microstructure alterations in adolescent rhesus monkeys exposed to early life stress: associations with high cortisol during infancy[4]

[4] Modified slightly from Howell BR, McCormack KM, Grand AP, Sawyer NT, Zhang X, Maestripieri D, Hu X, Sanchez MM. Brain white matter microstructure alterations in adolescent rhesus monkeys exposed to early life stress: associations with high cortisol during infancy. *Biology of Mood & Anxiety Disorders*. Submitted.

3.1 Abstract

Early adverse experiences, especially those involving disruption of the mother-infant relationship, are detrimental for proper socioemotional development in primates. Humans with histories of childhood maltreatment are at high risk for developing psychopathologies including depression, anxiety, substance abuse, and behavioral disorders. However, the underlying neurodevelopmental alterations are not well understood. Here we used a nonhuman primate animal model of infant maltreatment to study the long-term effects of this early life stress on brain white matter integrity during adolescence, its behavioral correlates, and the relationship with early levels of stress hormones. Using diffusion tensor imaging and tract-based spatial statistics, we found significant reductions in white matter structural integrity (measured as fractional anisotropy) in the corpus callosum, occipital white matter, external medullary lamina, as well as in the brainstem of adolescent rhesus monkeys that experienced maternal infant maltreatment. In most regions showing fractional anisotropy reductions, opposite effects were detected in radial diffusivity, without changes in axial diffusivity, suggesting that the alterations in tract integrity likely involve reduced myelin. Moreover, in most regions showing reduced white matter integrity this was associated with elevated plasma cortisol levels early in life, which was significantly higher in maltreated than control infants. Reduced fractional anisotropy in occipital white matter was also associated with increased social aggression. These findings highlight the long-term impact of infant maltreatment on brain white matter structural integrity, particularly in tracts involved in visual processing, emotional regulation, and somatosensory and motor integration. They also suggest a relationship between elevations in stress hormones detected in maltreated animals during infancy and long-term brain white matter structural effects.

3.2 Introduction

Childhood maltreatment is a serious health problem due to both adverse physical and psychopathological outcomes. Adverse outcomes associated with maltreatment include anxiety and mood disorders, substance abuse, conduct disorder, poor impulse control, increased aggression, and other social deficits (Glaser 2000; Teicher, Andersen et al. 2002; Teicher, Andersen et al. 2003; Gunnar and Quevedo 2007; Weber, Rockstroh et al. 2008). Infant abuse is not exclusive to humans, but also occurs in wild and captive populations of nonhuman primates, including macaques, chimpanzees, baboons and marmosets (Maestripieri and Carroll 1998). Studies in rhesus monkeys have shown that infant maltreatment also results in socioemotional and stress physiology deficits (McCormack, Sanchez et al. 2006; Sanchez 2006; McCormack, Newman et al. 2009; Sanchez and Pollak 2009; Sanchez, McCormack et al. 2010; Koch, McCormack et al. 2013) that resemble those seen in maltreated children.

The alterations in behavior and stress physiology exhibited by victims of maltreatment (both human and nonhuman) are hypothesized to be caused by stress-induced differences in brain development, particularly of neural circuits regulating those functions. Studies in humans utilizing MRI have shown alterations in the volumes of specific brain regions including the hippocampus, amygdala, and prefrontal cortex (PFC) in adults with histories of maltreatment (Bremner 2002; Bremner 2003; Bremner 2006; van Harmelen, van Tol et al. 2010; Dannlowski, Stuhrmann et al. 2012; Teicher, Anderson et al. 2012). Studies investigating alterations in children and adolescents are more inconsistent, and have found more diffuse neural alterations including reductions in temporal, frontal, and parietal cortical volumes as well as decreased corpus callosum (CC) and general cortical white matter (WM) volumes (Teicher, Andersen et al. 2003; Hanson, Chung et al. 2010; Edmiston, Wang et al. 2011; Hanson, Chung et al. 2012; De

Brito, Viding et al. 2013). This, and additional evidence, supports the view that maturation of brain WM is particularly sensitive to early life stress/adversity (Eluvathingal, Chugani et al. 2006; Choi, Jeong et al. 2009; Coplan, Abdallah et al. 2010; Govindan, Behen et al. 2010; Huang, Gundapuneedi et al. 2012), possibly due to the dramatic developmental changes in myelinated WM, and fiber tracts in general, that occur from childhood through adulthood in both humans (Schneider, Il'yasov et al. 2004; Barnea-Goraly, Menon et al. 2005; Lebel, Walker et al. 2008; Gao, Lin et al. 2009; Asato, Terwilliger et al. 2010; Giedd, Stockman et al. 2010; Westlye, Walhovd et al. 2010; Deoni, Mercure et al. 2011) and nonhuman primates (Gibson 1991; Malkova, Heuer et al. 2006; Shi, Short et al. 2013).

Diffusion tensor imaging (DTI) is a noninvasive, quantitative variation of structural magnetic resonance imaging (MRI) used to measure diffusion of water in the brain. When diffusion is unrestricted, the motion of the water molecules is isotropic, or equal in all directions. However, diffusion is restricted along the axons of myelinated WM tracts, resulting in anisotropic (preferential in one direction) diffusion. The strength of this directional diffusion can be quantified using measures such as fractional anisotropy (FA). Higher FA indicates an increase in the microstructural integrity of the tract, which can be due to several factors, such as increases in myelin thickness, axonal density/diameter, axon neurofilaments/microtubule density, and spread or coherence of fiber orientation in a given voxel (Beaulieu 2002; Mamata, Jolesz et al. 2004; Concha, Livy et al. 2010; Choe, Stepniewska et al. 2012). Other diffusion properties can be examined to complement investigations of FA, as they provide additional information regarding the mechanisms underlying microstructural differences (Bennett, Madden et al. 2010; Burzynska, Preuschhof et al. 2010; Bosch, Arenaza-Urquijo et al. 2012). In particular, radial diffusivity (RD), which quantifies water diffusion perpendicular to the axon and decreases with increased myelination (Keller and Just 2009; Zhang, Jones et

al. 2009; Bennett, Madden et al. 2010; Hu, Geng et al. 2011), and axial diffusivity (AD), which measures diffusivity parallel to the fibers and increases with axonal microorganization, density and caliber, but is not affected by myelin thickness (Kumar, Macey et al. 2010; Kumar, Nguyen et al. 2012), can provide valuable information when measured in parallel to FA.

Although the neurobiological mechanisms underlying differences in FA and its functional effects on axonal tract efficiency are not completely understood, there is strong evidence of overall increases in FA (i.e. tract integrity) in major brain fiber tracts during primate development, although the maturational rates are tract-specific (Schneider, Il'yasov et al. 2004; Barnea-Goraly, Menon et al. 2005; Lebel, Walker et al. 2008; Asato, Terwilliger et al. 2010; Westlye, Walhovd et al. 2010; Shi, Short et al. 2013). The role of brain WM tract integrity in behavioral control, particularly during development, is being recognized as an important mechanism underlying behavioral alterations (Fields 2008) due to its effects on timing and speed of intercellular communication; e.g. increased tract integrity via increased myelin can increase information transfer via faster conduction speed along the axon (Lang and Rosenbluth 2003; Paus 2010). Thus, increases in regional FA have been associated with behavioral training and learning (Keller and Just 2009; Scholz, Klein et al. 2009; Tang, Lu et al. 2010; Engvig, Fjell et al. 2011; Hu, Geng et al. 2011; Taubert, Villringer et al. 2012; Tang, Lu et al. 2012) and cognitive skills in typically developing children, so that, in general, increased FA has been related to improved behavioral performance (Paus 2010). FA alterations (both increases and decreases) have, instead, been linked to early stress/adversity (Eluvathingal, Chugani et al. 2006; Katz, Liu et al. 2009; Coplan, Abdallah et al. 2010; Govindan, Behen et al. 2010; Frodl, Carballedo et al. 2012; Howell, Godfrey et al. 2013) and with several psychopathologies including anxiety disorders

(Phan, Orlichenko et al. 2009; Thomason and Thompson 2011), major depression (Taylor, MacFall et al. 2004), and bipolar disorder (Kafantaris, Kingsley et al. 2009).

Prospective studies assessing the impact of childhood maltreatment on brain WM development and the potential mechanisms involved are difficult to perform in children. The goal of the present study was to use DTI to address these questions using a well-established rhesus monkey model of infant maltreatment. In particular we investigated the long-term effects of this adverse early experience on brain WM and behavior during adolescence, and its potential association with stress-induced elevations in cortisol during infancy. Infant maltreatment in this model is comprised of (1) physical abuse, operationalized as violent behaviors exhibited by the mother towards the infant, which reacts with overt signs of distress, and (2) high rates of infant rejection, which is a physically undamaging behavior consisting of pushing the infant away when it solicits contact from the mother, but that also causes infant distress (Maestripieri 1998; McCormack, Sanchez et al. 2006). Using this model we have previously reported increased emotional reactivity in maltreated infants and juveniles (Maestripieri, Higley et al. 2006; McCormack, Sanchez et al. 2006; McCormack, Newman et al. 2009) and social alterations including delayed independence from the mother and less play during infancy (Maestripieri and Carroll 1998; Maestripieri, Jovanovic et al. 2000), as well as increased social aggression during adolescence (Grand 2008). Alterations in the hypothalamic-pituitary-adrenal (HPA) stress neuroaxis have also been reported in this maltreatment model, including elevated basal plasma cortisol levels at one month of age, when abuse rates were highest (Sanchez 2006; McCormack, Newman et al. 2009), which in some cases remain elevated for the first year of life, in parallel with increased stress reactivity (Koch, McCormack et al. 2013), and pituitary changes (i.e. blunted adrenocorticotropic hormone [ACTH] responses to corticotropin-releasing hormone

[CRH] administration) that confirmed HPA axis overactivity during infancy (Sanchez, McCormack et al. 2010).

Given all this evidence, in this study we used DTI and tract-based spatial statistics (TBSS) to investigate the long-term effects of infant maltreatment on brain WM tract integrity during adolescence and whether they were related to the increased cortisol levels detected in maltreated animals during their first month of life. WM tract integrity was measured by FA, in parallel with RD and AD measures to aid with the interpretation of the local microstructural mechanisms involved (Keller and Just 2009; Wheeler-Kingshott and Cercignani 2009; Bennett, Madden et al. 2010; Burzynska, Preuschhof et al. 2010; Metwalli, Benatar et al. 2010; Shamy, Carpenter et al. 2010; Hu, Geng et al. 2011; Taubert, Villringer et al. 2012; Shi, Short et al. 2013; Tang, Lu et al. 2012). In order to assess potential functional correlates of maltreatment-related brain differences, we also examined the associations between brain WM tract integrity and measures of social behavior, in particular aggression, based on reports that it is increased in adolescent maltreated animals as compared to controls (Grand 2008). Given the associations reported between early adverse experiences and reduced brain WM tract integrity in children and adolescents, particularly in cortico-limbic tracts and association cortices, including prefrontal-temporal connections (Eluvathingal, Chugani et al. 2006; Choi, Jeong et al. 2009; Govindan, Behen et al. 2010; Hanson, Adluru et al. 2013; Howell, Godfrey et al. 2013), we hypothesized that maltreated monkeys would have lower FA in these tracts than control animals. Based on the role of these cortico-limbic tracts in social and emotional regulation, we also hypothesized that lower WM tract integrity would be associated with increased aggression.

3.3 Methods

3.3.1 Subjects and housing

Nineteen adolescent rhesus monkeys (*Macaca mulatta*) living in four large social groups were used in these studies. Each group consisted of 2-3 adult males and 18-49 adult females with their sub-adult and juvenile offspring. The groups were housed in outdoor enclosures with access to climate-controlled indoor housing areas located at the Yerkes National Primate Research Center (YNPRC) Field Station, in Lawrenceville GA. Subjects were given commercially available primate chow (Purina Mills Int., Lab Diets, St. Louis, MO) supplemented with fresh fruit twice daily, and water was available *ad libitum*. All procedures were approved by the Emory University Institutional Animal Care and Use Committee in accordance with the Animal Welfare Act and the U.S. Department of Health and Human Services "Guide for Care and Use of Laboratory Animals".

Of the 19 subjects in this study, 9 experienced maternal maltreatment in the form of physical abuse early in infancy (5 females and 4 males; see operational definition below and in previous publications)(McCormack, Sanchez et al. 2006; Sanchez, McCormack et al. 2010) and the other 10 subjects were non-maltreated controls (6 females and 4 males). Following behavioral definitions, observation protocols, and inclusion/exclusion criteria described in detail in previous publications using this same group of 19 animals (McCormack, Sanchez et al. 2006; McCormack, Newman et al. 2009), infant abuse was operationalized as at least three occurrences of the following violent behaviors by the mother towards the infant during the first three months of life: dragging the infant by the tail or leg while running or walking, crushing the infant against the ground with both hands, throwing the infant with one hand while standing or walking, stepping on the infant with one or both feet, sitting on the infant, roughly

grooming by forcing the infant to the ground and pulling out the infant's hair causing distress calls, or carrying the infant with one arm away from the mother's body not allowing the infant to cling (Maestripieri 1998; McCormack, Sanchez et al. 2006; Sanchez, McCormack et al. 2010). As mentioned in the Introduction, all of these abusive behaviors caused distress in the infants, who experienced an average of 1.5 events of abuse per hour during their first month of life (McCormack, Sanchez et al. 2006). Maltreated infants also experienced intense maternal rejection, which involved pushing away the infant when it solicited contact from its mother (McCormack, Sanchez et al. 2006), hence the use of the term maltreatment rather than simply abuse. Subjects in the control and maltreated groups were matched for age, sex, and maternal dominance rank whenever possible so that the two groups did not significantly differ in any of these variables.

3.3.2 HPA axis basal activity: cortisol in infancy

Basal blood samples were collected at sunrise from all subjects when they were one month old, coincident with the highest rates of abuse (McCormack, Sanchez et al. 2006), following published protocols (Sanchez, Noble et al. 2005; McCormack, Newman et al. 2009; Sanchez, McCormack et al. 2010). Plasma concentrations of cortisol were measured in duplicate 10μL aliquots by radioimmunoassay using commercially available kits (Diagnostic Systems Laboratories, DSL, Webster, TX). Although we have already reported elsewhere that maltreated animals have greater plasma cortisol levels at 1 month of age than controls (Sanchez 2006; McCormack, Newman et al. 2009), these cortisol concentrations were used in the current study to examine their correlations with brain structural measures during adolescence (see details below).

3.3.3 Behavioral data collection during adolescence

Social behavior was collected around 4 years of age (close to 48 months) from observation towers located in the corners above each subjects' social home compound. Data were collected between 7 and 11 AM, when animals are most active, using an established rhesus ethogram (Altmann 1962) with modifications (Maestripieri, Higley et al. 2006). This behavioral data was collected by three trained observers using binoculars and handheld computers (Palm IIIxe) programmed to collect durations, frequencies, and sequences of behavior (Graves and Wallen 2006). Inter-observer reliability was calculated prior to real time collection of behavior, by having each observer watch and record behavior from videos until percent agreement reached at least 90% and Cohen's Kappa was greater than 0.8.

Frequency of aggressive behaviors was measured using 5 hours of focal observations in each animal (5 separate sessions, 1 hour each). Behaviors categorized as aggression included biting, grabbing, pinning, threatening and chasing of others in the group. A composite score of the frequencies of all of these behaviors was used to calculate the frequency of total aggression used in the analysis as rates per hour. Although increased social aggression has been reported in these maltreated animals as a separate and more extensive study of affiliative and agonistic behavior in these animals (Grand 2008), total aggression rates per hour (average of contact and non-contact aggression) data were used in the current study to examine its associations with brain structural measures collected at similar ages (see details below).

3.3.4 *In vivo* neuroimaging

T1-weighted MRI acquisition and template construction

Imaging data was acquired during adolescence, beginning at 4 years of age (range: 48-55 months; mean± SEM scan ages were: maltreated animals=51.99 ± 0.6

months, controls=51.98 ± 0.57 months). The scanning age was not different between control and maltreated animals, as described in Results. Structural (T1-weighted MRI) images were acquired during the same scanning session as the DTI scans on a 3T Siemens Trio scanner (Siemens Medical Solutions USA, Inc., Malvern, PA) at the YNPRC Imaging Center using a transmit and receive volume coil (Siemens CP Extremity Coil) and a magnetization prepared rapid gradient echo (MPRAGE) sequence with the following parameters: TI/TR/TE=950/3000/3.3 ms; flip angle = 8 degree; total scan time=38 min; FOV=116mmx116mmx96mm, with a 192×192×160 matrix and 4 averages; voxel size: 0.6x0.6x0.6 mm^3. A T1 template was constructed from these scans using the methods described for rhesus monkeys by McLaren and colleagues (McLaren, Kosmatka et al. 2009). Briefly, first a single subject was affinely registered to the rhesus monkey atlas developed at the University of Wisconsin (McLaren, Kosmatka et al. 2009) resulting in a single subject in Wisconsin 112RM-SL rhesus atlas space (the target image), which is in the brain coordinate space of the Saleem-Logothetis rhesus stereotaxic atlas (Saleem and Logothetis 2012). Each of the other subjects was then affinely registered to the target image and all of these images (now in atlas space) were averaged. This first-run template was then used as the target for a second round of affine registrations and averages resulting in a 0.5x0.5x0.5 mm^3, study-specific average T1 image that was used as a template for the analyses described below (Fig. 3.1).

DTI data acquisition, preprocessing, and analysis

Whole brain DTI data was acquired using a dual spin-echo, segmented (multi-shot) diffusion-weighted echo-planar imaging (EPI) sequence with the acquisition parameters: TR/TE=6000/90 ms, 4 shots, b: 0, 1000 s/mm^2, FOV=96mm×96mm, slice thickness=1.5mm with zero gap, voxel size=1.5x1.5x1.5 mm^3, 30 slices, 64×64 matrix, 30 directions, and 4 averages.

The DTI data was corrected for B0 inhomogeneity-induced distortion (Jezzard and Balaban 1995) and eddy current effects (Mangin, Poupon et al. 2002) using the FSL software (FMRIB Centre, University of Oxford, Oxford, United Kingdom) (Smith, Jenkinson et al. 2004). FA, RD, and AD were calculated using the diffusion analysis tools in FSL (Smith, Jenkinson et al. 2004) (Fig. 3.2). The TBSS tool in FSL (Smith, Jenkinson et al. 2006) was used as a voxel-wise approach to identify the centers of all major WM tracts present in all subjects, therefore reducing the number of multiple comparisons. TBSS first nonlinearly registers each subject's FA image to the template image (the study specific T1 template produced as described above, resulting in a final image resolution of 0.5x0.5x0.5 mm^3). These images are then averaged to create a mean FA image from which a mean FA skeleton is created (see Fig. 3.2B) using a user defined FA threshold (0.2 in the current study). To reduce the effects of misregistration on the FA values contained within each subjects' skeletonized data, the TBSS software searches the voxels surrounding the mean FA skeleton in each subjects' registered FA image to assign the highest local FA value for each subject to the skeleton (for complete description see Smith, Jenkinson et al. 2006). This ensures that despite the fact that the mean FA skeleton does not exactly cover the same anatomical regions in all subjects, the FA values contained in each subject's skeletonized data do represent the centers of the major WM tracts of each individual subject. These FSL diffusion analysis tools have been previously applied with success to rhesus brain DTI data by our group (Hecht, Gutman et al. 2013; Howell, Godfrey et al. 2013) and others (Makris, Papadimitriou et al. 2007; Willette, Bendlin et al. 2010; Bendlin, Canu et al. 2011; Willette, Coe et al. 2012).

3.3.5 Statistical analyses

Statistical analysis of FA, RD, and AD data

A voxel-wise two-group t-test was performed on the skeletonized FA data using the Randomise tool in FSL (Smith, Jenkinson et al. 2004) to determine regions with significant differences between the maltreated and control groups. Results were considered significant at p-value less than 0.005 (uncorrected, but using a minimum cluster volume of 10μL, approximately 4.5 significant contiguous voxels in native diffusion space) due to the relatively low spatial resolution. Results (significant clusters of >4.5 contiguous significant voxels in native diffusion space) were displayed in the T1 study-specific template described above, registered to the Wisconsin 112RM-SL rhesus atlas (McLaren, Kosmatka et al. 2009; McLaren, Kosmatka et al. 2010), which is in the coordinate space of the Saleem-Logothetis rhesus brain stereotaxic atlas (Saleem and Logothetis 2012).

Binary masks were created for the clusters showing group FA differences. The mean RD and AD values were calculated within these regions, following previously published approaches (Smith, Jenkinson et al. 2004; Tang, Lu et al. 2012; Howell, Godfrey et al. 2013). A two-group t-test was performed on these values to determine the effects of infant maltreatment on RD and AD in those clusters with significant FA differences to aid in identifying the underlying microstructural mechanisms of the differences in tract integrity (significance level was set at $p<0.05$). The mean FA calculated for each cluster was also used to examine its correlations with infant cortisol and adolescence aggression data using Pearson correlation (see details below).

Correlations between FA and biobehavioral measures (cortisol and aggression)

Because we were interested in examining the associations between infant cortisol levels and long-term alterations in tract integrity (i.e. FA) detected as a consequence of

this early adverse experience, as well as functional correlates of FA group differences during adolescence, we performed Pearson product moment correlation analyses restricted to those regions (clusters) where group differences in FA were detected above. Control and maltreated groups were included together in the correlation analyses between FA and basal plasma cortisol levels at one month of age and aggression during adolescence. Statistical significance level was set to $p<0.05$.

3.4 Results

3.4.1 Group differences in FA

No differences in scanning age were detected between maltreated and control animals ($p=0.99$; Student t-test). Significantly lower FA ($p<0.005$, uncorrected, cluster volume ≥10μL) was observed in maltreated animals, in comparison to controls, in 6 clusters: (1) one in WM located in the lateral portion of the medial midbody of the CC (Sanchez, Hearn et al. 1998) (Fig. 3.3); (2) one in right occipital WM (Fig. 3.4A); (3) two clusters in left occipital WM (Figs. 3.4B and 3.4C), which, along with the cluster located in right occipital WM could include the inferior longitudinal fasciculus (ILF) or possibly short intra-occipital fiber systems; (4) one in the WM dorsal to the left hippocampus and lateral to the pulvinar nucleus, which could correspond to the external medullary lamina (EML) (Fig. 3.5); and (5) one in the brainstem, in a location that matches the position of central tegmental tract (CTT) (Fig. 3.6). No regions were found in which the maltreated animals had significantly higher FA than controls.

3.4.2 Group differences in RD and AD in regions with significant FA effects

The mean RD and AD values were calculated for each of the clusters with significant group differences in FA. In all clusters, except for the brainstem cluster, decreased FA was accompanied by an increase in RD, suggesting that the difference in FA was due to decreased myelin (Song, Sun et al. 2002; Song, Sun et al. 2003; Keller and Just 2009; Zhang, Jones et al. 2009; Bennett, Madden et al. 2010; Hu, Geng et al. 2011; Choe, Stepniewska et al. 2012). No differences in AD were observed in any of the clusters with FA effects.

3.4.3 Correlations of biobehavioral measures with FA

As mentioned above, our group has previously reported elevated plasma cortisol levels during infancy (at 1 month of age) (McCormack, Newman et al. 2009), as well as increased aggression towards group mates during adolescence (at approximately 4 years of age) in the maltreated animals, which are the focus of this study, in comparison to controls (Sanchez 2006; Grand 2008; McCormack, Newman et al. 2009). Therefore, only results of the correlations between FA and these biobehavioral measures are presented here. The mean FA value of each cluster in which significant group differences in this measure were found was correlated with infant basal cortisol and frequency of aggressive behaviors during adolescence. Neither RD nor AD values were included in the correlation analyses because they are components of, and thus correlated with, FA.

Negative correlations between FA and infant cortisol were found in all clusters except for the one in the brainstem (see Table 1). A negative correlation (Table 1) between aggression and FA was also found in one of the clusters in left occipital WM (Fig. 3.4C), but in none of the other clusters examined.

3.5 Conclusions

The main goal of this study was to examine the long-term consequences of infant maltreatment on brain WM tracts of adolescent rhesus monkeys and to determine whether they were related to the elevated cortisol levels reported in these maltreated animals during infancy (Sanchez 2006; McCormack, Newman et al. 2009). We also examined whether alterations in brain WM microstructure were related to the increased aggressive behavior previously reported in the maltreated animals during adolescence (Grand 2008). To do this we used measures of microstructural integrity, specifically FA, RD, and AD, calculated from DTI scans. We chose this technique because of its sensitivity to changes in WM microstructure, such as myelin thickness and axon/microtubule density (Beaulieu 2002; Mamata, Jolesz et al. 2004; Concha, Livy et al. 2010). These are neuronal characteristics that can affect the timing and speed of intercellular communication (Lang and Rosenbluth 2003; Paus 2010), and can therefore affect behavior (Fields 2008). FA increases significantly in brain WM tracts throughout primate development, and is accompanied by decreases in RD and few changes in AD (Schneider, Il'yasov et al. 2004; Barnea-Goraly, Menon et al. 2005; Lebel, Walker et al. 2008; Asato, Terwilliger et al. 2010; Shi, Short et al. 2013). These developmental changes in measures of axon microstructure suggest a global increase in tract integrity mainly due to increases in myelin from childhood to adulthood. Brain region-specific increases in FA are also observed after training on visuo-motor tasks (Scholz, Klein et al. 2009) and with acquirement of new cognitive skills, such as reading and math, in parallel to decreases in RD, but no changes in AD (Keller and Just 2009; Paus 2010; Engvig, Fjell et al. 2011; Hu, Geng et al. 2011). This suggests that these experience-related and region-specific increases in FA are due to increases in myelin and underlie behavioral and cognitive improvements. In contrast, reduced FA, associated in most

regions with elevated cortisol during infancy and with increased concurrent aggression in one of the clusters, was detected here in adolescent rhesus monkeys that experienced infant maltreatment. Our findings are consistent with previous reports in human individuals that experienced childhood maltreatment (Choi, Jeong et al. 2009; Huang, Gundapuneedi et al. 2012) or other forms of early life stress (Eluvathingal, Chugani et al. 2006; Govindan, Behen et al. 2010) and in other nonhuman primate models of adversity (Coplan, Abdallah et al. 2010), as well as in several mood and anxiety disorders (Drevets 2000; Ayling, Aghajani et al. 2012), with significant overlap with the regions affected in the current study.

To our knowledge, this is the first DTI study to examine the long-term effects of infant maltreatment on brain WM tract integrity in a nonhuman primate model. It is also the first to examine the associations of brain structural alterations with infant cortisol elevations and concurrent social behavior. Our findings show alterations in brain WM tract integrity measured using DTI in adolescent rhesus monkeys with histories of infant maltreatment. Decreased WM integrity (i.e. FA) was found in maltreated subjects in the CC, occipital WM, EML, and brainstem, in comparison to controls. These regional FA decreases were paralleled by increases in RD, but no changes in AD, suggesting that the alterations in tract microstructural integrity in these brain regions were likely due to reduced myelin (Song, Sun et al. 2002; Song, Sun et al. 2003; Keller and Just 2009; Zhang, Jones et al. 2009; Bennett, Madden et al. 2010; Hu, Geng et al. 2011; Choe, Stepniewska et al. 2012). An exception was the brainstem cluster, where no RD differences were found between groups. Basal plasma cortisol levels measured when the individuals were one month old, when abuse rates were highest (McCormack, Sanchez et al. 2006), were negatively correlated with FA in all regions except for the brainstem cluster. This suggests that maltreatment at that early age caused stress-induced elevations in cortisol that could have potentially contributed to the long-term brain WM

alterations reported. However, future studies are needed to examine causality in this relationship.

One of the clusters with lower FA in maltreated animals than controls was located in the lateral aspect of the medial midbody of the CC (Sanchez, Hearn et al. 1998). The CC is the largest WM tract in the brain conveying interhemispheric fibers important for integration of information between cortical regions in both hemispheres (Schmahmann and Pandya 2006). Because these fibers are some of the last to myelinate (Malkova, Heuer et al. 2006; Gao, Lin et al. 2009; Deoni, Mercure et al. 2011; Shi, Short et al. 2013), finding alterations in the CC is consistent with the view that areas undergoing active myelination or other protracted developmental processes are especially vulnerable to environmental experience (Davison and Dobbing 1966; Rice and Barone Jr 2000). Alterations in the CC have also been reported in several studies of maltreated children, with reduced CC volume reported in maltreated children (De Bellis, Keshavan et al. 2002; Teicher, Dumont et al. 2004), a difference that appears to be related to a failure to show the typical age-related increase in volume (De Bellis and Keshavan 2003). Reduced CC size has also been reported in adults with histories of childhood maltreatment (Kitayama, Brummer et al. 2007), suggesting that these CC alterations are persistent. Decreased FA in the CC of maltreated children (Jackowski, Douglas-Palumberi et al. 2008) and adults that experienced various forms of early life stress (Paul, Henry et al. 2008) has also been reported. The findings of the current study are also consistent with findings of reduced CC size in other nonhuman primate models of adverse early experience (Sanchez, Hearn et al. 1998). Our findings of reduced WM integrity in the CC medial midbody region, which carries some prefrontal but mostly frontal motor and somatosensory fibers (Lamantia and Rakic 1990), could result in group differences in integration of motor and somatosensory information. The reduced interhemispheric integration reported here and in human studies of childhood maltreatment could

contribute to behavioral alterations and psychopathology, an idea supported by similar CC alterations reported in anxiety and mood disorders (Drevets, Price et al. 2008).

The location of the three clusters identified in occipital WM suggest that the tracts affected could include short intra-occipital fiber systems (possibly part of the forceps major, an interhemispheric tract that connects occipital cortices in both hemispheres), and/or the caudal portion of the ILF, a long cortico-cortical association tract that courses through occipital, parietal, and temporal cortices (Schmahmann and Pandya 2006). However, this can't be corroborated without running additional tractography analyses. Interestingly, reduced FA has been reported in the forceps major of adolescents with histories of child maltreatment (Huang, Gundapuneedi et al. 2012) and in the caudal portion of the ILF in adolescents that witnessed domestic violence as children (Choi, Jeong et al. 2012). The ILF is part of the ventral visual pathway which is important for object identification (Mishkin, Ungerleider et al. 1983), face processing (Fox, Iaria et al. 2008), and emotional memory (Habib 1986; Ross 2008). Along these lines, alterations in WM microstructure of the ILF have been observed in several mood and anxiety disorders. For example, decreased FA in the ILF at the level of the occipital lobe has also been found in patients with depression (Versace, Almeida et al. 2010; Liao, Huang et al. 2013) and bipolar disorder (Bruno, Cercignani et al. 2008; Zanetti, Jackowski et al. 2009). Thus, it is possible that decreases in microstructural integrity of occipital WM, likely involving the ILF, could affect visual and face processing, as well as emotional responses to stimuli.

The negative correlation of FA with aggressive behavior detected in occipital WM is difficult to explain. Most neuroimaging studies involving neural substrates of aggression implicate structural and/or functional abnormalities in frontal brain circuits (Dolan 2010; Hoptman and Antonius 2011),although many of these studies have been done in patients with schizophrenia. Decreased FA in the anterior commissure (AC) has

also been reported in violent youth with bipolar disorder, and FA in the AC was negatively correlated with aggression (Saxena, Tamm et al. 2012). However, this study was done in a clinical population making it difficult to integrate with the findings reported here. Increased occipital WM volume has been reported in adult violent offenders (Tiihonen, Rossi et al. 2008), but to our knowledge no other occipital alterations have been associated with aggression. Interestingly, a recent study comparing neural systems supporting social cognition in chimpanzees and bonobos reported that chimpanzees (known to be more aggressive than bonobos) had higher FA in occipital WM and bigger occipital GM volumes than bonobos (Rilling, Scholz et al. 2012), suggesting a potential association between aggression and FA in occipital WM in these species. The discrepancy of the directionality of the correlation with our findings could be explained by factors such as species-specific differences in neural substrates of aggression, or age at measurement. Given the paucity of research on the neural substrates of aggression, particularly in children, the interpretation of our findings is difficult. The visual cortices located near the cluster in which FA and aggression were correlated are part of attentional networks (Bisley 2011), and thus alterations in these circuits could reflect more general alterations in attention that might be better reflected by other behaviors not measured in the current study.

The WM cluster located lateral to the pulvinar thalamic nucleus and dorsal to the hippocampus seems to be the EML based on rhesus brain atlases (Saleem and Logothetis 2012). The EML contains both thalamo-cortical and cortico-thalamic fibers connecting the thalamus with parietal, temporal, occipital, cingulate, motor and PFC (Schmahmann and Pandya 2006). Although without tractography it is difficult to identify the specific thalamic nuclei and cortical regions affected, although based on the rostro-caudal location of this cluster the fibers affected are likely occipital or temporal (Schmahmann and Pandya 2006). Interestingly, thalamo-cortical systems modulate amygdala activity,

and are involved in the perception of fear (Das, Kemp et al. 2005). Cortico-thalamic circuits are implicated in the pathogenesis of mood disorders (Price and Drevets 2012). Thus, our findings of reduced structural integrity in EML suggest potential alterations in cortico-thalamic and thalamo-cortical circuits that could contribute to deficits in emotional regulation reported above in maltreated animals.

The brainstem cluster where FA was lower in maltreated animals than controls was difficult to identify anatomically due to the low MRI contrast in this region. However, as described above, its location matches the position of the CTT (Snider and Lee 1961). The CTT is a pathway containing descending fibers from midbrain nuclei that project to the olivary complex, as well as ascending fibers originating in the pontine and medullary reticular formation that project to the thalamus (Carpenter and Sutin 1983). These are brainstem pathways that carry and coordinate somatosensory and somatomotor information. MRI studies report lesions in the CTT in neurodegenerative and neurodevelopmental disorders, linked with motor and cognitive deficits (Shioda, Hayashi et al. 2011). This was the only region where group differences in FA (lower in maltreated subjects than in controls) were not related to the increased levels of cortisol during infancy in the maltreated animals, suggesting that the effects of maltreatment on this WM could be associated with other aspects of the early experience.

There are limitations to the DTI method as applied here. Most are due to the low spatial resolution of the diffusion data acquired in the relatively small rhesus brain. At this resolution partial voluming effects can make interpreting or finding results difficult. The TBSS analysis applied here addresses this limitation by using only voxels from the centers of large WM tracts in individual subjects. Partial voluming can also make registration difficult, which is another reason why we used the nonlinear registration built into the TBSS processing pipeline to perform our voxelwise analyses. Although the low angular resolution, especially when combined with the low spatial resolution

acquired, also makes accurate probabilistic tractography difficult, tractography would be helpful in future studies to determine the exact tracts affected in the clusters with group differences, although it would not help in determining the directionality of the affected fibers.

The results of the current study suggest that early life stress in the form of infant maltreatment has long-term effects on brain WM in regions previously reported as vulnerable to childhood maltreatment in humans, and that are also altered in anxiety and mood disorders. One possible mechanism could be through the effects of elevated levels of glucocorticoids (GCs), in this case cortisol, on the development of WM (Jauregui-Huerta, Ruvalcaba-Delgadillo et al. 2010). Oligodendrocytes that form the myelin sheath express both intracellular glucocorticoid and mineralocorticoid receptors (Bohn, Howard et al. 1991), and recent evidence suggests that GCs suppress proliferation of oligodendrocyte precursor cells in GM and WM (Alonso 2000). Developmental studies also provide evidence that GCs modulate oligodendrocyte differentiation and myelogenesis via regulation of key oligodendroglial proteins such as myelin basic protein (MBP) (Kumar, Cole et al. 1989), and that the effects of synthetic GCs differ as function of gestational age, with decreases in MBP immunoreactivity and numbers of oligodendrocytes associated with younger ages of GC exposure(Antonow-Schlorke, Helgert et al. 2009). Taken together, these studies suggest that myelination is sensitive to GCs during development, making it possible for early life stress, via elevated cortisol levels, to affect brain WM development. The associations detected in our studies between decreased FA and basal cortisol level at one month are consistent with this possibility, although the causality of this relationship needs to be tested in future studies. Due to the strong role of brain WM in behavioral control (e.g. Nagy, Westerberg et al. 2004), GC-induced alterations in brain WM development could potentially lead to the alterations reported in maltreated monkeys, including increased aggression. Our findings also

open new questions and hypotheses that need to be empirically tested. Does maltreatment lead to altered function of the affected circuits? When do these differences emerge and how they unfold? Prospective, longitudinal studies beginning at birth are necessary to address these important developmental questions in the context of maltreatment to determine the most beneficial timing and type of potential treatments, as well as intervention and prevention strategies.

Table 3.1

Correlations of FA, one month cortisol, and total aggression in adolescence.

Pearson correlation analysis was used. *$p<0.05$ was considered significant.

	Month 1 Cortisol		**Total Aggression**	
Cluster location	*r*	*p*	*r*	*p*
Corpus callosum	-0.512	0.025*	-0.31	0.181
Right occipital	-0.561	0.012*	-0.113	0.645
Left occipital 1	-0.483	0.036*	-0.281	0.244
Left occipital 2	-0.479	0.038*	-0.465	0.045*
EML	-0.637	0.003*	-0.254	0.293
Brainstem	-0.315	0.189	-0.317	0.118

Figure 3.1

Study specific template of 4.5 year old rhesus monkeys produced using iterative affine registrations and averaging as previously described McLaren, Kosmatka et al. 2009.

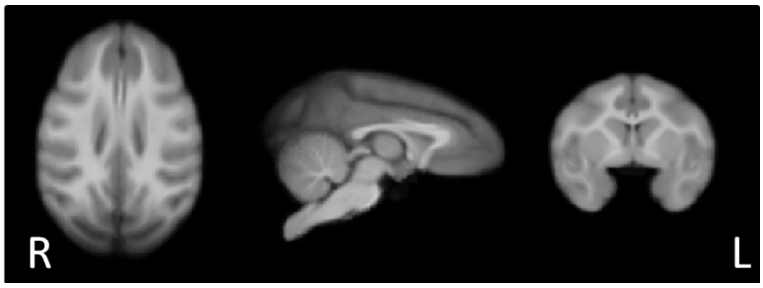


Figure 3.2

(A) Representative FA color map of a 4.5 year old rhesus monkey. Red represents left-right oriented fibers, blue represents dorsal-ventral oriented fibers, and green represents anterior-posterior oriented fibers. (B) Mean FA skeleton displayed on study specific template.

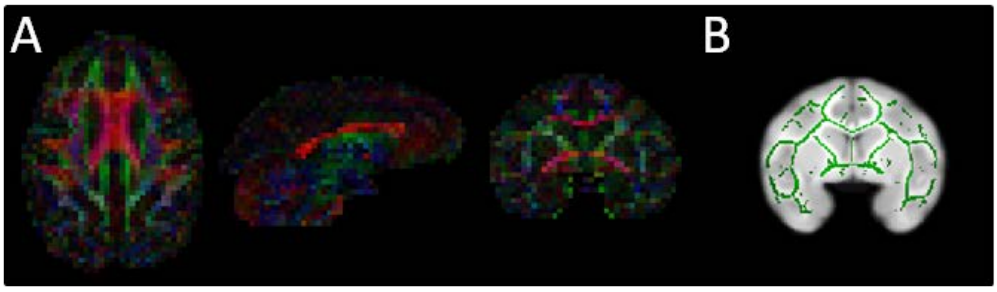


Figure 3.3

Maltreated animals have reduced FA in the corpus callosum. Red cluster of voxels in right corpus callosum represents the region where maltreated animals had significantly lower FA than controls ($p<0.005$, uncorrected, ≥10μL volume). In insets, green represents the mean FA skeleton.

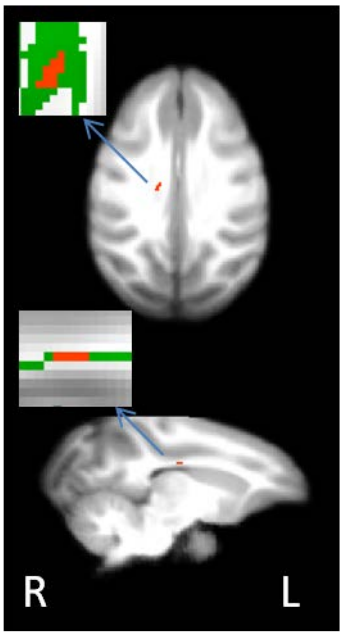


Figure 3.4

Maltreated animals have reduced FA in occipital WM. Three clusters of voxels (red) in occipital WM where maltreated animals had significantly lower FA than controls ($p<0.005$, uncorrected, ≥10μL volume). (A) Right occipital cluster. (B) Left occipital cluster 1. (C) Left occipital cluster 2. In insets, green represents the mean FA skeleton.

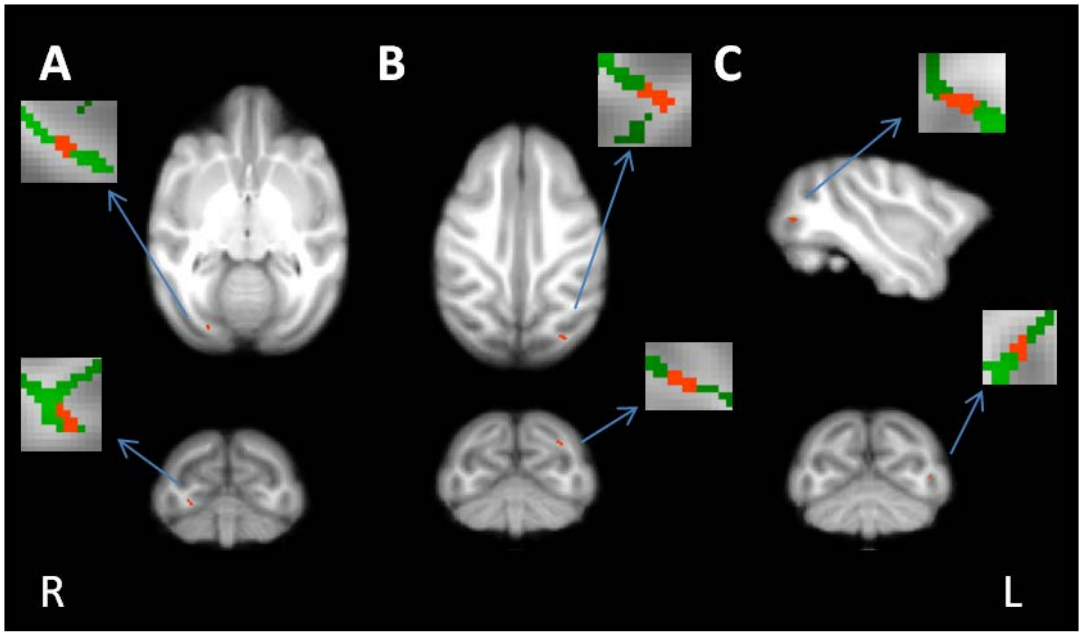


Figure 3.5

Maltreated animals have reduced FA in WM dorsal to the hippocampus and lateral to the pulvinar nucleus. The location of the cluster of voxels (red) where maltreated subjects had significantly lower FA than controls ($p<0.005$, uncorrected, ≥10μL) seems to correspond to the external medullary lamina (EML). In insets, green represents the mean FA skeleton.

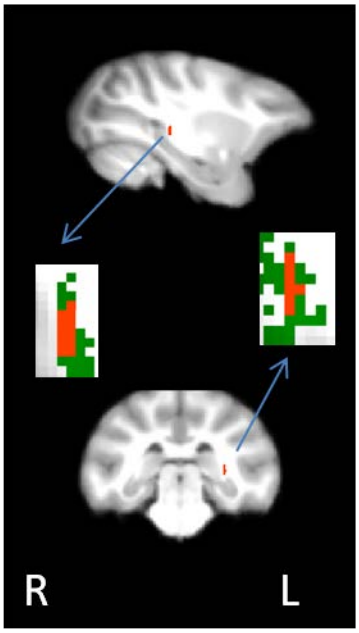
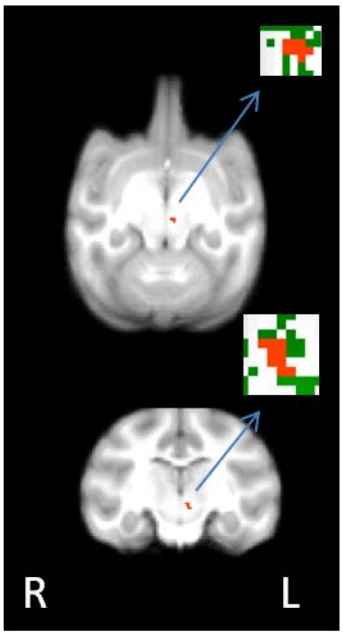


Figure 3.6

Maltreated animals have reduced FA in brainstem WM. The cluster of voxels (red) in left brainstem WM where maltreated animals had significantly lower FA than controls ($p<0.005$, uncorrected, ≥10μL) could correspond to the central tegmental tract (CTT). In insets, green represents the mean FA skeleton.



Chapter 4 : Longitudinal effects of infant maltreatment from birth using a cross-foster paradigm: experiential effects on brain white matter and emotional reactivity during infancy

4.1 Abstract

Child maltreatment has been consistently linked to increased risk for psychopathology, especially affective disorders. This increased risk is thought to be due to alterations in the brain resulting from repeated activations of the stress response. The primate brain undergoes rapid developmental changes early in life, and prolonged exposure to glucocorticoids (cortisol in primates) during brain development is one possible mechanism through which the chronic stress of child maltreatment might exert its effects on the brain. This prolonged exposure to elevated cortisol is thought to be particularly damaging to prefrontal-limbic circuits because of their prolonged development, sensitivity to glucocorticoids, and roles in behaviors altered in maltreated children. Evidence from cross-sectional studies supports this hypothesis, but without prospective longitudinal studies it is impossible to determine how these effects unfold during development. It is also impossible to disentangle the effects of heritable factors in human studies. The current study addresses both of these limitations by using a naturalistic model of spontaneous maternal maltreatment in rhesus monkeys. A cross-fostering design was utilized to control for the possible effects of heritable factors, and data was collected longitudinally during the first 6 months of life. To determine whether maltreated infants were exposed to chronically high levels of cortisol, hair was collected and assayed for accumulated cortisol. Physical growth measurements were also collected to investigate the effects of infant maltreatment on physical development. Diffusion tensor imaging (DTI) was used to investigate the effects of infant maltreatment at 2 weeks, 3 and 6 months of age on brain white matter (WM) microstructural integrity. Emotional reactivity was assessed using both observations in the social group and the Human Intruder (HI) paradigm. Maltreated infants had higher cortisol accumulation

than controls over the first 6 months of life, when they were receiving physical abuse and high rates of maternal rejection. Despite evidence in humans that maltreatment is related to deficits in physical growth, no group differences were detected in any of the measures of physical growth collected. Behavioral alterations included increased emotional reactivity in the social group, but decreased reactivity in response to threat measured during the HI. Group differences in WM integrity, measured as fractional anisotropy (FA) were detected at all ages scanned. At 2 weeks decreases in FA in maltreated infants were detected in cerebellar, frontal, and middle temporal-occipital WM, as well as in the internal capsule (IC). Maltreated infants also had higher FA than controls in medial occipital WM at 2 weeks. At 3 months increases in FA were detected in frontal WM and the IC. At 6 months decreases in FA were detected in the corpus callosum (CC), and prefrontal and middle temporal-occipital WM. Taken together these results provide evidence that infant maltreatment is related to chronically elevated cortisol levels, alterations in socioemotional behavioral and brain WM development.

4.2 Introduction

4.2.1 Why studying childhood maltreatment is important

Early life stress (ELS) such as childhood maltreatment, including physical abuse and neglect, is a much more pervasive issue than most people are aware of. In 2011 there were approximately 700,000 substantiated victims of childhood maltreatment (U.S. Department of Health and Human Services, Youth and Families, 2012), and estimated lifetime cost (including medical care, child welfare, criminal justice, and special education costs, and productivity losses) per victim of childhood maltreatment is estimated to be $210,012 (Fang, Brown et al. 2012). The high medical costs are not only

due to the acute care needed as a direct result of maltreatment, but also to the poor long-term outcomes associated with this adverse early experience. These outcomes include increased incidence of psychopathology (e.g. anxiety and depression, post-traumatic stress disorder, PTSD), behavioral disorders, substance abuse, cognitive and language deficits and obesity (Glaser 2000; Teicher, Andersen et al. 2002; Teicher, Andersen et al. 2003; Gunnar and Quevedo 2007; Weber, Rockstroh et al. 2008; Danese and Tan 2013). Furthermore, the earlier the exposure to maltreatment the more severe the psychological and behavioral outcomes (Keiley, Howe et al. 2001; Kaplow and Widom 2007). This becomes especially important given that more than 25% of victims of maltreatment are under the age of three years (U.S. Department of Health and Human Services, Youth and Families, 2012). Given the poor behavioral and health outcomes, and the number of children affected by this problem, it is critical to determine how these experiences get under the skin to affect the brain and behavior.

4.2.2 Effects of maltreatment on emotion regulation and social behavior

Childhood maltreatment has been linked to alterations in emotion regulation and social behavior that at first glance appear to be contradictory (Cicchetti and Toth 2005). For example, maltreated children show increased incidence of both internalizing and externalizing symptomatology (Kim and Cicchetti 2010), and thus exhibit dysregulated emotion patterns (Maughan and Cicchetti 2002; Kim and Cicchetti 2010). Children exposed to this type of ELS are prone to increased reactive aggression (Shields and Cicchetti 1998) and increased risk-taking (Weller and Fisher 2013), but also show increases in internalizing behaviors such as social withdrawal, anxiety, and depression (Kaufman 1991; Bolger and Patterson 2001; Cicchetti and Toth 2005). The heterogeneity of behavioral alterations reported in maltreated children could be explained by differences in the type, severity, or timing of maltreatment, as well as sex (Sanchez and

Pollak 2009). Maltreated children also show deficits in distinguishing between emotions. Physically abused children use more liberal criteria for defining angry faces and neglected children use broader criteria for defining sad faces (Pollak, Cicchetti et al. 2000; Pollak 2008). In physically abused children this broader definition of anger combined with an attentional bias towards angry faces (Pollak and Tolley-Schell 2003) could result in the alterations in socioemotional behavior described above via alterations in emotional learning during development (Pollak 2003). It is thus important to determine the neurodevelopmental underpinnings of these effects to gain a better understanding of how these effects are related to design effective treatment strategies.

4.2.3 Prefrontal-amygdala circuits regulate behaviors altered in maltreatment

Prefrontal-amygdala circuits are likely affected in maltreated populations because of their role in emotional regulation and inhibitory control of behavior. These circuits include several subdivisions of prefrontal cortex (PFC) including orbitofrontal cortex (oPFC, areas 11, 13, 14, and the region caudal to area 13 referred to as proisocortex, in monkeys (Carmichael and Price 1994)) and medial prefrontal cortex (mPFC, areas 24, 25, and 32 in monkeys (Carmichael and Price 1994)), and their connections (i.e. white matter [WM] tracts) with the amygdala. In primates, these prefrontal regions are not only reciprocally connected with each other but are also connected with the dorsolateral PFC (dlPFC) (Barbas 1995), which plays a strong role in top-down cognitive control of emotions. As discussed above, maltreated children have difficulty with inhibitory control of actions and emotional regulation (Shields and Cicchetti 1998; Stein 2008), causing them to be more impulsive and aggressive, and to be at higher risk to develop anxiety and mood disorders. These deficits are likely related to alterations in prefrontal-limbic circuits. For example, populations with impulsive

aggression show dysfunction in oPFC-amygdala circuitry (Coccaro, McCloskey et al. 2007).

Individuals with histories of childhood maltreatment also exhibit alterations in emotional reactivity and internalizing disorders such as anxiety and depression as discussed above (Bremner 2003). The mPFC and oPFC both have reciprocal connections with the amygdala, regulate emotionality and stress responses and are altered in behavioral disorders such as depression and anxiety (Barbas 2000; Drevets, Price et al.2008). Electrical stimulation of the WM tracts connecting these regions is currently used to treat depression and other psychopathologies (Gutman, Holtzheimer et al. 2009; Haber and Brucker 2009). Orbital PFC is implicated in the representation of emotional information and the regulation of emotional processes (Dolan 2007), and thus oPFC damage leads to socioemotional deficits, including emotional outbursts, impulsivity, difficulty with goal-directed behavior and failure to follow social norms (Tucker, Luu et al. 1995; Hartikainen, Ogawa et al. 2000; Bechara and Van Der Linden 2005). These connections with the amygdala play a key role in emotion, partly due to the amygdala's involvement in the regulation of fear, anxiety and other responses to threat. This role is consistent with amygdala alterations reported in psychopathologies such as anxiety disorders and depression (Phillips, Drevets et al. 2003; Shekhar, Truitt et al. 2005; Sibille, Wang et al. 2009).

Interestingly, studies using magnetic resonance imaging (MRI) have shown alterations in the volumes of the PFC (decreased volume) and amygdala (increased volume) in adults with histories of childhood maltreatment, as well as in the hippocampus (decreased volume, although not all studies report differences) (Bremner 2002; Teicher, Andersen et al. 2002; Bremner 2003; Teicher, Andersen et al. 2003; Bremner 2006). Some studies investigating brain structural alterations in maltreated children and adolescents have not detected these regional volumetric changes, but more

diffuse alterations such as reductions in cortical WM and corpus callosum (CC) volumes (Teicher, Andersen et al. 2003). Other studies utilizing both structural MRI and diffusion tensor imaging (DTI) also support the idea that maturation of WM is sensitive to ELS/adversity (Eluvathingal, Chugani et al. 2006; Choi, Jeong et al. 2009; Coplan, Abdallah et al. 2010; Govindan, Behen et al. 2010; Huang, Gundapuneedi et al. 2012; Howell, Godfrey et al. 2013), possibly due to the dramatic developmental changes in myelinated WM, and fiber tracts in general, that occur from childhood through adulthood in both humans (Lebel, Walker et al. 2008; Asato, Terwilliger et al. 2010) and nonhuman primates (Shi, Short et al. 2013). It is this protracted development of brain WM in primates, especially in regions involved in emotion regulation, such as prefrontal and temporal regions (Lenroot and Giedd 2006; Lebel, Walker et al.2008), that is thought to make these regions particularly vulnerable to ELS.

Not much is known about human PFC-amygdala circuit development. The basic architecture of the human amygdala is present at birth (Humphrey 1968) and volumetrically appears to develop early in life, reaching adult levels in girls by age 4 (Giedd, Vaituzis et al. 1996). In human PFC maximum synapse density is reached at around 15 months of age and then decreases through midadolescence (Huttenlocher and Dabholkar 1997). Myelination of WM tracts that connect these regions begins during childhood and doesn't reach maturity until young adulthood, around 20 years old (Gibson 1991). More specific information on the development of these circuits has been gained through studies of rhesus monkeys. At birth the macaque cerebral cortex contains an excess of neurons, dendrites, axons and synapses that are selectively pruned throughout development following appropriate time courses that can be defined both structurally and functionally (Hayashi 1992). Synaptic density increases rapidly through postnatal month 4 when it begins to decline, reaching adult levels at 3 years (Rakic, Bourgeois et al.1986). The peak in limbic synapse density coincides with the onset of

limbic function (e.g., memory, social fear) at postnatal month 2 (Rakic and Nowakowski 1981) and reaches full functional maturity at 4 months (Bachevalier and Mishkin 1984; Kalin and Shelton 1989). Other maturational processes such as axon elimination and myelination also occur at different times in different regions, again in parallel with emergence of specific behaviors. For example amygdala afferents from portions of temporal cortex that relay visual information mature at week 3 when an animal first begins to respond appropriately to social cues, while efferents from these same temporal regions to oPFC do not become mature until 2 months when curiosity and frustration become apparent (Machado and Bachevalier 2003). It is also during this time that the amygdala undergoes the fastest changes in volume with rates stabilizing around 8 months (Payne, Machado et al. 2010). PFC development occurs over a much longer period, not becoming mature until approximately 3-4 years (Machado and Bachevalier 2003). An example of how maturation and myelination of the PFC is reflected behaviorally is in the transition from infancy to early childhood, when the capacity for effortful behavioral control becomes apparent (Dawson, Panagiotides et al. 1992; Maestripieri and Carroll 1998). These examples highlight the close connections between the development of prefrontal-amygdala circuits and emergence of behaviors they control, and lead to questions about what happens when these closely coupled processes are altered.

4.2.4 Early life stress lead to alterations in the development of limbic regions: possible mechanisms?

Childhood maltreatment is an early stressful experience that results in repeated activation of main stress response systems such as the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (Van Voorhees and Scarpa 2004; Tarullo

and Gunnar 2006; Chrousos 2009). Stressful stimuli cause the release of corticotropin releasing hormone (CRH) from the hypothalamus, which then stimulates release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary. ACTH binds to cell receptors on the adrenal cortex inducing synthesis and release of the glucocorticoid (GC) cortisol in primates (Sanchez, Ladd et al. 2001; de Kloet, Joels et al. 2005). Cortisol then binds to intracellular receptors (glucocorticoid receptors [GRs] and mineralocorticoid receptors [MRs]), which work as transcription factors in target tissues to affect gene expression. Thus, one potential mechanism by which ELS experiences such as maltreatment can affect neurodevelopment is through the ability of stress-induced elevations in cortisol to alter gene expression. This is especially pertinent to the development of limbic regions and PFC in primates because GRs and MRs are expressed at high levels in these regions (Sanchez, Young et al. 2000; Sanchez 2006). This, combined with their protracted development, makes them particularly vulnerable to stress-induced elevations of cortisol. In fact in rodents, chronic stress is related to increases in dendritic arborization in the amygdala and decreases in dendritic arborization in the hippocampus, another limbic region important in regulating the HPA axis (Vyas, Mitra et al. 2002).

Not only is HPA axis activation thought to be an underlying mechanism for maltreatment-induced neurodevelopmental alterations in prefrontal-limbic circuits (as described above), but the HPA axis is strongly regulated by these prefrontal-limbic circuits, which are also altered in maltreated populations (Herman, Ostrander et al. 2005; Cerqueira, Almeida et al. 2008). This is thought to lead to a feed-forward cascade of effects. In fact, alterations in HPA axis function (basal and stress reactivity) have been reported in victims of maltreatment (Sanchez 2006; Tarullo and Gunnar 2006). In animal models of ELS the HPA axis response to stress is increased, as are behavioral

responses such as fear, anxiety and acoustic startle (Sanchez, Ladd et al. 2001; Sanchez, Noble et al.2005) suggesting alterations in these prefrontal-limbic modulatory circuits.

4.2.5 Nonhuman primate model of infant maltreatment

Despite the evidence of neurobehavioral alterations reported in human adults and children with histories of childhood maltreatment, very little is known during the infant period or regarding the emergence and time course of neurodevelopmental alterations. This is partially due to difficulties of doing prospective, longitudinal studies in at risk children and the possibility that these alterations may not result from ELS experiences, but are transmitted transgenerationally via genetic/epigenetic mechanisms. The current study utilized a translational model of infant maltreatment in socially housed rhesus monkeys to address these difficult questions. The overall goal was to test the hypothesis that ELS in the form of maltreatment is, indeed, stressful and leads to alterations in PFC-amygdala circuits that emerge with age and are related to alterations in social and threat-response behavior. Based on evidence that maltreatment can be perpetuated from generation to generation in human and nonhuman primates, and that genetic and epigenetic mechanisms can result in behavioral traits associated with maltreatment (Maestripieri 2005; Huizinga, Haberstick et al. 2006; Tarullo and Gunnar 2006; Franklin, Russig et al. 2010), it is important to disentangle the role of ELS experience from that of heritability on these outcomes. This was accomplished in the current study by cross-fostering infants at birth using random assignment to either control mothers, or mothers with a history of maltreating their infants (see Section 4.3.3 below for details). To confirm that infant maltreatment is a stressful experience, cortisol in the hair (accumulated during the first 6 months of life) was measured in addition to behavioral measures of infant distress. Emotional reactivity was measured using the Human Intruder paradigm at 3 and 6 months of age based on previous reports of the

development of behaviors during this task (Kalin and Shelton 1989; Kalin and Shelton 1998; Raper, Wallen et al. 2013), in addition to social behaviors collected in the social group throughout the first 6 months of life. Diffusion tensor imaging (DTI) was used to investigate the effects of this adverse early experience on brain WM development throughout the first 6 months of life. Due to the rapid development early in infancy of the amygdala and the protracted development of PFC, DTI data were collected at 3 time points during the first 6 months of life: 2 weeks, 3 months, and 6 months of age. Because sex differences exist in brain development in the PFC-amygdala circuits hypothesized to be affected (Giedd, Vaituzis et al. 1996; Giedd, Blumenthal et al. 1999), the effects of maltreatment on the brain (De Bellis and Keshavan 2003 ; Elton, Tripathi et al. 2013), stress systems (Sanchez, Noble et al. 2005; Doom, Cicchetti et al.2013), and vulnerability to develop psychopathology (Shea, Walsh et al. 2005), the current study also investigated whether the outcome measures collected varied by sex of the infant.

4.3 Methods

4.3.1 Subjects and housing

Subjects for this study were 40 infant rhesus monkeys (*Macaca mulatta*) studied from birth through 6 months of age, reared with their mothers in complex social groups 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. Each social group had a stable matrilineal structure and dominance hierarchy. Water was available *ad libitum* and standard high fiber, low fat monkey chow (Purina Mills Int., Lab Diets, St. Louis, MO) was provided twice daily. Additionally, fresh fruit

and enrichment items were provided daily. All procedures described below were approved by the Emory Institutional Animal Care and Use Committee and were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Successful cross-fosters (90% success rate; see Section 4.3.3 for cross-fostering details) across 4 breeding seasons resulted in 40 infant monkey subjects, 10 (4 male, 6 female) born between April and May 2009, 13 (9 male, 4 female) born between April and June 2010, 12 (6 male, 6 female) born between April and June 2011, and 4 (2 male, 2 female) born between March and May 2012. After observations of maternal care from birth to confirm group assignment (see Section 4.3.4 for details), the sample used for this study included 11 subjects (6 male, 5 female) in the control-to-control cross-fostered group (i.e. biological infant of a control mother cross-fostered to a control mother), 9 subjects (3 male, 6 female) in the maltreating-to-control group (i.e. biological infant of a maltreating mother cross-fostered to a control mother), 12 subjects (9 male, 3 female) in the control-to-maltreating group (i.e. biological infant of a control mother cross-fostered to a maltreating mother), and 8 subjects (5 male, 3 female) in the maltreating-to-maltreating group (i.e. biological infant of a maltreating mother cross-fostered to a maltreating mother). To control for the possible confounding effects of social rank, the mother's dominance rank (high, medium, low) was also considered for assignment in order to balance it within each experimental group as much as possible. Social ranks were based on existing data at the YNPRC on aggression and submission collected in these groups as in previous studies (Bernstein 1976). In the maltreated group there were 7 high ranking, 9 middle ranking, and 4 low ranking animals, and in the control group there were 5 high ranking, 8 middle ranking, and 7 low ranking animals (see Section 4.3.3 for details on group assignments and Table 4.2 for a summary of the subjects).

4.3.2 Training and capture

Prior to the beginning of this study, all potential foster mothers were trained and habituated to the procedures for capture. These included moving on command from the outdoor enclosure to an indoor capture unit, and from the capture unit to a transfer box, following previously published procedures (Laule, Bloomsmith et al. 2003). For all capture procedures following a successful cross-foster, the mothers were accessed in their groups just as they had been during training, except now carrying their cross-fostered infants with them.

4.3.3 Experimental design: cross-foster design

In order to control for the potential contribution of heritable traits and prenatal experience, all infants were cross-fostered the day of birth to an unrelated female (counterbalanced for competent or maltreating maternal care and dominance rank), with the exception of a few infants cross-fostered within 48 (n=3) or 72 hours (n=1) of birth. Previous studies have demonstrated that cross-fostering is feasible in rhesus monkeys if done close to birth using the methods described below without affecting the foster mothers' maternal behavior (Maestripieri, Megna et al. 2000; Maestripieri 2005; Maestripieri, Lindell et al. 2007). Rhesus monkeys are seasonal breeders, with breeding occurring in the fall and births occurring between March and June at the YNPRC, increasing the likelihood of two mothers giving birth close to each other for successful cross-fostering (i.e. to avoid adoption failure). Potential foster mothers were multiparous females identified using YNPRC and laboratory records based on their prior history of maternal care, either competent care (potential control mothers) or physically abusive and neglectful care (potential maltreating mothers). Extremely abusive females (i.e. history of abuse leading to serious injury or death of the infant) are removed from breeding by the YNPRC, and were thus excluded from the study due to ethical concerns

and to avoid confound effects of physical trauma on our measures. Multiparous females were also chosen to control for differences in maternal behavior related to parity (Seay 1966; Maestripieri and Carroll 1998). Prior to each birth season, sonograms were collected to verify pregnancies, estimate birth dates, and monitor fetal health. Cross-fostering was done within a few hours of parturition (first day of life) for most infants, except for those exceptions listed above, between females living in different social groups. Mother-infant separations were never more than 5 minutes during the cross-fostering procedure (Maestripieri, Megna et al. 2000; Maestripieri 2005). This short separation and the integral involvement of YNPRC veterinary and colony management staff have been key factors in successfully cross-fostering rhesus monkeys at YNPRC, in addition to performing this procedure as close to birth as possible.

The assignment of each infant as either control or maltreated was further confirmed post cross-fostering via focal observations of the mother-infant pair (see section 4.3.4 for focal observation details). In this study maternal maltreatment was operationalized using two maternal behaviors: physical abuse and early infant rejection. Maternal abuse was defined as any of the following aberrant, violent, infant-directed actions: dragging, crushing, rough grooming, throwing, stepping or sitting on, or roughly carrying the infant (Maestripieri 2005; McCormack, Sanchez et al. 2006) (see Table 4.3 for definitions of these actions). In order to be included in the maltreated group at least three occurrences of physical abuse had to be recorded during the first three months of life. Maternal rejection was defined as the mother preventing contact or infant access to the her ventrum by holding the infant at a distance with an arm or leg, passively blocking the chest with an arm, or twisting her torso away from the infant (McCormack, Sanchez et al. 2006).

4.3.4 Behavioral data collection in the social group

Focal observations of each infant (including mother-infant interactions to characterize maternal care) were collected during the first 6 months postpartum by four experienced coders from observation towers situated over each social compound. An adaptation of a well established rhesus monkey ethogram (Altmann 1962) was used, following previously published modifications and procedures (Maestripieri 1998; McCormack, Sanchez et al. 2006) (see Table 4.4 for complete ethogram). Prior to data collection, reliability was reached among the 4 observers, such that percent agreement exceeded 90% and Cohen's Kappa exceeded 0.8. Each observation lasted 30 minutes, and occurred five times per week in the first month postpartum, twice per week in the second month, and once per week in the third month. This observation schedule was chosen in order to best document early maternal care received by the infant, including the occurrence of infant maltreatment, as the frequency of physical abuse is highest in the first month and decreases steadily thereafter (Maestripieri 1998; McCormack, Sanchez et al. 2006). This schedule was also chosen to fully capture the rapid developmental changes in the infant and the mother-infant relationship during the first 3 months of life. After the third month, four 30 min observations were collected at 3, 4, 5 and 6 months of age. Observations were done between 7:00 and 11:00 AM, when the animals are most active. All animals in the group were locked outdoors during observation sessions. Whenever possible no more than one observation per subject was conducted in a single day and no observations were collected on the same day as other procedures (e.g. the HI task). Data was collected on either laptop (Lenovo IdeaPad S10) or hand held computers (Palm IIIxe) using in-house behavioral software designed to collect the initiator, behavior, recipient and time of each behavior observed (Graves and Wallen 2006).

4.3.5 Hair cortisol

Cortisol accumulates in the shaft of hair that is being produced during exposure to an experience (e.g. during a stressful event) (Cone 1996; Davenport, Tiefenbacher et al. 2006). On the second day following cross-fostering a patch of hair was shaved from the sub-occipital region of each infant to measure hair cortisol accumulation. This was done to assess baseline hair cortisol levels at birth and examine potential prenatal stress exposure. At 6 months of age the hair that had grown in the same region since the original removal was shaved, and assayed to measure cortisol accumulation that had occurred between birth and 6 months of age to assess potential chronic exposure to cortisol during the first 6 months of life. Analysis of the hair samples for cortisol accumulation was done according to the methods described in Davenport et al. (2006). Briefly, each sample was washed twice in isopropanol to remove external contamination, dried and ground to a find powder using a Retsch ball mill. The powder was extracted overnight with methanol, and cortisol assayed in duplicate using a sensitive and specific enzyme immunoassay (Salimetrics, State College, PA).

4.3.6 Physical growth

To assess the potential effects of maltreatment on physical development, as previously reported in human populations (Pears and Fisher 2005; Johnson, Bruce et al. 2011), measures of physical growth were collected at the following ages: 2 days, 2 weeks, 3 months, and 6 months. Measures included head circumference (cm), crown-rump length (cm), crown-heel length (cm), and weight (kg).

4.3.7 Human Intruder paradigm

The HI paradigm (Kalin and Shelton 1989) was collected at 2 ages (3 and 6

months) to determine whether maltreated animals differed from control animals in the development of their behavioral responses to threat. These two ages were selected based on previous literature describing the ontogeny of responses to threat in which organized behavioral responses begin to appear at 3 months of age and continue to develop throughout the adolescent period (Kalin and Shelton 1998; Raper, Wallen et al. 2013). All 40 subjects were tested at 3 months, however one animal (control female) was not tested at 6 months of age due to a camera malfunction. Immediately following capture and prior to this task a baseline blood sample was obtained for a separate study to examine stress-induced cortisol plasma increases in response to the task (pre- versus post-test cortisol levels; data not presented here). Animals were accessed as described in Section 4.3.2, transported to a novel testing room, and then transferred to a stainless steel cage with one side made of clear plexiglass to allow video recording. The test lasted approximately 30-min, and consisted of three conditions (alone, profile [referred to as "no eye contact" in other publications], and stare) presented in the same order for all animals. The intruder wore a rubber mask (different for each age) depicting a male face with cutouts at the eyes to allow the monkey to view the experimenter's eyes. The animal was first left alone in the cage for 10 minutes (alone condition), then the intruder entered the room and sat approximately two meters from the clear wall of the test cage for 10 minutes while presenting his/her profile to the animal (profile condition). After the profile condition, the intruder turned to make direct eye contact with the animal for 10 minutes (stare condition). Immediately following the test, monkeys were reunited with their mothers and returned to their social groups.

Animals' behavior during the HI paradigm was video recorded and later coded using a detailed ethogram based on previous studies (Kalin and Shelton 1989; Machado and Bachevalier 2008; Raper, Wallen et al. 2013) (Table 4.5). Digital videos were coded using the Observer XT program (v10.5, Noldus, Inc., Netherlands) by one experimenter.

The coder had an average inter-rater reliability of Cohen's Kappa = 0.845 with other trained experimenters who coded a representative set of videos from similar studies with rhesus infants (Raper, Wallen et al. 2013).

4.3.8 DTI data

4.3.8.1 DTI acquisition

Subjects were transported from their social group with their mothers to the YNPRC Imaging Center the day before the scans, which were performed at 2 weeks, 3 months, and 6 months of age. Due to health concerns and social group stability issues, some infants were not scanned at every age, and due to image artifacts not all data collected could be used. The final subjects included at each age were: 2 weeks- 16 maltreated (10 males and 6 females) and 17 controls (6 males and 11 females); 3 months - 17 maltreated (11 males and 6 females) and 17 controls (8 males and 9 females); 6 months - 17 maltreated (12 males and 5 females) and 18 controls (8 males and 10 females). A DTI MRI scan was acquired using a 3T Siemens Magnetom TRIO system (Siemens Med. Sol., Malvern, PA) and an 8-channel phase array coil. All animals were scanned supine in the same orientation, achieved by placement and immobilization of the head in a custom-made head holder using ear bars and a mouth piece. A vitamin E capsule was taped on the right temple to mark the right side of the brain. Scans were acquired under isoflurane anesthesia (0.8-1% to effect, inhalation), following initial induction with telazol (5mg/kg, i.m.) and endotracheal intubation. Animals were fitted with an oximeter, ECG, rectal thermistor and blood pressure monitor for physiological monitoring, an i.v catheter to administer dextrose/NaCl (0.45%) to maintain normal hydration, and an MRI-compatible heating pad. Upon completion of the scans and full

recovery from anesthesia, each infant was returned to its mother, and the mother-infant pair returned to their social group.

DTI scans were acquired following published protocols by our group for rhesus monkeys (Hecht, Gutman et al. 2013; Howell, Godfrey et al. 2013), using a single-shot dual spin-echo echo planar imaging (EPI) sequence with GRAPPA (R=3), voxel size=1.3x1.3x1.3 mm^3 with zero gap, 60 directions, TR/TE=5000/86ms, FOV= 83mm, b:0, 1000s/mm^2, and 12 averages. T1-MRI scans were also acquired for registration purposes using a 3D magnetization prepared rapid gradient echo (3D-MPRAGE) parallel imaging sequence with GRAPPA (R=2), voxel size=0.5x0.5x0.5 mm^3, TI/TR/TE=950/3000/3.49ms, FOV=96mm, 8 averages.

4.3.8.2 DTI image analysis

Preprocessing

Diffusion weighted images were upinterpolated to an isotropic 0.65 mm resolution using windowed sinc interpolation. Diffusion tensors were computed using weighted least squares fitting (Goodlett, Fletcher et al. 2009) via the NAMIC DTI Process software suite (http://www.nitrc.org/projects/dtiprocess). Eigenvalues and corresponding eigenvectors were calculated to obtain diffusion properties, including FA, axial diffusivity (AD), and radial diffusivity (RD), as described in Shi et al. (2012). Skull stripping was performed by applying a binary brain mask to the diffusion property maps.

FA, in combination with AD and RD, characterizes the local microorganization of brain WM and has been previously used in DTI studies describing developmental changes of brain tracts (Shi, Short et al. 2013), effects of lesions (Shamy, Carpenter et al. 2010) in this species, as well as effects of experience (Tang, Lu et al. 2012) and ELS (Eluvathingal, Chugani et al. 2006; Govindan, Behen et al. 2010; Frodl, Carballedo et al. 2012; Huang, Gundapuneedi et al. 2012) in humans. This is because changes in FA (calculated as the ratio of diffusion parallel to the fibers to the diffusion perpendicular to

the fibers) can be due to either changes in perpendicular diffusion along the tract (measured as RD, which decreases with increased axonal myelination (Keller and Just 2009; Zhang, Jones et al. 2009; Bennett, Madden et al. 2010)) or to changes in parallel diffusivity (measured as AD, which increases with axonal density, caliber, and microtubular packing and organization (Kumar, Macey et al. 2010; Kumar, Nguyen et al. 2012)), evidence also supported by combined DTI and histological studies performed in rodent and nonhuman primate brains (Song, Sun et al. 2002; Song, Sun et al. 2003; Choe, Stepniewska et al. 2012). Thus, higher FA values in the presence of decreased RD, but no AD changes, is generally interpreted as increased WM tract integrity due to increased myelin, whereas increased FA in parallel to increased AD, without RD changes, indicates increased fiber tract organization.

Tract-based Spatial Statistics (TBSS)

The Centre for Functional MRI of the Brain (FMRIB, Oxford, UK) Software Library (FSL) (Smith, Jenkinson et al. 2004; Woolrich, Jbabdi et al. 2009)) TBSS tool (Smith, Jenkinson et al. 2006) was used to identify the centers of all major WM tracts present in all subjects. In our study, the data was registered to study- and age-specific atlases produced by Dr. Martin Styner's group at the University of North Carolina, Chapel Hill, NC. (as described in Shi et al. 2012), resulting in a final resolution of 0.65x0.65x 0.65 mm^3 (1 voxel has volume 0f approximately 0.27μL). The mean FA image was then skeletonized and thresholded (only voxels with FA>0.2 were included) so that only the centers of major WM tracts are included in the analysis, excluding small peripheral tracts that may confound findings due to anatomic individual variability and partial volume effects. Each individual subjects' local maximum FA values were then projected onto the mean FA skeleton (see Smith, Jenkinson et al. 2006 for details). Skeletonized-FA data significantly minimizes the number of voxels included in the voxel-wise statistical analysis (described below in Section 4.3.9.1), cutting down multiple

comparisons, and is less dependent on the accuracy of the initial registrations. The neuroanatomical locations and possible origins/destinations of fibers included in the resulting clusters with statistically significant group differences were identified using available rhesus brain and fiber pathway atlases (Schmahmann and Pandya 2006; Saleem and Logothetis 2012).

4.3.9 Statistical analyses

4.3.9.1 Statistical analysis of behavioral, growth and cortisol measures

All analyses were done using IBM SPSS v19 statistical software. Data were analyzed using a repeated measures analysis of variance (rmANOVA) with maternal care (control or maltreating) and sex (male or female) as between subjects factors, and age (months 1-3 for abuse and rejection measures used for group classification; months 1-6 for behaviors recorded in the social group; 3 or 6 months for HI data) as the within subjects factor. Each measure was first tested for normality using the Shapiro-Wilk test. If the data were not normally distributed a log base 10 transform was applied. If the measure had any zero values the formula was used to account for these zero values. Results for data that were still not normally distributed after the log transform was applied are reported with the Greenhouse-Geisser correction. Effects were considered significant where $p \leq 0.05$, and post-hoc comparisons were conducted, when necessary, using a Bonferroni correction for multiple comparisons. Results are presented as mean ± standard error of the mean (SEM) of untransformed data. To examine the potential effects of inherited characteristics, a separate repeated measures analysis of covariance (ANCOVA) was performed with biological mother (control or maltreating) included as a covariate.

Behavioral measures were analyzed individually or as composite scores (see Table 4.6 for behaviors analyzed from observations in the social group and Table 4.7 for behaviors analyzed from the HI paradigm). Behaviors coded as frequencies were measured as occurrences per minute and those coded as durations were measured as proportion of observation the subject was observed doing the behavior.

For the HI data an initial rmANOVA was performed that included the factors listed above as well as condition (alone, profile, stare) as a second within-subjects factor. Because there is already abundant literature on significant condition effects on the behavioral responses of rhesus infants (Kalin and Shelton 1989; Machado and Bachevalier 2008; Raper, Wallen et al. 2013) and the main focus of the current study was differences due to early experience, and no maternal care-by-condition interaction effects were detected with this 4 factor model, each condition was analyzed separately for the human intruder paradigm to increase power to detect effects of the remaining factors.

4.3.9.2 Statistical analysis of DTI measures

A voxel-wise, two-way analysis of variance (ANOVA) was run to examine the effects of maternal care (control, maltreating) and sex (male, female) on skeletonized FA data using a general linear model and the Randomise tool in FSL (Smith, Jenkinson et al. 2004; Woolrich, Jbabdi et al. 2009) for each scan age. Clusters were considered significant at $p<0.05$, uncorrected, with a cluster-threshold correction of at least 50 contiguous voxels, similar to previously published thresholds (Willette, Bendlin et al. 2010; Howell, Godfrey et al. 2013).

Binary masks were created for the regions where group differences in FA were detected (i.e. clusters of voxels with statistically significant FA differences detected in the voxel-wise ANOVA for maternal care and/or sex effects). The mean RD and AD were calculated within these clusters, following previously published approaches (Smith,

Jenkinson et al. 2004; Tang, Lu et al. 2012; Howell, Godfrey et al. 2013). A two-way ANOVA was run in SPSS to examine the effects of maternal care and sex on RD and AD in those clusters with significant FA differences, with significance level set at $p<0.05$.

4.4 Results

4.4.1 Maternal behavior: physical abuse and rejection during the first 3 months of life

Maltreated foster infants received rates of abuse and rejection similar to those previously reported in infants raised by their biological mothers (McCormack, Sanchez et al. 2006). Thus, rates of physical abuse and rejection received were significantly higher for infants fostered to maltreating mothers across the first 3 months of life (maternal care main effect for abuse: $F_{(1, 36)}=25.829$, $p= 0.00001$, $\eta_p^2=0.418$,Fig. 4.1A; maternal care main effect for rejection: $F_{(1,36)}=46.652$, $p= 5.487E-8$, $\eta_p^2=0.564$,Fig. 4.1B). Males were rejected more than females across the first 3 months of life (sex main effect: $F_{(1,36)}=5.242$, $p=0.028$, $\eta_p^2=0.127$). A main effect of age was detected for abuse ($F_{(1.808,65.079)}=4.223$, $p=0.022$, $\eta_p^2=0.105$), with rates at month 1 being significantly greater than month 3 ($p=0.021$). A significant maternal care-by-age interaction was also detected for physical abuse ($F_{(4.054, 65.079)}=4.054$, $p=0.025$, $\eta_p^2=0.101$), with rates being significantly higher in the maltreated as compared to the control group at months 1 ($p=2.665E-5$) and 2 ($p=0.007$). No other main or interaction effects were detected. Including biological mother as a covariate did not affect the maternal care effects reported above.

4.4.2 Cortisol accumulation in hair

A main effect of maternal care was detected with maltreated infants having significantly greater levels of accumulated cortisol than control infants ($F_{(1,32)}$=5.843, p=0.022, η_p^2=0.154, Fig. 4.2). Month 6 hair cortisol was significantly lower than day 2 (age main effect: $F_{(1,32)}$=518.703, p=2.431E-21, η_p^2=0.942, Fig. 4.2). No other main or interaction effects of care, sex, or age were detected. When biological mother was included as a covariate the main effects of maternal care remained, and a significant maternal care-by-age interaction was detected (F(1, 31)=4.203, p=0.049, η_p^2=0.119), with maltreated infants having significantly greater levels of hair cortisol accumulated during the first 6 months of life than control infants (p=0.007), but not prenatally.

4.4.3 Physical growth

No main effects of maternal care were found for any of the measures of physical growth collected; however a main effect of age was detected for all measures (age main effect for weight: $F_{(1.812,63.434)}$=472.612, p= 9.914E-38, η_p^2=0.931, Fig. 4.3A; age main effect for crown-rump length: $F_{(2.529,85.982)}$=382.209, p= 5.027E-47, η_p^2=0.918 Fig. 4.3B; age main effect for crown-heel length: $F_{(1.943,66.048)}$=787.640, p= 1.862E-46, η_p^2=0.959 Fig. 4.3C; age main effect for head circumference: $F_{(2.514,72.913)}$=242.266, p= 1.08E-35, η_p^2=0.893, Fig. 4.3D), with all measures increasing with age. No other main or interaction effects of care, sex, or age were detected. There were no significant effects of biological mother when it was included as a covariate.

4.4.4 Behavior in social group

4.4.4.1 Emotional reactivity

Maltreated infants showed higher rates of tantrums and screamed more than controls across the first 6 months of life (maternal care main effect for tantrums: $F_{(1, 35)}$=7.291, p=0.011, η_p^2=0.172, Fig. 4.4A; maternal care main effect for screams: $F_{(1,35)}$=14.688, p=0.001, η_p^2=0.296, Fig. 4.4B). A main effect of age was also detected for tantrums ($F_{(4.344,152.03)}$=4.704, p=0.001, η_p^2=0.118) with rates decreasing across time. A significant care-by-age interaction was detected for screams (maternal care-by-age effect: $F_{(4.055, 141.93)}$=2.828, p=0.026, η_p^2=0.075), with maltreated infants screaming at higher rates than controls at months 1 (p= 5.696E-5), 3 (p=0.01), and 6 (p=0.002). No other main or interaction effects of care, sex, or age were detected. The inclusion of biological mother as a covariate did not affect any of the effects of maternal care reported above.

4.4.4.2 Anxiety and interactions with others

A main effect of age was found for anxiety-like behaviors ($F_{(4.120,144.189)}$=9.663, p= 4.448E-7, η_p^2=0.216, Fig. 4.5A), avoidance of others ($F_{(3.128,109.477)}$=21.771, p= 2.034E-11, η_p^2=0.383, Fig. 4.5B), play solicits ($F_{(3.841,134.422)}$=21.324, p= 2.721E-13, η_p^2=0.379, Fig. 4.6A), and social play ($F_{(3.953,138.346)}$=23.249, p=1.329E-14, η_p^2=0.399, Fig. 4.6B) with rates of all of these behaviors increasing across time. A main effect of sex was also detected for play solicit ($F_{(1,35)}$=6.345, p=0.016, η_p^2=0.153) and social play ($F_{(1,35)}$=4.296, p=0.046, η_p^2=0.109), with males soliciting others for play more frequently and spending more time playing than females across the first 6 months of life. A significant maternal care-by-age-by-sex interaction for play solicits ($F_{(3.841, 134.422)}$=3.228, p=0.016, η_p^2=0.084), where maltreated females soliciting others for play less than controls at

month 2 (p=0.017), but not at the other ages examined. There were no main or interaction effects of care, sex, or age detected for affiliative behaviors (duration of contact with and groom of others). No other main or interaction effects of care, sex, or age were detected. The inclusion of biological mother as a covariate did not alter any of the effects of maternal care reported above.

4.4.5 Human Intruder paradigm

4.4.5.1 Alone condition

No main effects of maternal care were detected in any of the behaviors collected when the subjects were brought from their social groups and left alone in the novel testing environment. Significant main effects of age were detected for the rates (measured as events per minute) of screams ($F_{(1,35)}$=19.074, p= 0.0001, η_p^2=0.353, Fig. 4.7A), aggression ($F_{(1,35)}$=5.522, p= 0.025, η_p^2=0.136, Fig. 4.8A), and atypical behaviors ($F_{(1,35)}$=8.108, p= 0.007, η_p^2=0.188, Fig. 4.9A), with all of these behaviors decreasing from 3 to 6 months. A significant maternal care-by-age interaction effect was detected for rate of screams ($F_{(1,35)}$=6.415, p= 0.016, η_p^2=0.155, Fig. 4.7A), with maltreated infants screaming less frequently than controls at 6 months (p=0.037). A significant maternal care-by-age interaction effect was also detected for rate of anxiety behaviors ($F_{(1,35)}$=4.718, p= 0.037, η_p^2=0.119, Fig. 4.10A); although a posteriori tests did not yield significant differences, maltreated infants showed higher rates of anxiety behaviors than control infants at 3 months, with an opposite pattern at 6 months (i.e. maltreated infants had lower rates of anxiety behaviors than controls). Significant sex-by-age interaction effects were found for rates of screams ($F_{(1,35)}$=7.370, p= 0.010, η_p^2=0.174, Fig. 4.7A), aggression ($F_{(1,35)}$=8.336, p=0.007, η_p^2=0.192, Fig. 4.8A), and anxiety ($F_{(1,35)}$=5.437, p= 0.026, η_p^2=0.134, Fig. 4.10A), with females exhibiting all of these behaviors at greater

rates than males at 3 months of age (screams p=0.035, aggression p=0.015, and anxiety p=0.021). When biological mother was included as a covariate several age (but not care or sex) effects reported above changed, specifically the aggression and atypical behaviors main age effects were no longer significant. No other main or interaction effects were detected.

4.4.5.2 Profile condition

When the subjects were presented with a potential threat (the intruder's profile) only main effects of age were detected. Significant age effects were detected for rates of screams ($F_{(1,35)}$=5.811, p= 0.021, η_p^2=0.142, Fig. 4.7B) and withdrawal ($F_{(1,35)}$=4.103, p= 0.05, η_p^2=0.105, Fig. 4.11B), as well as for duration of freezing ($F_{(1,35)}$=6.604, p= 0.015, η_p^2=0.159, Fig. 4.12B), with all of these measures decreasing from 3 months to 6 months. Significant age effects were also detected for duration of total explore ($F_{(1,35)}$=7.855, p= 0.008, η_p^2=0.183, Fig. 4.13B) and frequency of urination or defecation ($F_{(1,35)}$=5.470, p= 0.025, η_p^2=0.135, Fig. 4.14B), with these measures increasing from 3 to 6 months. When biological mother was included as a covariate the age effects reported above for freezing and urination/defecation were no longer significant. No other main or interaction effects were detected.

4.4.5.3 Stare condition

No main effects of maternal care were detected when the intruder turned to make direct eye contact with the subjects, a direct threat; however several main effects of sex and age were detected. Significant main effects of sex were detected for withdrawal ($F_{(1,35)}$=4.418, p= 0.043, η_p^2=0.112, Fig. 4.11C), urination/defecation ($F_{(1,35)}$=7.367, p= 0.010, η_p^2=0.174, Fig. 4.14C), and tooth grind ($F_{(1,35)}$=4.902, p= 0.033, η_p^2=0.123, Fig. 4.15C), with males withdrawing and tooth grinding more than females, and females urinating/defecating more than males. Significant main effects of age were detected for

rates of screams ($F_{(1,35)}$=6.751, p= 0.014, η_p^2=0.162, Fig. 4.7C), and durations of freezing ($F_{(1,35)}$=5.687, p= 0.023, η_p^2=0.14, Fig. 4.12C) and locomotion ($F_{(1,35)}$=5.193, p= 0.029, η_p^2=0.129, Fig. 4.16C), with all of these measures decreasing from 3 months to 6 months. Significant age effects were also detected for rates of aggression ($F_{(1,35)}$=13.294, p= 0.001, η_p^2=0.275, Fig. 4.8C), anxiety ($F_{(1,35)}$=9.846, p= 0.003, η_p^2=0.22, Fig. 4.10C), and urination/defecation ($F_{(1,35)}$=11.995, p= 0.001, η_p^2=0.255, Fig. 4.14C), as well as for durations of turn away ($F_{(1,35)}$=4.731, p= 0.036, η_p^2=0.119, Fig. 4.17C), with all of these measures increasing from 3 to 6 months. Significant maternal care-by-age interaction effects were detected for rates of screams ($F_{(1,35)}$=10.065, p= 0.003, η_p^2=0.223, Fig. 4.7C) and atypical behaviors ($F_{(1,35)}$=7.535, p= 0.009, η_p^2=0.177, Fig. 4.9C). Although a posteriori comparisons of the means did not yield significant differences, maltreated animals had higher rates of screams than controls at 3 months and lower rates at 6 months. The opposite pattern was found for atypical behaviors, with maltreated animals having lower rates than controls at 3 months, and higher rates than controls at 6 months. Significant sex-by-age interactions were found for rates of screams ($F_{(1,35)}$=4.357, p= 0.044, η_p^2=0.111, Fig. 4.7C) and urination/defecation ($F_{(1,35)}$=5.012, p= 0.032, η_p^2=0.125, Fig. 4.14C), with females urinating/defecating more than males at 6 months. No other main or interaction effects of care, sex, or age were detected. When biological mother was included as a covariate the main effects of age reported above for locomotion, freezing, and aggression were no longer significant.

4.4.6 DTI: White matter microstructure

Two-way ANOVA of the TBSS results at each age identified several regions of the FA skeleton that exceeded the criteria for significance ($p<0.05$, cluster-corrected for clusters >50 significant, contiguous, voxels).

4.4.6.1 Two weeks

A main effect of maternal care was found in six clusters at 2 weeks of age: (1) in the right anterior limb of the internal capsule (ICa), most likely frontal fibers (cluster 1, 50 voxels, Fig. 4.18A); (2) in WM underlying somatosensory cortex in the right hemisphere (cluster 2, 69 voxels, Fig. 4.18B); (3) in the WM underlying medial occipital cortex of the right hemisphere (cluster 3, 51 voxels, Fig. 4.18C); (4) in the WM underlying middle temporal-occipital cortex of the left hemisphere (cluster 4, 52 voxels, Fig. 4.18D); and (5) two clusters in cerebellar white matter, one in each hemisphere (left cluster 5, 53 voxels, Fig. 4.18E, and right cluster 6, 283 voxels, Fig. 4.18F). In all but cluster 3 the maltreated animals had decreased FA as compared to controls. In clusters 1, 2, 5, and 6, the lower FA in maltreated animals was paralleled by higher RD (see Table 4.8 for details of statistical results), with no difference in RD detected in clusters 3 and 4. No differences in AD were detected for any of these clusters. No significant main effects of sex were found in any clusters. Significant maternal care-by-sex interaction effects were found in a single cluster in the WM of the left hemisphere of the cerebellum (cluster 7, 55 voxels, Fig. 4.19). In this cluster maltreated females had lower FA than control females, while maltreated males had higher FA than control males, with no differences in RD or AD.

4.4.6.2 Three months

A significant main effect of maternal care was found in four clusters at 3 months of age: (1) in frontal WM underlying left dorsal premotor cortex (cluster 8, 56 voxels, Fig. 4.20A); (2) in WM underlying right primary motor cortex (cluster 9, 67 voxels, Fig. 4.20B); (3) two in the posterior limb of the internal capsule (ICp), one in each hemisphere (left, cluster 10, 95 voxels, Fig. 4.20C, and right cluster 11, 81 voxels, Fig. 4.20D). In all of these clusters maltreated animals had higher FA than controls, but only

cluster 11 (right ICp) had a significant parallel decrease in RD. No significant differences in AD were detected (see Table 4.8 for details of statistical results). No significant main effects of sex or maternal care-by-sex interaction effects were detected.

4.4.6.3 Six months

A significant main effect of maternal care was found in three clusters at 6 months old: (1) in the genu of the corpus callosum (CC) (cluster 12, 52 voxels, Fig. 4.21A); (2) in prefrontal fibers (cluster 13, 88 voxels, Fig. 4.21B); and (3) in middle temporal-occipital cortex in the left hemisphere (cluster 14, 74 voxels, Fig. 4.21C). In all of these clusters maltreated animals had lower FA than controls, and in clusters 12 and 13 this decreased FA was paralleled by an increase in RD (see Table 4.8 for details of statistical results). A significant main effect of sex was detected in two clusters: one in the left ICp, or possibly cortico-cortical fibers just lateral to the ICp (cluster 15, 61 voxels, Fig. 4.22A), and the other in the genu and anterior portions of the midbody of the CC (cluster 16, 254 voxels, Fig. 4.22B). In cluster 15 females had higher FA than males, and in cluster 16 females had lower FA than males. In both of these clusters there were significant opposite differences in RD, such that in cluster 15 females had lower RD (higher FA) than males, and in cluster 16 females had higher RD (lower FA) than males (see Table 4.8 for details of statistical results).

4.5 Discussion

4.5.1 Cross-fostering does not affect maternal behavior: maltreatment is a maternal trait

In this study a naturalistic model of infant maltreatment was used to investigate the developmental consequences of this ELS on socioemotional behavior, stress hormones, physical growth and brain white matter. A cross-fostering design was used to control for the potentially confounding effects of heritable factors and prenatal environment on the outcome measures of interest. The current cross-foster study replicated findings in previous studies using biological mother-infant pairs, including (1) rates of infant abuse and rejection exhibited by maltreating foster mothers were similar to those reported in maltreating biological mothers and (2) both foster and biological infants of maltreating mothers show higher rates of screams and tantrums than control infants during the first 6 months of life (Maestripieri 1998; McCormack, Sanchez et al. 2006). Female monkeys with histories of maltreatment, including both abuse and rejection during the first 3 months of life, abused their foster infants while mothers with histories of competent care did not. Previous cross-fostering studies in this model have demonstrated that the intergenerational transmission of abusive maternal care is primarily experiential, that is, those females that experienced abuse as infants were more likely to abuse their own infants regardless of their biological mother's behavior (Maestripieri 2005). Cross-fostering studies in rats have also reported no effects of cross-fostering on maternal care (i.e. mothers displayed similar rates of care regardless of whether pups were fosters or biological offspring), as well as experiential transmission of maternal care (Francis, Diorio et al. 1999). The results of the current study provide further evidence that maternal abuse and early rejection in rhesus monkeys are maternal

phenotypes, and that there does not seem to be anything inherently different about the infants born to maltreating mothers that triggers abuse or rejection by the foster mother.

4.5.2 Infant maltreatment is a chronic stressor

To examine whether infant maltreatment is related to chronic elevations in cortisol, hair cortisol accumulation was measured in hair grown prenatally and collected on day 2 of life, and again in hair grown between day 2 and month 6, both in maltreated and control infants. Maltreated infants had higher levels of cortisol accumulation at both day 2 and month 6, an effect that appears to be driven by the much greater levels detected in the maltreated infants at 6 months (which are the months when infants experienced abuse and rejection). This provides *direct* evidence that the experience of infant maltreatment is a chronic stressor that led to increased activation of the HPA axis, resulting in increased exposure to cortisol. Long-term alterations in the HPA axis are reported in children exposed to maltreatment and other forms of ELS (Tarullo and Gunnar 2006), overall suggesting heightened HPA axis activity as a result of the experience. Results are not consistent from study to study and there is no direct evidence of chronic exposure to elevated cortisol during the adverse experience. Decreased morning cortisol is thought to reflect long-term consequences of chronic stress through a down regulation of the HPA axis in response to repeated activations (Fries, Hesse et al. 2005). Studies of morning cortisol in maltreated children have found both decreases and increases in morning cortisol, inconsistencies that could be explained by the number, type, severity, and/or chronicity of maltreatment experienced, as well as when the cortisol was collected (Cicchetti and Rogosch 2001; Bruce, Fisher et al. 2009). Findings from animal models of adverse early experience have also found decreased morning cortisol (Sanchez, Ladd et al. 2005), which fits with the hypothesis that chronic stress leads to a down regulation of the HPA axis and thus decreased basal levels of cortisol.

4.5.3 Infant maltreatment does not affect physical growth in this model

There were no differences between maltreated and control infants during the first 6 months of life in any of the measures of physical growth collected. These data suggest that maltreatment does not lead to alterations in infant growth; however, several studies have found alterations in growth in maltreated and post-institutionalized children (Gunnar and Vazquez 2001; Johnson, Bruce et al. 2011). For example, in a study of preschool aged children placed in foster care a negative correlation was found between being removed for emotional abuse and neglect and height, while head circumference was also negatively associated with number of maltreatment types experienced (Pears and Fisher 2005). This suggests that different types of maltreatment, and cumulative exposure, may have differential effects on physical growth. Thus, it is possible that the maltreated infants in this study were not exposed to severe enough forms of maltreatment to cause deficits in physical growth. Children adopted from orphanages often show stunted physical growth that can be alleviated with placement in a family (Gunnar and Vazquez 2001; Johnson, Bruce et al. 2011), not only providing additional evidence that physical growth is particularly sensitive to specific types of adverse early experiences, but also that positive changes in the environment can improve physical growth outcomes. It is key then to note that bouts of maltreatment are alternated with competent maternal care, including nursing, in our model, so that infant nutrition is unaffected (Maestripieri 1998). Maltreating mothers do not differ on rates of positive maternal behaviors, only on rates of negative infant-directed behaviors (unpublished data) providing a possible explanation for the lack of differences reported in this model. It is also possible that these effects are due to qualitative differences between human and macaque maltreatment.

4.5.4 Infant maltreatment increases emotional reactivity in the social group

The current study replicated previous findings of increased emotional reactivity in maltreated infants raised by their biological mothers (McCormack, Sanchez et al. 2006) as evidenced by higher rates of behavioral signs of distress (tantrums and screams) during the first 6 months of life. Previous studies have demonstrated that abuse precedes these behavioral signs of distress (Maestripieri, Jovanovic et al. 2000), but abuse is rarely observed beyond 3 months (Maestripieri 1998; McCormack, Sanchez et al. 2006), suggesting this increase in screams and tantrums represents a general increase in emotional reactivity, although the possibility remains that these behaviors are in response to the higher rates of maternal rejection received by the maltreated infants throughout the first 6 months of life. Maltreatment did not seem to affect social interactions during the first 6 months of life. In contrast, at 18 months of age maltreated animals exhibit reduced affiliative and play behavior with other juveniles (Vratanina-Smoot 2013), suggesting that social deficits may not emerge until the juvenile, prepubertal period in rhesus monkeys. In contrast, maltreated toddlers do tend to interact less with peers (Cicchetti, Lynch et al. 1992), and school-aged maltreated children are reported as being more socially withdrawn and having difficulties developing and maintaining friendships (Salzinger, Feldman et al. 1993; Dodge, Pettit et al. 1994; Parker and Herrera 1996). This discrepancy could be due to differences in the contexts being studied, i.e. all of the human data was collected in school or day care settings, not in the home, while all of the monkey data was collected in the social group, an effect supported by differences in teacher and parent reports of social behavior for maltreated preschoolers (Smith and Walden 1999).

4.5.5 Infant maltreatment decreases emotional reactivity during the HI paradigm

The HI paradigm (Kalin and Shelton 1989) was used to assess whether maltreated infants also showed increased emotional reactivity (as they did in their social groups) in a different context: a laboratory task involving differing levels of threat. The maltreated animals didn't differ in their overall ability to modulate their behavior based on level of threat as assessed using the HI paradigm, as shown by the lack of significant condition-by-maternal care interaction effects, but they did differ in other aspects of their responses. The overall development of organized responses to threat have been previously reported to occur by 3 months of age, both in animals reared by their mothers in single cages (Kalin, Shelton et al. 1991) and in animals with atypical surrogate-peer rearing (Raper, Wilson et al. 2013). The age effects reported here suggest that interesting developmental changes occur through 6 months, and the loss of these effects with the inclusion of biological mother as a covariate suggests that some of the differences between 3 and 6 months may be due to heritable factors. Heritability of behavior has been exploited by humans by selective breeding for centuries (Plomin 1990), and twin studies in humans suggest stability and heritability in a diverse range of behaviors consistent with the current findings (Saudino, Ronald et al. 2005).

Maltreated animals did not differ from controls drastically in their responses to the HI task. In the least threatening condition (when animals were left alone in the testing room), the only group differences detected were lower rate of screams in maltreated infants at 6 months, and a maternal care-by-age interaction effect for anxiety-like behaviors, with maltreated animals showing a trend for more anxiety than controls at 3 months and less anxiety than controls at 6 months, paralleling the decreased rate of screams and suggesting less reactivity. Previous studies of the ontogeny of the behavioral responses to the HI suggest adult-like patterns of responses appear

beginning around 3 months of age in rhesus monkeys (Kalin, Shelton et al. 1991), thus the decreased screaming exhibited by the maltreated infants at 6 months suggests a decrease in reactivity perhaps above and beyond the species-typical decrease in vocalizations that occurs with age (Kalin and Shelton 1998).

When the intruder was present, but was not making direct eye contact (a *potential* threat), there were no differences between maltreated and control infants. Species-typical decreases in screams and increases in freezing with age (Kalin, Shelton et al. 1991; Kalin and Shelton 1998) were observed during this portion of the task. Extreme manipulations of PFC-amygdala circuits, such as lesioning the oPFC or amygdala, have found alterations in fear behaviors, e.g. freezing, during the profile condition of the HI (Kalin, Shelton et al. 2004; Kalin, Shelton et al. 2007; Raper, Wilson et al. 2013). These findings suggest that maternal maltreatment may not have affected these circuits in such a way as to manifest such overt behavioral differences, or that those effects may emerge with age as previously reported for rhesus monkeys with amygdala lesions (Raper, Wallen et al. 2013). Studies of fear responses in other models of ELS have reported early experience-dependent alterations in the fear response (Sanchez, Noble et al. 2005; Macrì and Würbel 2006), although the discrepancy between those studies and our findings could be explained by the different type of early adversity measure (e.g. social isolation, maternal separation, etc.), measures/tasks employed or the age at which the effect was observed. During the most threatening portion of the task, when the intruder turned and maintained direct eye contact with the animal, maltreated infants differed in their rates of screams and atypical behaviors in an age-dependent manner, screaming less than controls, and exhibiting higher rates of atypical behaviors than controls at 6 months. This suggests a decrease in reactivity in the maltreated animals between 3 and 6 months, but with a concurrent increase in atypical behavior. Similar emergence of blunted reactivity with age has been previously reported in rhesus monkeys that received

neonatal amygdala lesions, during the juvenile period but not during infancy (Raper, Wallen et al. 2013), suggesting that infant maltreatment could have affected amygdala circuits.

Maltreated infants showed increased reactivity in the social group, but decreased reactivity during the HI paradigm, which suggests a context-dependent manifestation of behavioral reactivity that could be explained by a combination of alterations in how maltreated infants perceive and respond to potential threats depending on their ethological relevance. Maltreated children show alterations in patterns of emotional reactivity, and can be both underregulated, i.e. insufficiently regulated (elevated signs of both positive and negative emotionality with no apparent goal), and overregulated (no overt signs of emotionality and the inhibition of behavioral signs of distress) depending on the context (Maughan and Cicchetti 2002). It is unclear why these context-dependent differences in behavior exist, but it may be related to alterations in the perception of threat in the maltreated children. Alterations in attention bias to threat have been reported in maltreated children, including attentional bias *away* from threat (Pine, Mogg et al.2005), and increased attentional bias *towards* threat (shown as angry facial expressions) (Pollak and Tolley-Schell 2003; Shackman, Shackman et al. 2007). Thus one possibility, although speculative, is that maltreated animals have increased attentional bias away from threat during the HI causing them to appear to be emotionally blunted, and increased attentional bias to threat (or other stressful stimuli) in the social group causing the increase in emotional reactivity reported. It is interesting to note that alterations in the threat perception system, which includes PFC, amygdala, temporal, and parietal circuits, are thought to underlie this attentional bias and subsequent alterations in behavior (Cicchetti and Toth 2005), and are supported by reports of alterations in the function of components of these systems in children with adverse early experience (Teicher, Andersen et al. 2003; Loman and Gunnar 2010).

Alterations in attention have also been suggested as possible mediators of the increased reactivity in maltreated children (Shields and Cicchetti 1998).

4.5.6 Sex differences in emotional reactivity during the HI

Sex-by-age interactions were detected for screams, aggression, and anxiety-like behaviors during the alone condition, with females exhibiting all of these behaviors at higher rates than males at 3 months. Sex differences in emotional reactivity during the HI are not always detected in developmental studies (Kalin and Shelton 1998; Suarez-Jimenez, Hathaway et al. 2013); however one study did find increased screams during the alone condition in infant female monkeys that had received neonatal amygdala lesions (Raper, Wallen et al. 2013). This suggests that amygdala dysfunction may play a role in the behavioral alterations observed in maltreated females (Fig. 4.7A). This supports the hypothesis that maltreatment could have altered amygdala circuits, although perhaps in a sex-dependent manner. Males exhibited higher rates of withdrawal and tooth grinding and females more urination/defecation during the stare condition, the most threatening condition. The increase in tooth grinding in males in the current study is consistent with sex differences reported in Raper et al (2013). Sex differences in the effects of maltreatment on behavior have also been reported in human children with boys having increased rates of externalizing problems as compared to girls (Manly, Kim et al. 2001). A longitudinal study of maltreated children also found that internalizing behaviors mediated the link between maltreatment and externalizing behaviors in girls, while a direct link between maltreatment and externalizing behaviors was detected in boys, suggesting complex effects of maltreatment on behavior modulated by sex (Maschi, Morgen et al. 2008). The results of the current study support a role of sex in the behavioral outcomes of infant maltreatment, and highlight the need for studies

designed to specifically address the underlying mechanisms of these complex relationships.

4.5.7 Alterations in brain WM development

Differences between maltreated and control infants were detected in WM microstructure (measured as FA) at all three ages, providing evidence that infant maltreatment does alter the development of brain WM. Although unexpected, some of these structural alterations emerge very early, so that at 2 weeks prefrontal, temporal, and cerebellar WM were affected by maltreatment, with all but the one cluster in right medial occipital WM (cluster 3) showing lower FA in maltreated animals than controls. Most decreases in FA were accompanied by increases in RD, suggesting that the reduced WM integrity is a result of less myelinated WM in maltreated infants (Song, Sun et al. 2002; Song, Sun et al. 2003; Keller and Just 2009; Zhang, Jones et al. 2009; Bennett, Madden et al. 2010; Zhu, Wang et al. 2011; Choe, Stepniewska et al. 2012). In contrast, at 3 months maltreated infants had higher FA (with only cluster 11 in ICp having a parallel reduction in RD) in all four clusters where group differences were found. These included clusters in bilateral frontal WM and bilateral ICp. At 6 months two clusters in PFC WM and one in occipital WM were found to have lower FA in the maltreated infants, with only the two PFC clusters having parallel increases in RD (suggesting decreased myelin in these clusters).

Cortical WM undergoes significant increases in myelination during infancy in humans, generally progressing from medial structures (i.e. the CC) outward, and caudo-frontally (Gao, Lin et al. 2009; Deoni, Mercure et al. 2011). The ratio of human to rhesus monkey age is approximately 1:4, thus the timing of the scans in the infant monkeys in the current study can be compared roughly to 2 months, 1 year, and 2 years old in human children. Myelin maturation begins around 2.5 months in human infants (Gao, Lin et al.

2009), and has been shown to begin in the cerebellum, pons, and internal capsule (Deoni, Mercure et al. 2011). In the remainder of the first year of life all major WM systems have begun myelination, beginning in the splenium of the CC and optic radiations around 3-4 months, the occipital and parietal lobes around 4-6 months, and finally the genu of the CC, frontal, and temporal lobes around 6-8 months (Deoni, Mercure et al. 2011). Axonal development, including myelination, continues between 1 and 2 years of age (Gao, Lin et al. 2009), throughout childhood, and in some regions, including the PFC, into early adulthood (Giedd, Rumsey et al. 1996; Barnea-Goraly, Menon et al. 2005; Clayden, Jentschke et al. 2012; Kochunov, Williamson et al. 2012). This general pattern is mirrored in rhesus monkeys (Malkova, Heuer et al. 2006; Shi, Short et al. 2013). Cerebellar, temporal and prefrontal tract myelination occurs during infancy, and the cerebellum achieves adult levels of myelin between 3 and 6 months of age, while WM of some commissural and association tracts only reach adult levels of myelination at 3.5 years (Gibson 1991). Axon elimination in the CC is complete by 6 months in monkeys, but myelination continues throughout the juvenile period (LaMantia and Rakic 1990). This is in contrast to the WM of the anterior commissure (AC), which completes axon elimination at 3 months and reaches adult levels around 1 year (LaMantia and Rakic 1994). The maltreatment effects on FA detected at 2 weeks, 3 months, and 6 months, seem to follow a similar spatiotemporal pattern (in general) as the developmental pattern described above (Fig. 4.23), supporting the hypothesis that the adverse experience impacts brain regions undergoing significant developmental changes during the experience. The importance of considering the age at the time of measurement when trying to interpret the effects of early experience on brain development is also reflected in studies of the human amygdala and hippocampus (Tottenham and Sheridan 2009). The current findings may also suggest that early life stress may alter patterns of WM maturation, and perhaps that it is not the final outcome

in terms of differences in brain structure that really matters for functional outcomes, but rather how those differences occur over time that matters; however this hypothesis remains to be statistically tested in an appropriately large sample.

There is a paucity of research on the effects of maltreatment on brain WM in human infants, but several studies have looked at outcomes at later ages during childhood, adolescence, and adulthood, with significant overlap with the findings of this investigation. Maltreatment-related differences in cerebellar WM were relatively large, and only detected at 2 weeks of age, a developmental time point in monkeys comparable to the time when myelination occurs in this region in humans, around 3 to 4 months (Deoni, Mercure et al.2011). Although recent studies of WM microstructure and the effects of early life stress do not report alterations in cerebellar white matter in children (Govindan, Behen et al.2010) or young adults (Choi, Jeong et al. 2009), cerebellar development has been shown to be particularly sensitive to experience (Giedd, Schmitt et al.2007) and decreased cerebellar volumes have been reported in maltreated children (De Bellis and Kuchibhatla 2006; Bauer, Hanson et al. 2009). Cerebellar damage is related to the “Cerebellar Cognitive Affective Syndrome”, whose symptoms include impairment of executive functions like planning and set shifting, difficulties with spatial cognition, and blunting of affect or disinhibited and inappropriate behavior (Schmahmann and Sherman 1998). These effects are thought to reflect subsequent disruption of the connections between the cerebellum, prefrontal, posterior parietal, superior temporal, and limbic cortices (Schmahmann and Sherman 1998). It is through these connections with the cerebral cortex that the cerebellum is thought to maintain a sort of homeostatic behavioral baseline, modifying behavioral outputs depending on context (Schmahmann, Weilburg et al. 2007b). Although speculative at this point and difficult to confirm anatomically (i.e. it is difficult to determine what WM in the cerebellum constitutes a particular connection), the alterations in the cerebellum

detected in the current study could potentially be related to the altered emotional reactivity also reported in this model and in maltreated children. The cerebellum also plays a role in sensorimotor processing (Manto, Bower et al. 2012). Alterations in cerebellar WM could reflect alterations in this function, which would be consistent with the alterations in other sensorimotor WM reported in this study.

Alterations in the IC and middle temporal-occipital WM were detected at both 2 weeks and 6 months of age in the current study. The IC can be split into two parts, an anterior limb (ICa) and a posterior limb (ICp), with the ICa containing prefrontal, rostral cingulate, and motor fibers and the ICp containing motor, caudal cingulate, parietal, temporal, and occipital fibers (Schmahmann and Pandya 2006). Decreased FA in the IC has been reported in adult macaques with histories of ELS (Coplan, Abdallah et al. 2010), but not in studies of humans exposed to ELS; however studies of WM alterations in depression have shown decreased FA in the IC (Jia, Huang et al. 2010; Zhu, Wang et al. 2011; Guo, Liu et al. 2012), suggesting a link between WM microstructural integrity in this region and the increased risk for mood disorders observed in victims of childhood maltreatment. A recent report has also linked increased cortisol with increased FA in the IC in prepubescent rhesus monkeys (Tromp, Fox et al. 2012), supporting a potential link between the increase in FA observed in maltreated animals in the current study and the exposure to elevated cortisol levels resulting from this ELS. The decreased FA detected in this region in maltreated animals at 2 weeks may be due to the chronicity of stress exposure (i.e. at younger ages the effects of stress act to decrease FA, but with longer exposure stress increases FA), although this is speculative due to the paucity of longitudinal studies of WM development in maltreated populations. It is also possible that although these clusters are in similar anatomical regions the alterations observed may actually be present in different axon populations that are near each other.

To our knowledge no other studies of infant maltreatment have reported alterations in medial occipital, middle temporal-occipital, sensorimotor, premotor, or primary motor WM. The location of the temporal-occipital clusters suggests that the tracts affected could include the inferior longitudinal fasciculus (ILF), a long cortico-cortical association tract that courses through occipital, parietal, and temporal cortices (Schmahmann and Pandya 2006). However, this can't be corroborated without running additional tractography analyses. Interestingly, reduced FA has been reported in the caudal portion of the ILF in adolescents that witnessed domestic violence as children (Choi, Jeong et al. 2012). The ILF is part of the ventral visual pathway which is important for object identification (Mishkin, Ungerleider et al. 1983), face processing (Fox, Iaria et al. 2008), and emotional memory (Habib 1986; Ross 2008). Along these lines, alterations in WM microstructure of the ILF have been observed in several mood and anxiety disorders. For example, decreased FA in the ILF at the level of the occipital lobe has also been found in patients with depression (Versace, Almeida et al. 2010; Liao, Huang et al. 2013) and bipolar disorder (Bruno, Cercignani et al. 2008; Zanetti, Jackowski et al. 2009). Thus, it is possible that decreases in microstructural integrity of occipital WM, likely involving the ILF, could affect visual and face processing, as well as emotion/mood processes. Thus alterations in this WM tract could contribute to the context-dependent alterations in emotional reactivity reported here. One might expect more general behavioral alterations given the alterations in FA detected in sensorimotor, premotor, and motor cortex. This hypothesis remains to be tested using tasks or behaviors known to be related to the function of these regions.

Alterations in WM microstructure in more anterior regions (e.g. frontal and prefrontal WM and anterior portions of the CC) were observed at 2 weeks, 3 and 6 months in the current study, with the effects in the most anterior regions (prefrontal cortex) only detected at 6 months of age. This is consistent with the spatiotemporal

pattern of myelination observed in humans and monkeys (Malkova, Heuer et al. 2006; Gao, Lin et al. 2009; Deoni, Mercure et al. 2011; Shi, Short et al. 2013) and the view that areas undergoing active myelination (or other developmental processes), or regions with protracted development such as the PFC, are especially vulnerable to environmental insult (Davison and Dobbing 1966; Rice and Barone Jr 2000). Alterations in the CC have been described in several studies of maltreated children, including decreased FA (Jackowski, Douglas-Palumberi et al. 2008), decreased volume (Teicher, Ito et al.1997), and failure to show the typical age-related increase in volume (De Bellis and Keshavan 2003). Similar findings of reduced CC volumes are reported in adolescents (De Bellis, Keshavan et al. 2002; Teicher, Dumont et al. 2004) and adults (Kitayama, Brummer et al. 2007), and decreases in FA in the genu of the CC in adults with histories of ELS (Paul, Henry et al. 2008). Taken together these alterations in the CC could result in alterations in integration of information between the hemispheres that could be related to the behavioral differences detected between control and maltreated animals.

In the current study PFC WM alterations (decreased FA) were only detected at the oldest age studied, 6 months. Decreased FA in WM connecting PFC and amygdala has been observed in children and adolescents exposed to early adversity (Eluvathingal, Chugani et al. 2006; Choi, Jeong et al. 2009; Govindan, Behen et al. 2010). Decreased FA in these prefrontal tracts, particularly the uncinate fasciculus (UF), which connects PFC with medial temporal structures including the amygdala, has been associated with behaviors that are altered in maltreated children (Govindan, Behen et al. 2010), specifically inattention and hyperactivity. The cortical regions connected by this fiber tract show decreased activation in children exposed to early deprivation (Chugani, Behen et al. 2001). Based on its anatomical location, the prefrontal WM where maltreatment-related alterations in FA were found could include the UF (Schmahmann and Pandya 2012), which is consistent with the findings of WM alterations in the UF in children

exposed to ELS highlighted above, and could be related to the alterations in emotional reactivity reported.

Maltreatment-by-sex interaction effects were only detected in a single cluster in the cerebellum at 2 weeks of age. The cerebellum has been shown to be larger in men than women in adulthood (Raz, Gunning-Dixon et al. 2001), but not much is known about the role of sex early in development in this region. Sex-dependent differences in WM microstructure were only observed at the oldest age studied, 6 months, and included increased FA in parietal WM and decreased FA in a large portion of the anterior portion of the midbody of the CC in females. The CC has been shown to be a sexually dimorphic region in structure (Allen, Richey et al. 1991; Ardekani, Figarsky et al. 2012) and in development (Cowell, Allen et al. 1992; Aboitiz, Rodriguez et al. 1996). The current findings are consistent with a previous study of environmental influence on CC structure in rats that demonstrated fewer myelinated axons in the CC of female rats, regardless of environment (Juraska and Kopcik 1988), as well as sex-dependent regional differences in size (Berrebi, Fitch et al. 1988). The concordance between the sex effects and interactions detected in the current study and previous studies highlight the importance of considering sex when analyzing and interpreting the effects of infant maltreatment on brain structure.

The findings of elevated hair cortisol concentrations in maltreated infants suggest chronic exposure to higher circulating levels of cortisol than in controls, and supports the view that maltreatment was a stressful experience, but with no concurrent effects on physical growth. Increased emotional reactivity in the social group, but not in response to a threatening laboratory task, was observed with no alterations in peer interactions. Alterations in brain WM were detected in several brain regions at 2 weeks, 3 and 6 months of age. These results are consistent with previous findings of brain alterations,

including alterations in WM microstructure, in maltreated children, and with the longitudinal design used provides direct evidence of the importance of developmental context in interpreting results. These data support the hypothesis that infant maltreatment affects brain circuits involved in emotional regulation, including PFC circuits, and suggests regional-specific alterations depending on the scanning age. Consistent group differences were also detected in sensorimotor regions, suggesting that the experience of maltreatment could result in alterations of sensory integration with motor output, a hypothesis that can be tested in future studies by looking at correlations between FA in these regions and specific sensorimotor behaviors/tasks. The context-dependent effects on emotional reactivity are consistent with reports of behavioral alterations in maltreated children, and highlight the complex behavioral outcomes that result from adverse early experiences such as maternal abuse and rejection. Future studies are needed to clearly identify the ontogeny of the relationships between the alterations of brain structure and behavioral outcomes in infant maltreatment.

Table 4.1

Summary of subjects split by foster and biological mother, sex, and rank. Foster mother is indicated by either MALTREATED or CONTROL in the first column. Biological mother is indicated by the single letter in the second column, C=control, and M=maltreating.

		GENDER		RANK		
		FEMALE	MALE	HIGH	MIDDLE	LOW
CONTROL	M	6	3	3	5	1
	C	5	6	2	3	6
MALTREATED	C	3	9	5	4	3
	M	3	5	2	5	1

Table 4.2

Definitions of abusive maternal behavior.

Behavior	Operational Definition
Drag	Drags infant while walking or running
Crush	Pushes infant against the ground with hands
Throw	Throws the infant ahead while walking or sitting
Step or sit on	Steps or sits on infant
Rough groom	Holds infant on the ground and pulls out infant's hair with force
Abusive carry	Carries infant with one arm away from her body, infant unable to cling

Table 4.3

Definitions of behaviors collected in the social compound

Behavior	*Definition*
attack	common definition
avoidance	turning or moving away in response to animal approach within 1m
bite	common definition
body shake	shaking fur like a wet dog, (mother during first two months)
chase	pursuit in agonistic context, not in play
contact	body contact initiated
on ventrum	infant is in ventral contact, but not nipple contact
on dorsal	infant is on dorsum
other contact	code when infant is in any other form of contact than those listed
infant coo	cooing by infant
cradle	ventro-ventral contact with the other animals arms wrapped around infant
display	infant bounces up and down, shakes equipment.
dorsal carry	mother carries infant on her back during the first month of life
eating/drinking	animal is eating or drinking water
gential inspec	inspection of another animal's genitals (to or from infant)
groom solicit	presenting flank or rump to solicit grooming
grimace	bared-teeth display
groom	picking and spreading fur of other animal
hit	mother slaps infant
harass	pulls, drags, or hits another
kidnap infant	other individual prevents infant from returning to mother (>1 min.). There must be obvious signs of distress on the part of the

	mother or the kid. Do not score behaviors during kidnap, other than kidnap scream.
kidnap scream	infant screams during a kidnap
kidnap coo	infant coos during a kidnap
limb carry	mother carries infant on her limb, with infant parallel to her arm, clinging, during first month of life
lipsmack	repeated lip movements
mount	common definition
passive	animal is sitting or standing passively, and does not fit into other categories
present	animal orients hindquarters toward other with raised tail
proximity	animal enters and stays within 1 ft diameter of a stationary animal.
reject	prevent contact or infant access to nipple by holding the infant at a distance with an arm, passively blocking the chest with an arm, or twisting torso away
restrain	actively prevent infant from breaking contact by pulling its leg or tail
scratch	common definition
infant scream	acute vocalization by infant, not coos
self-groom	picking and spreading one's fur
social play	rough and tumble, chase play with another animal
play solicit	attempt to play with another, without success
solitary play	play with objects, or running, spinning, and gamboling
tantrum	infant's body shakes, while infant geckers or screams
threat	open mouth, stare with/without woofing
touch	other individual touches infant, or vice versa
vocalize	vocalization of any kind, other than a scream, coo, or threat made by the infant, or towards the infant (ex: gurney, grunt)
yawn	common definition

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

Table 4.4

Definitions of behaviors recorded during the Human Intruder Paradigm.

Behavior	Operational Definition
	Fearful/Defensive
freeze	Motionless, except for slow/small head movements, with tense body posture; hanging or on ground.
crouch freeze	Similar to Freezing but subject has all 4 limbs on floor of the test cage, ventrum usually pressed down to ground and lowers head to within 1 head-width of the floor (may look as though subject is ducking).
withdrawal	Quick, jerky motion backwards, away from intruder.
fear grimace	Lips pulled back exposing clenched teeth; ears pulled back.
tooth grind	Repetitive, audible rubbing of upper and lower teeth.
turn away	Subject turns away from intruder/fruit, with back towards stimulus; not looking.
look away/avert	Visual avoidance- facing the intruder/fruit with tense body posture avoiding eye contact with the stimulus.
scream/screech	High pitched vocalization. New bout after 3 second pause (Frequency).
coo	Soft “call” vocalization made by rounding and pursing the lips; medium pitch and intensity.
tantrum	Subject’s body shakes, while geckering or screaming.
urination/defecation	Common definitions
	Displacement/Self-Directed
self groom	Picking and spreading own fur using hands, feet or mouth.
yawn	Common definition.
scratch	Subject uses hands or feet to scratch an area of body; usually rapid strokes.
body shake	Shaking head and body like a wet dog.
	Aggressive
threat	Open mouth stare (w/ or w/o vocalization); includes head bob; eyes wide open; code also “raised eyebrow” (stare w/o open mouth)]. Without contact.

threat bark	Vocalization made by forcing air from abdomen through vocal chords, producing a short, rasping, low frequency “hoot” sound w/ threat.
lunge	Quick movement towards intruder with direct eye contact.
display	Vigorous shaking of cage/equipment; Subject bounces on all fours,shaking, biting, hitting, slapping cage.end display
crooked tail	Common definition.
Submissive	
lipsmack	Repeated lips movements, pressing them together with soft clucking/smacking noises; ears pulled back.
present	Orienting hindquarters towards stimulus with raised tail.
Exploration	
explore	visual: visual inspection of surroundings tactile/oral: oral or tactile exploration of the cage or room. Not cage agitation (i.e. display) or locomotion. Includes reaching out of cage.
explore intruder*	Fixated gaze on intruder. Does not include visual scanning of intruder.
visual scanning	Subject makes quick shifts in gaze, avoiding direct eye contact.
Activity	
locomotion	Self-induced movement that results in change of location; includes walking, bouncing, running, climbing, jumping and dropping from cage.
sleep	Inactive, with eyes closed
passive/stationary	Inactive (still and calm); not explore/sleep, or other behavior
motor stereotypy	Repetitive motor pattern (e.g. circling, rocking, pacing, head turning, jumping) that occurs 3 or more consecutive times.
Other	
out of view	Common definition.
depressive-like	Head down, self-clutching, rocking.
atypical*	Species-atypical behaviors, bizarre posture, self-directed (self-bite, self-clasp, etc.)
errection	Common definition.

masturbation	Common definition, but also includes oral manipulation (self-sex).
coprophagia	Eating feces.
run into panel	Running into front clear panel with head or arms (not backing into the panel).

Table 4.5

Behaviors and composite scores analyzed from behavior collected in social group.

Behavior	Components (if applicable)	Frequency or Duration
scream/screech	N/A	F
tantrums	N/A	F
anxiety	sum of yawns, scratches, bodyshakes, and self groom	F
avoidance	N/A	F
social play	N/A	D
play solicit	N/A	F
affiliation	sum of contact and groom	D

Table 4.6

Behaviors and composite scores analyzed for the Human Intruder paradigm.

Behavior	Components (if applicable)	Frequency or Duration
explore (total)	sum of tactile/oral and visual explore	D
locomotion	N/A	D
freezing (total)	sum of freeze and crouch freeze	D
withdrawal*	N/A	F
turn away*	N/A	D
scream/screech	N/A	F
tooth grind	N/A	D
coo	N/A	F
urination/defecation	N/A	F
aggression	sum of threats, threat barks and displays	F
anxiety	sum of yawns, scratches, bodyshakes, and self groom	F
atypical	sum of coprophagia, atypical, and run into panel	F

*Only recorded and analyzed for the Profile and Stare conditions.

Table 4.7

Statistical results of the ANOVA performed on the mean FA, RD, and AD calculated in each cluster found to have significant maternal care and/or sex effects in the TBSS analysis.

					FA		RD	
Cluster	**Location**	**Laterality**	**Age**	**Effect**	**F**	***p***	**F**	***p***
1	Internal capsule	Right	2 weeks	Maternal care	9.72	0.00	4.21	0.05
2	Frontal WM	Right	2 weeks	Maternal care	10.01	0.00	10.33	0.00
3	Medial occipital WM	Right	2 weeks	Maternal care	11.44	0.00	N/A	N/A
4	Middle temporal-occipital WM	Left	2 weeks	Maternal care	14.03	0.00	N/A	N/A
5	Cerebellar WM	Left	2 weeks	Maternal care	10.27	0.00	6.16	0.02
6	Cerebellar WM	Right	2 weeks	Maternal care	22.49	0.00	12.11	0.00
7	Left cerebellar WM	Left	2 weeks	Maternal care by sex	7.84	0.01	N/A	N/A
8	Frontal WM	Left	3 months	Maternal care	10.43	0.00	N/A	N/A
9	Frontal WM	Right	3 months	Maternal care	17.79	0.00	N/A	N/A
10	Internal capsule	Left	3 months	Maternal care	8.20	0.01	N/A	N/A
11	Internal capsule	Right	3 months	Maternal care	6.98	0.01	4.53	0.04
12	Corpus callosum and prefrontal WM	Left	6 months	Maternal care	11.52	0.00	9.39	0.00
13	Prefrontal WM	Left	6 months	Maternal care	15.48	0.00	5.40	0.03
14	Middle temporal-occipital WM	Left	6 months	Maternal care	11.60	0.00	N/A	N/A
15	Internal capsule	Left	6 months	Sex	7.56	0.01	5.46	0.03
16	Corpus callosum	Center	6 months	Sex	20.76	0.00	24.94	0.00

Figure 4.1

Mean rates (±SEM) of abuse (A) and rejection (B) recorded during the first 3 months of life.*significant effect of maternal care (p≤0.05)

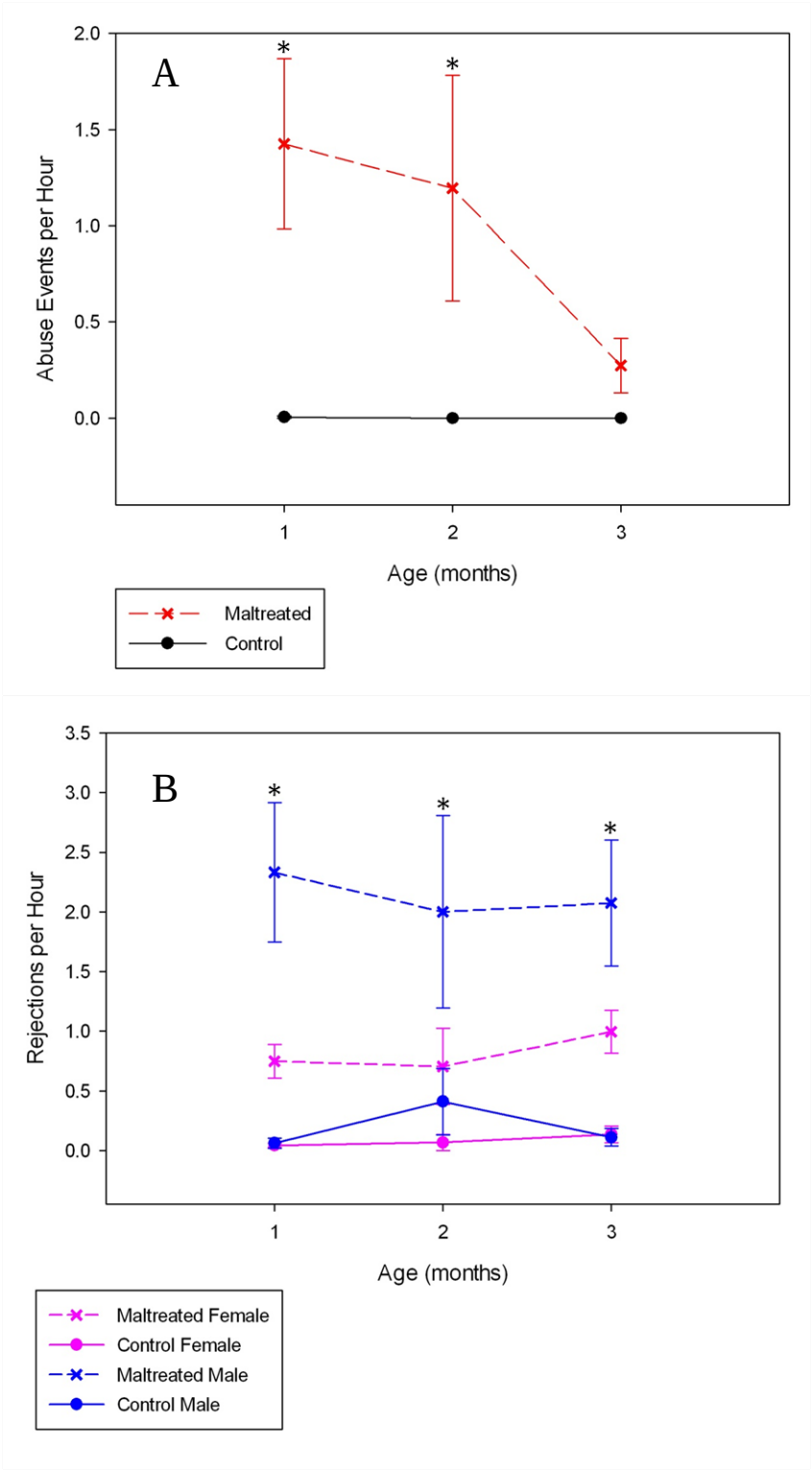


Figure 4.2

Mean (±SEM) cortisol accumulate in hair. * significant effect of maternal care (p≤0.05).

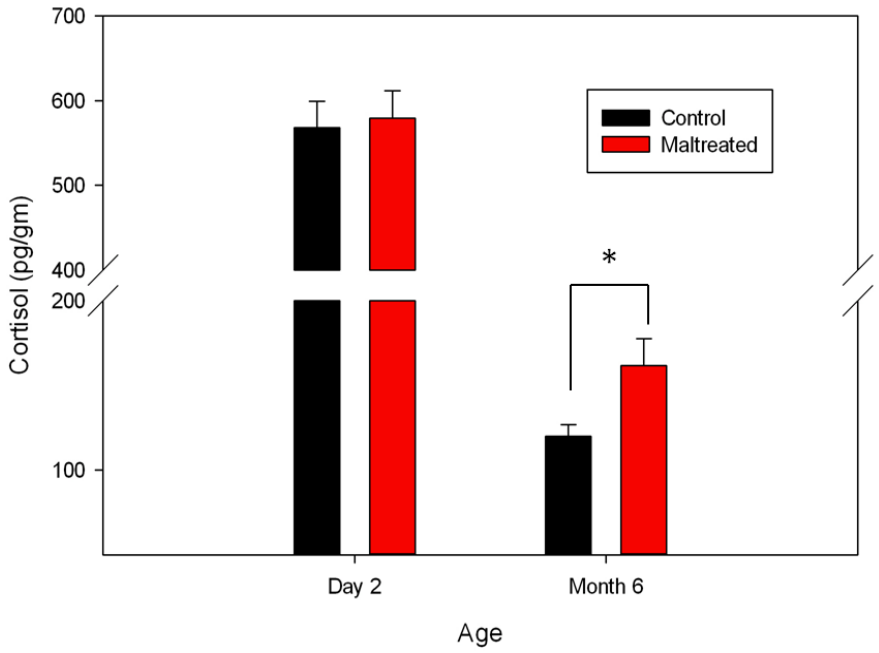


Figure 4.3

Mean (±SEM) weight (A), crown-rump length (B), crown-heel length (C), and head circumference (D).

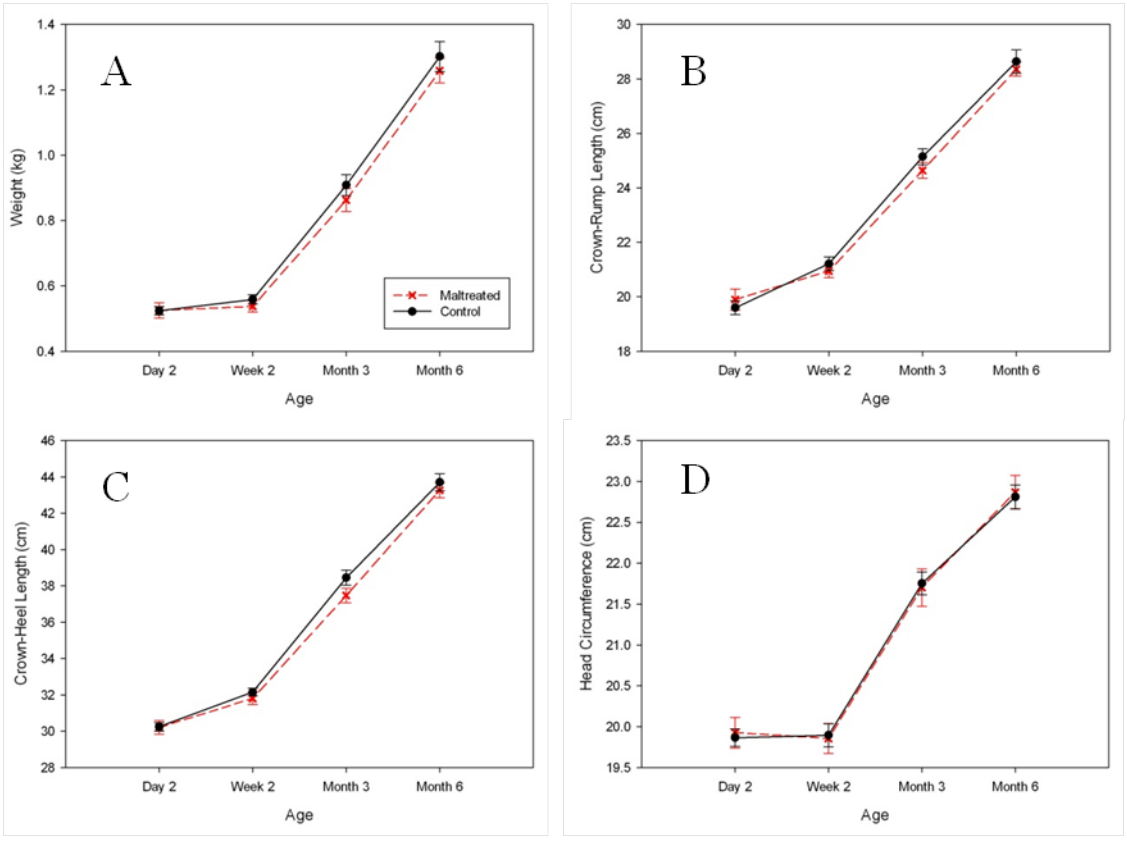
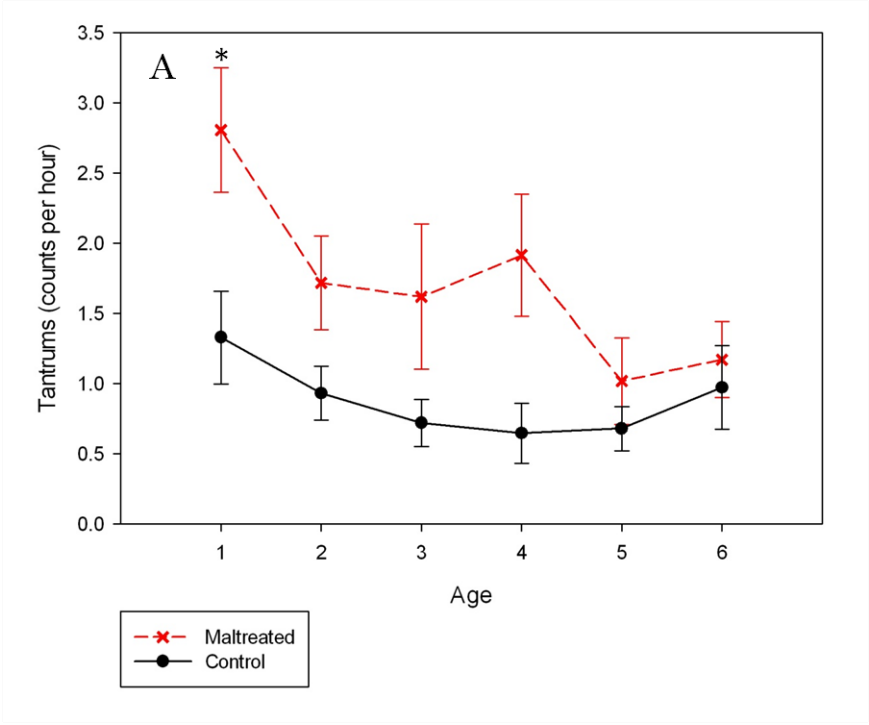


Figure 4.4

Emotional reactivity: Mean rates (±SEM) of tanrums (A) and screams (B) recorded in the social group during the first 6 months of life.*significant effect of maternal care ($p \leq 0.05$)



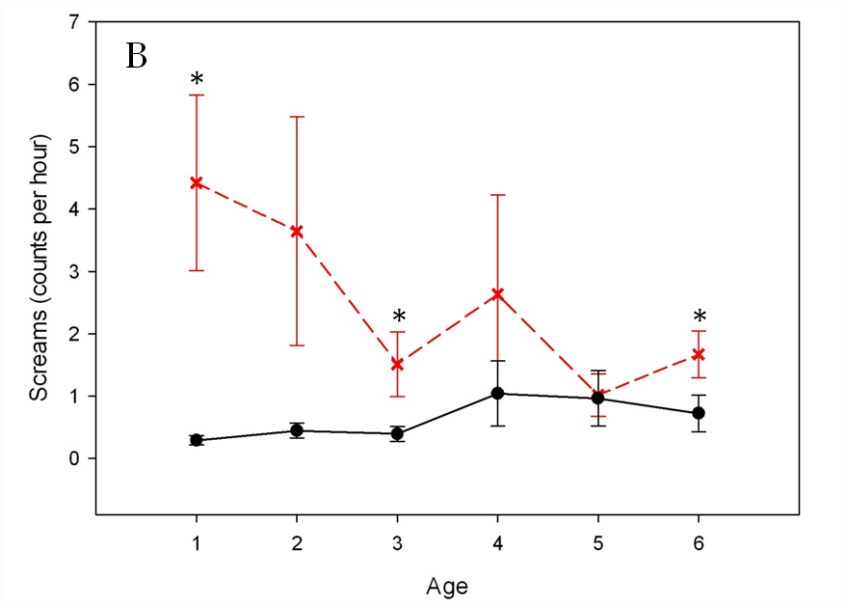
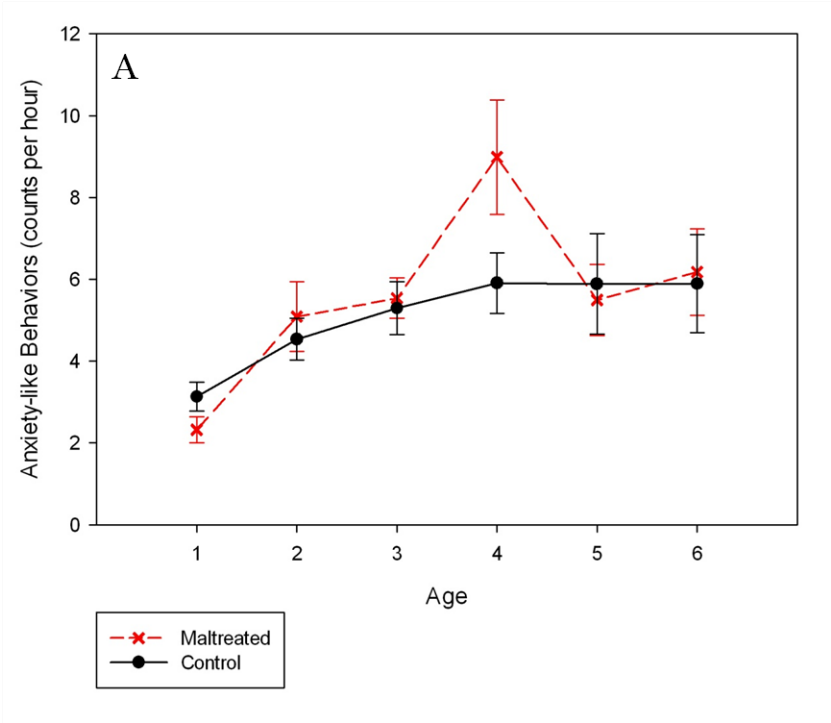


Figure 4.5

Mean rates (±SEM) of anxiety (A) and avoidance (B) recorded in the social group during the first 6 months of life.



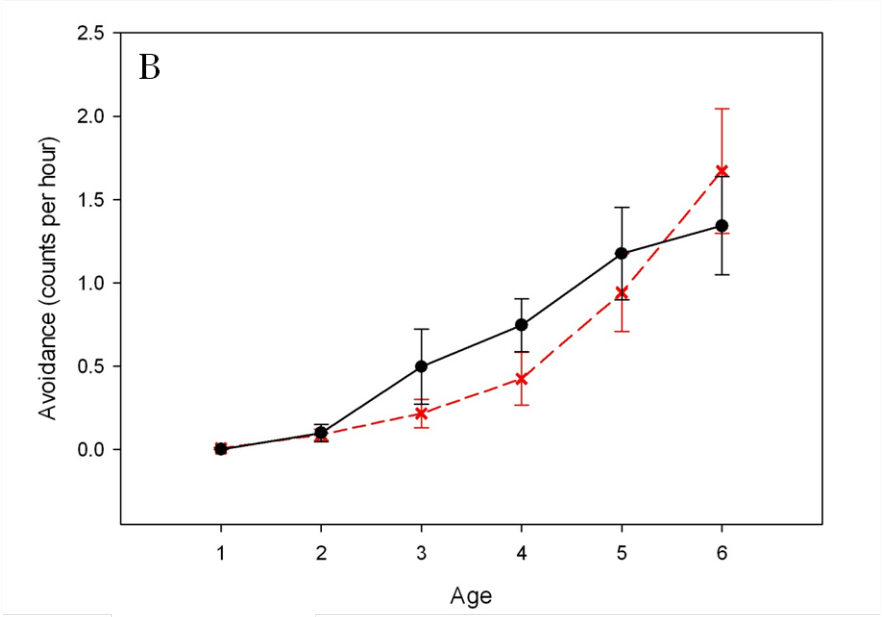


Figure 4.6

Mean rates (±SEM) of play solicit (A) and social play (B) recorded in the social group during the first 6 months of life. +significant effect of sex (p≤0.05)

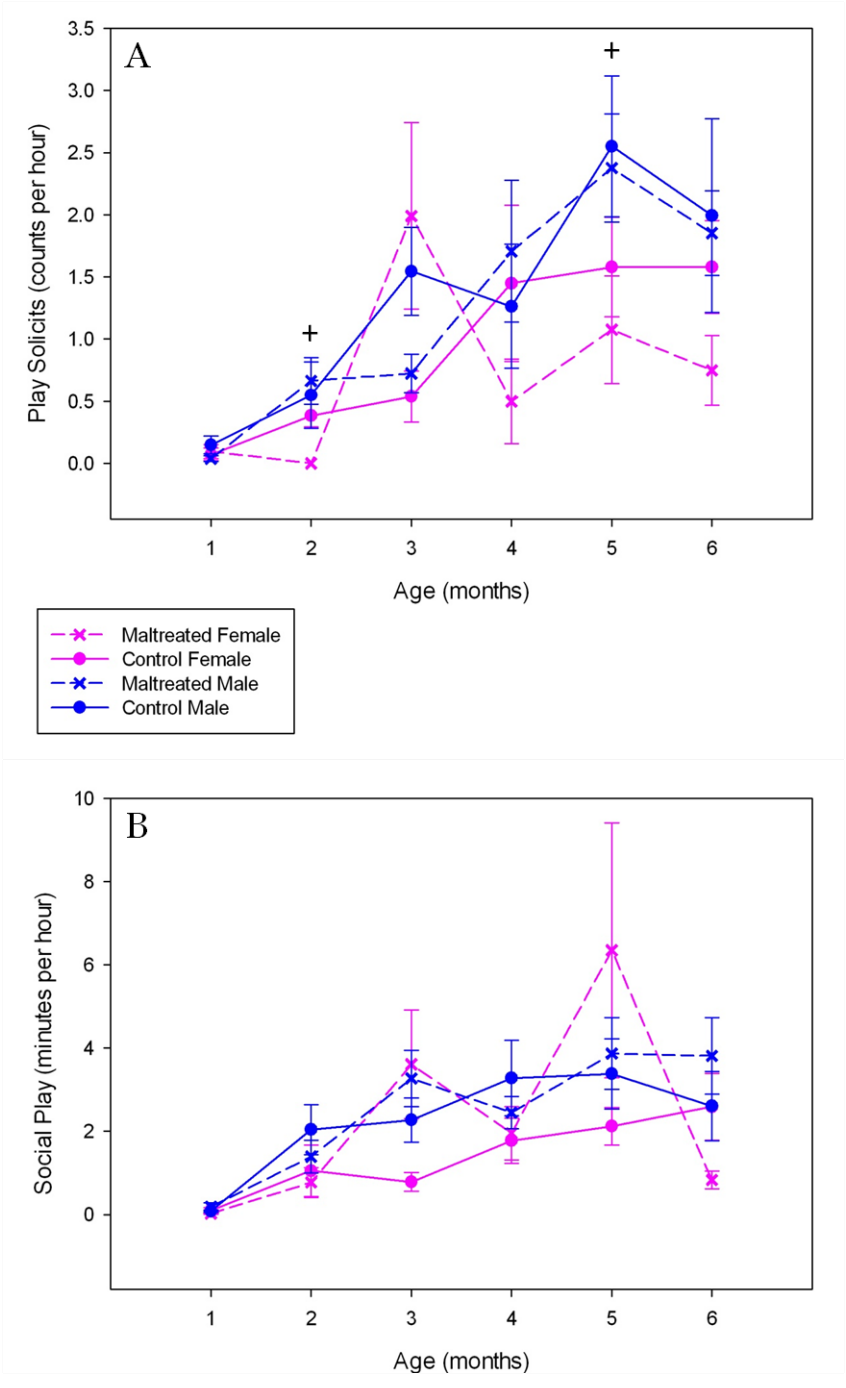


Figure 4.7

Human Intruder: Mean rates (±SEM) of screams recorded during the Alone (A), Profile (B), and Stare (C) conditions at 3 and 6 months old. .*significant effect of maternal care ($p \leq 0.05$); +significant effect of sex ($p \leq 0.05$)

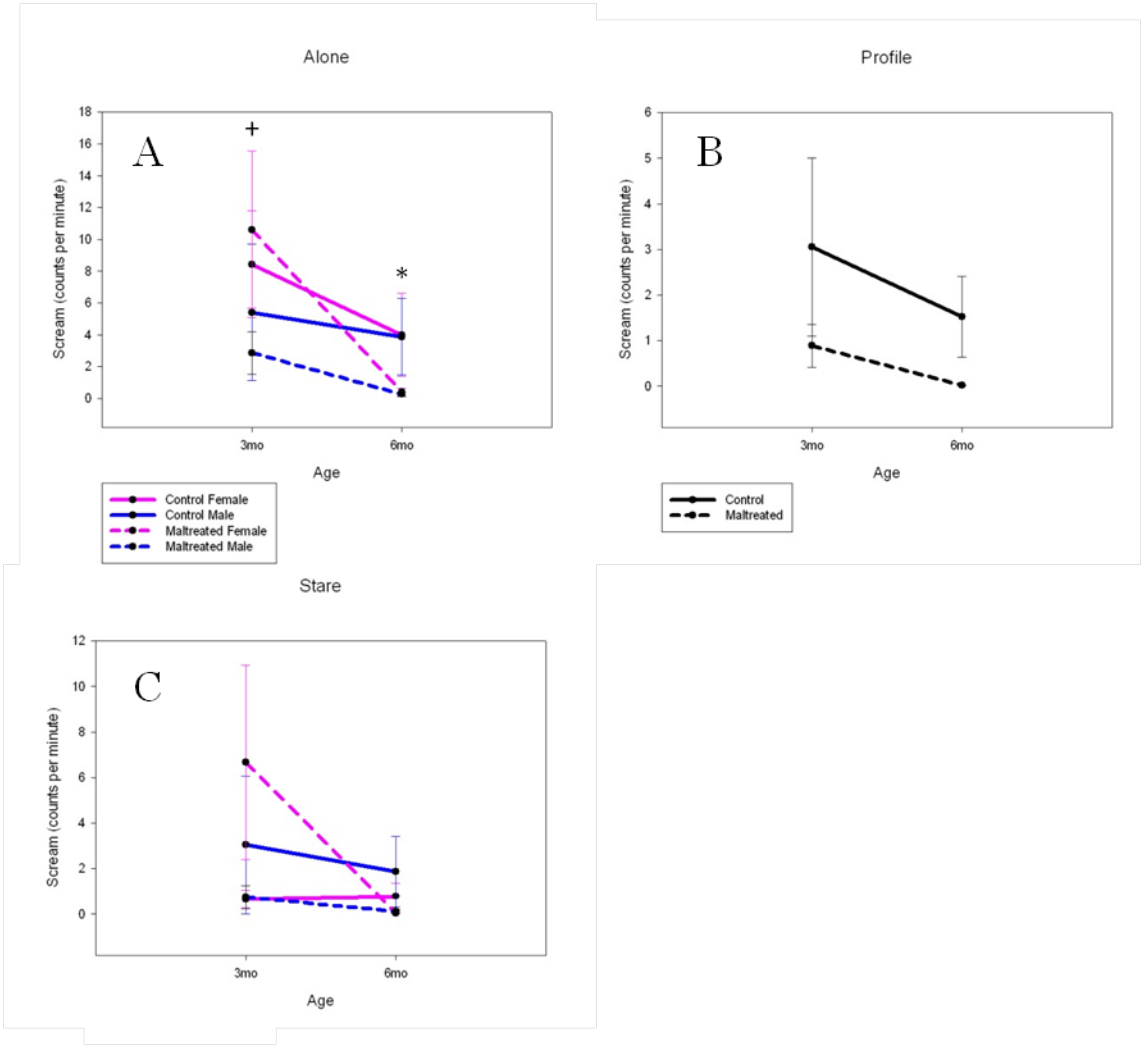


Figure 4.8

Human Intruder: Mean rates (±SEM) of total aggression recorded during the Alone (A), Profile (B), and Stare (C) conditions at 3 and 6 months old. +significant effect of sex ($p \leq 0.05$)

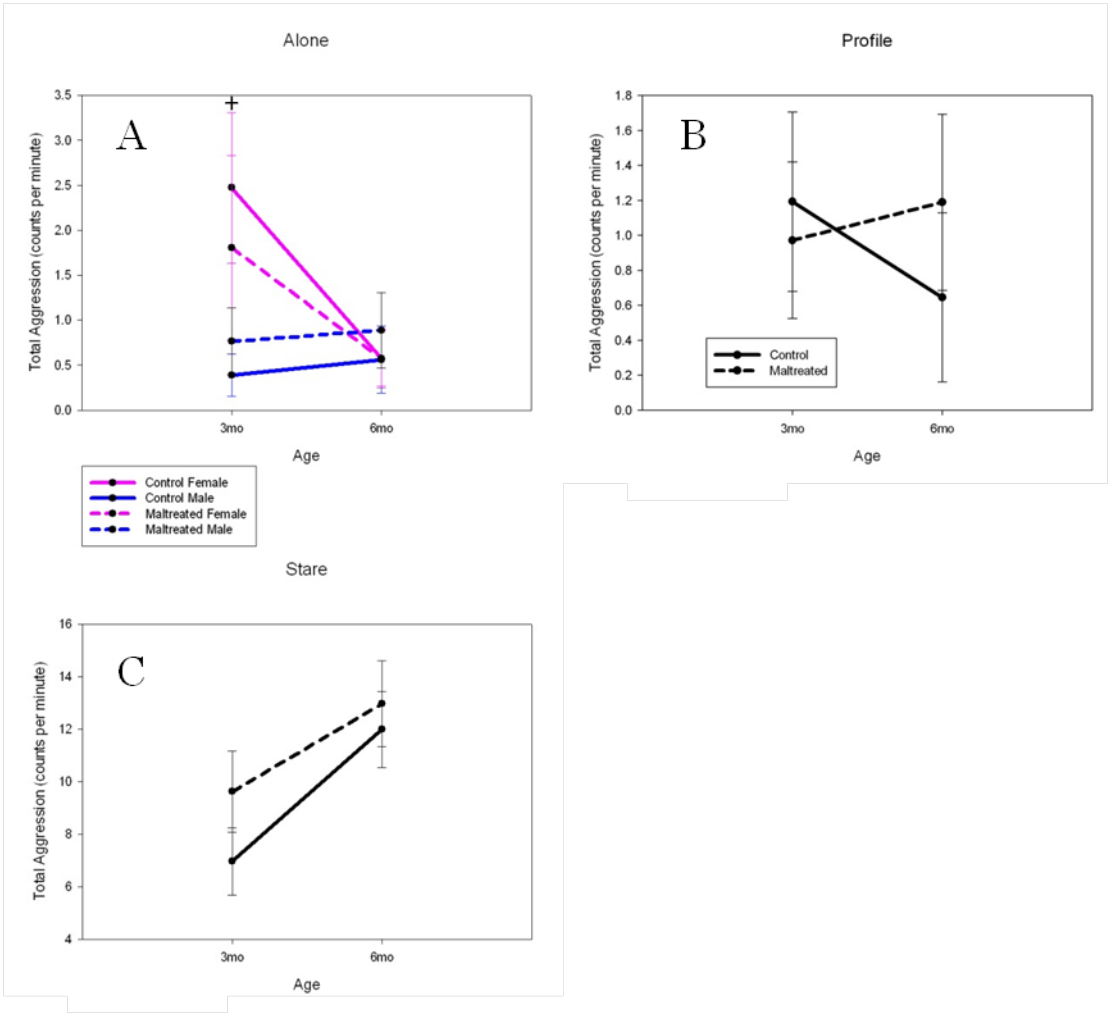


Figure 4.9

Human Intruder: Mean rates (±SEM) of atypical behaviors recorded during the Alone (A), Profile (B), and Stare (C) conditions at 3 and 6 months old.

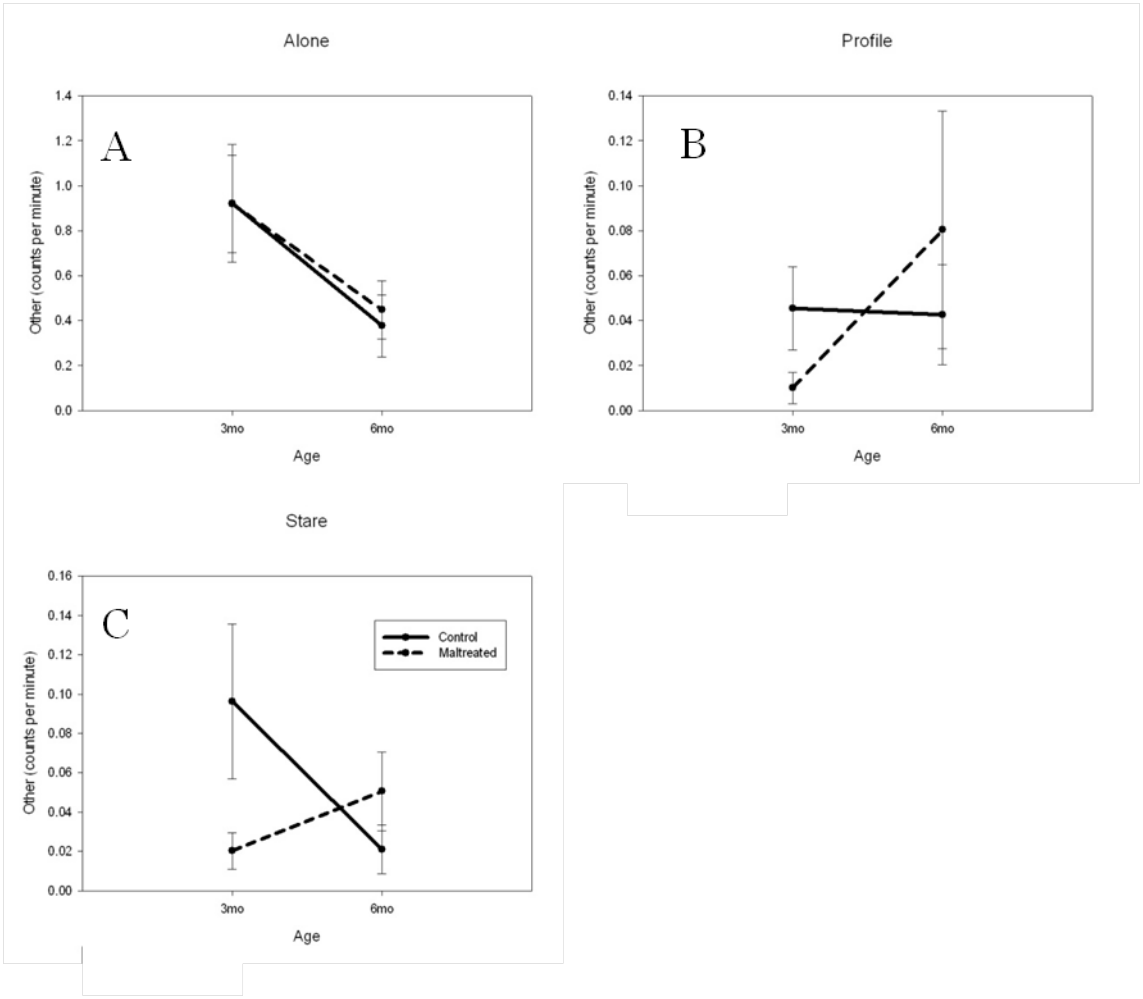


Figure 4.10

Human Intruder: Mean rates (±SEM) of anxiety-like behaviors recorded during the Alone (A), Profile (B), and Stare (C) conditions at 3 and 6 months old. +significant effect of sex ($p \leq 0.05$)

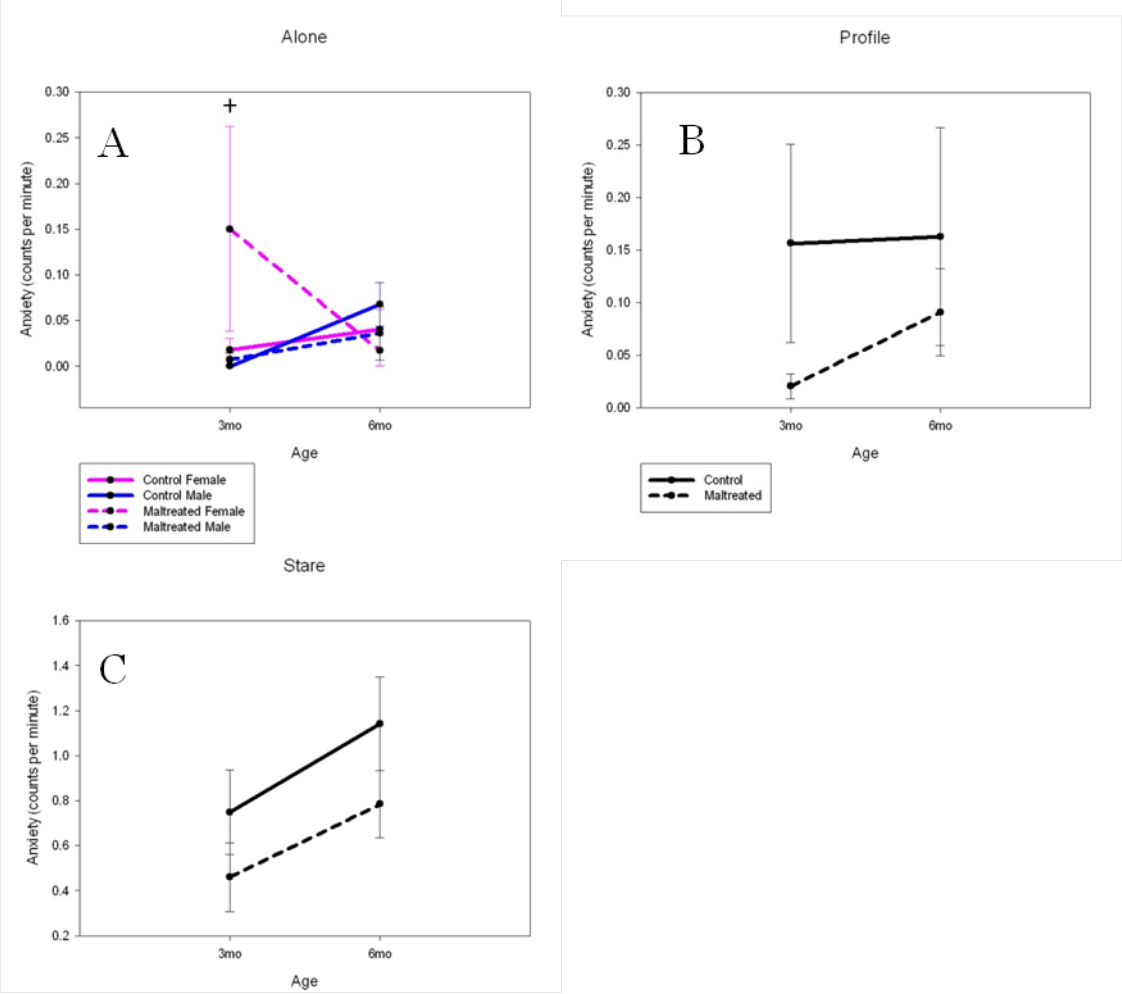


Figure 4.11

Human Intruder: Mean rates (±SEM) of withdrawal recorded during the Profile (A) and Stare (B) conditions at 3 and 6 months old.

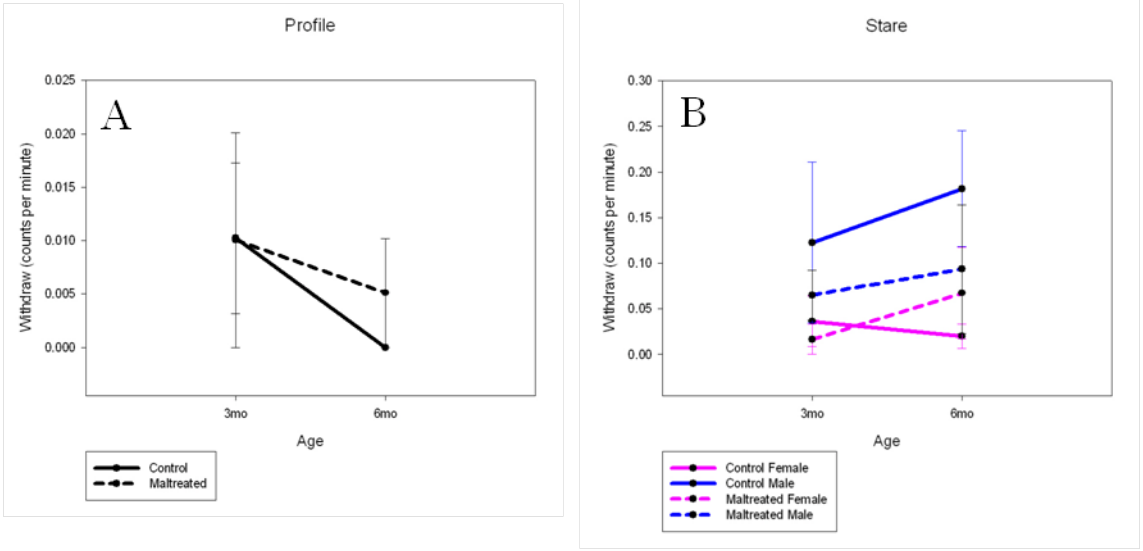


Figure 4.12

Human Intruder: Mean proportion of observation (±SEM) of total freezing recorded during the Alone (A), Profile (B), and Stare (C) conditions at 3 and 6 months old.

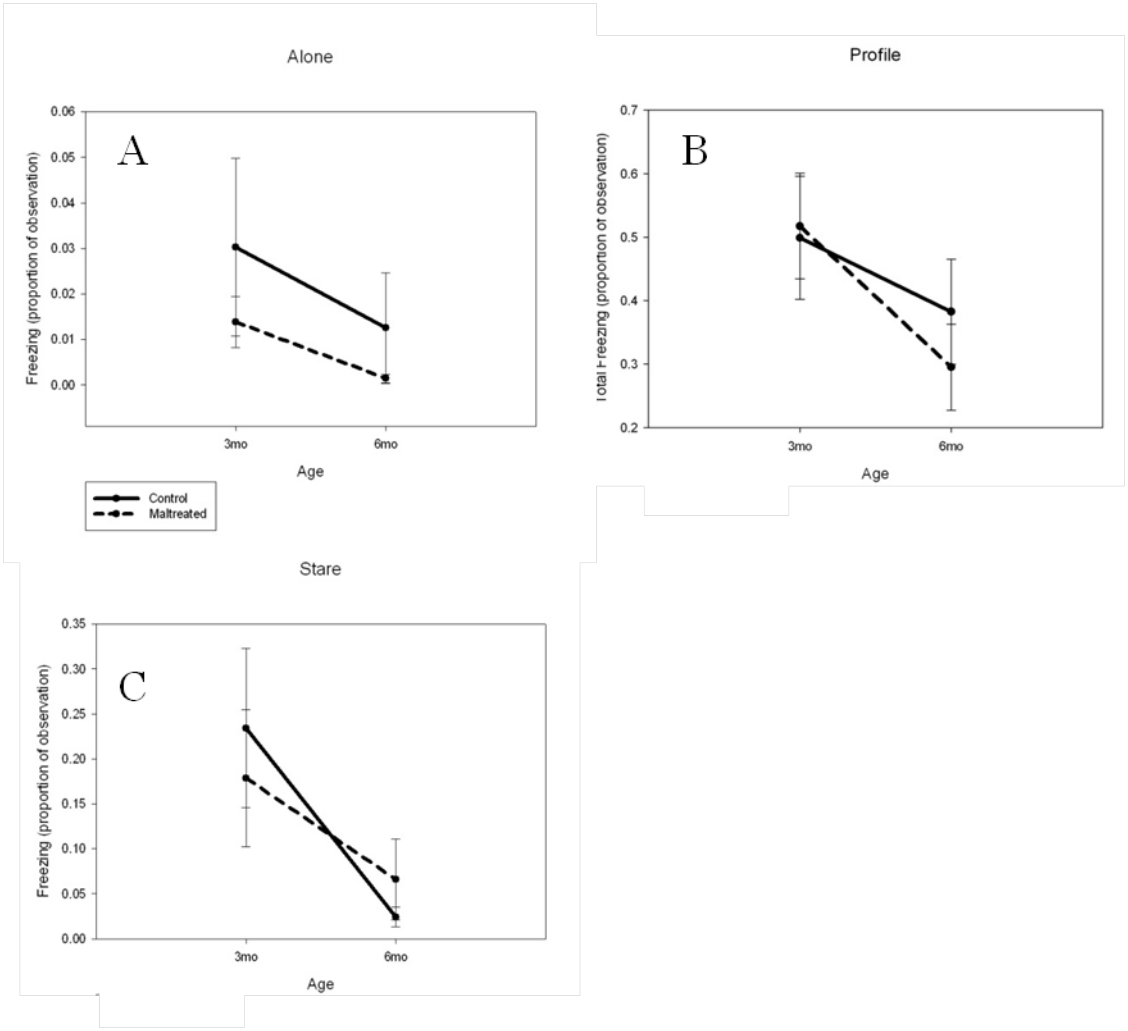


Figure 4.13

Human Intruder: Mean proportion of observation (±SEM) of total explore recorded during the Alone (A), Profile (B), and Stare (C) conditions at 3 and 6 months old.

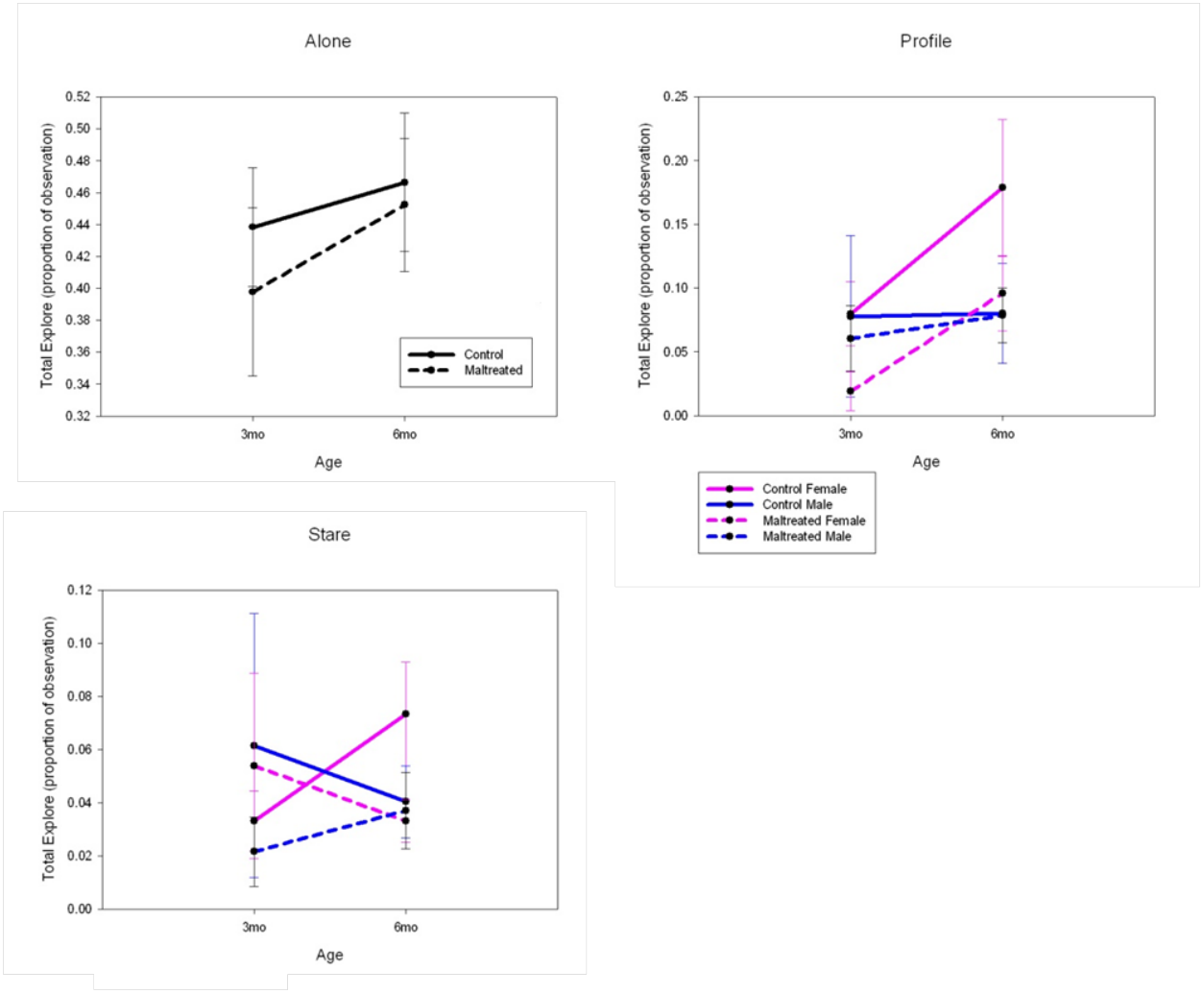


Figure 4. 14

Human Intruder: Mean rates (±SEM) of urination/defecation recorded during the Alone (A), Profile (B), and Stare (C) conditions at 3 and 6 months old. +significant effect of sex ($p \leq 0.05$)

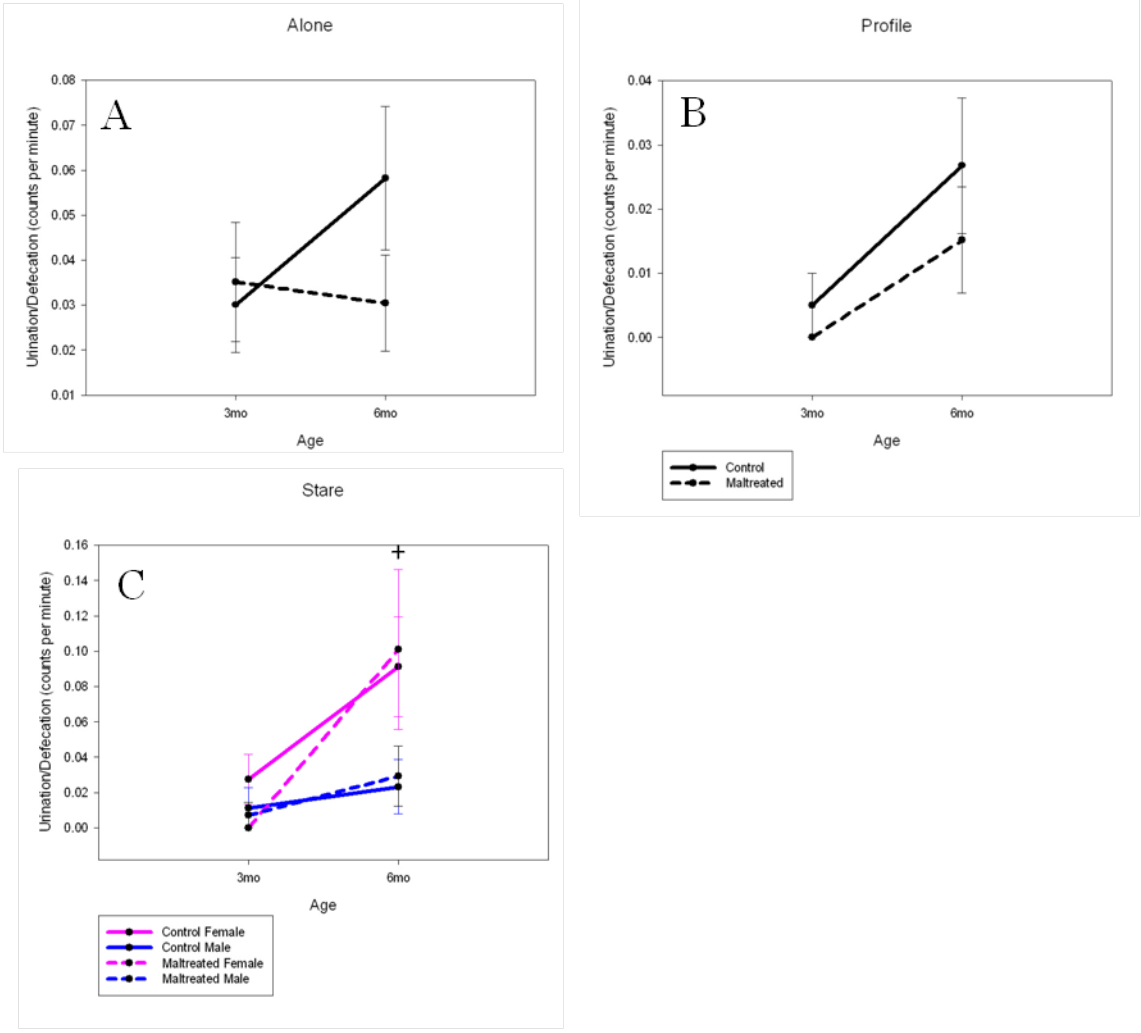


Figure 4.15

Human Intruder: Mean proportion of observation (±SEM) of tooth grind recorded during the Alone (A), Profile (B), and Stare (C) conditions at 3 and 6 months old. +significant effect of sex (p≤0.05)

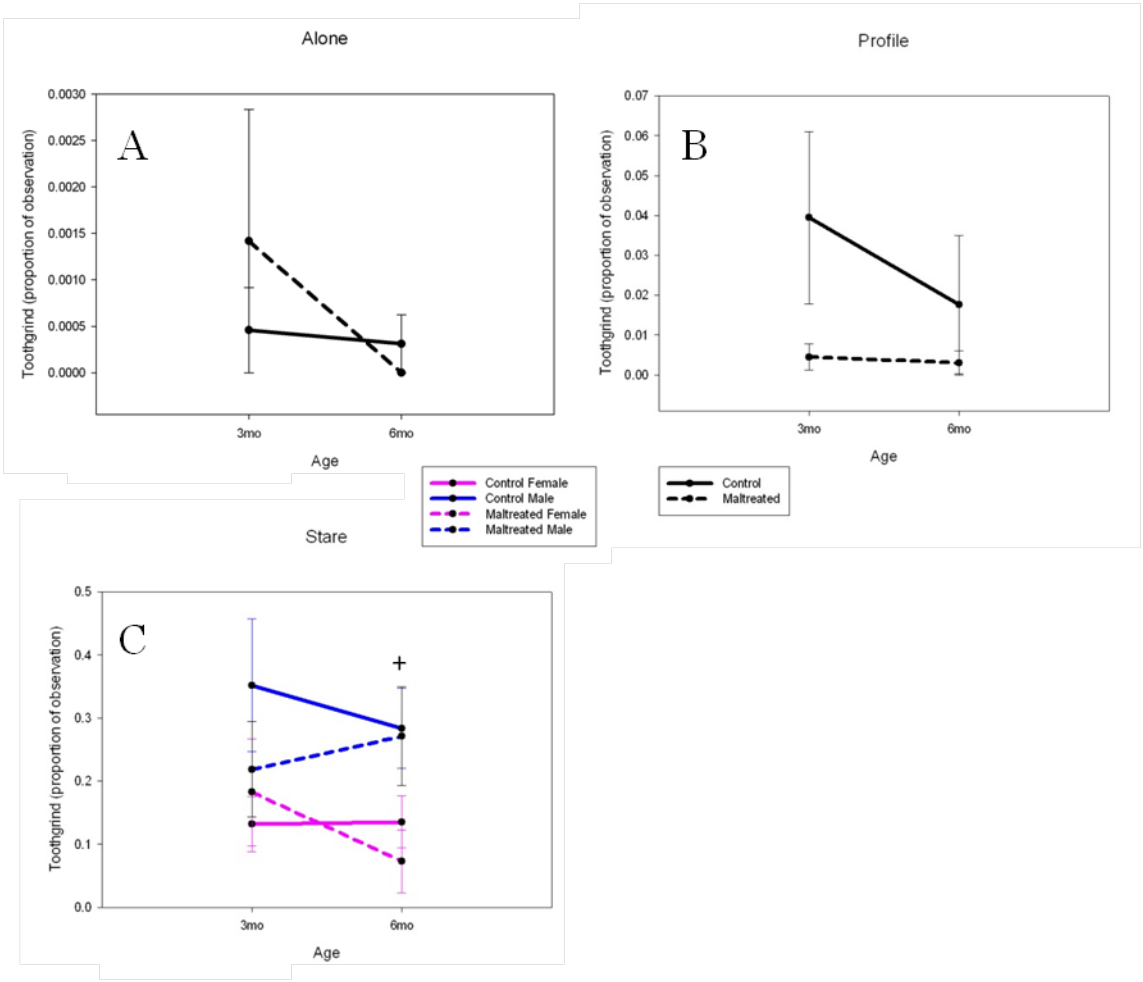


Figure 4.16

Human Intruder: Mean proportion of observation (±SEM) of locomotion recorded during the Alone (A), Profile (B), and Stare (C) conditions at 3 and 6 months old.

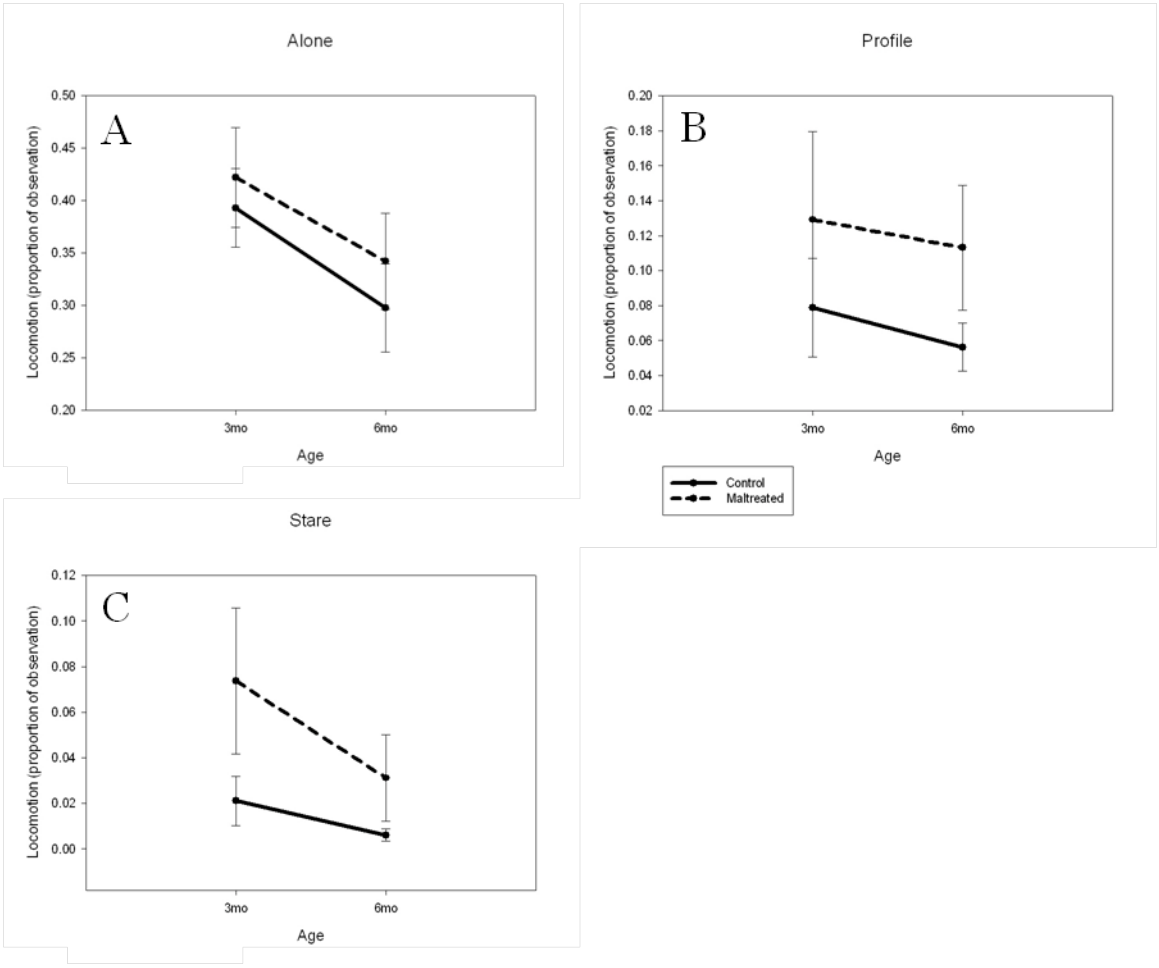


Figure 4.17

Human Intruder: Mean proportion of observation (±SEM) of turn away recorded during the Profile (A) and Stare (B) conditions at 3 and 6 months old.

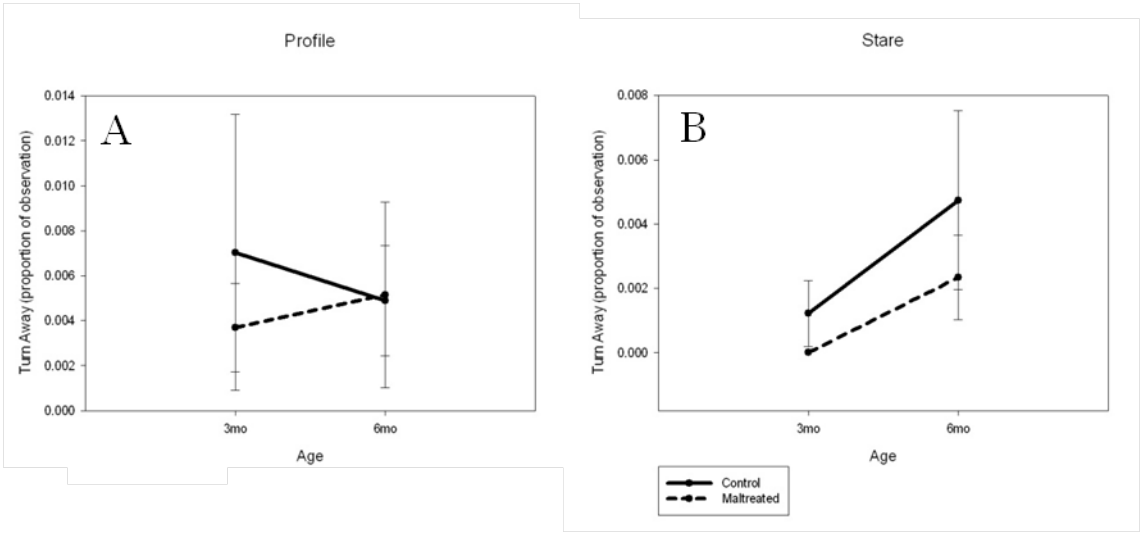


Figure 4.18

Results of the DTI analysis at 2 weeks: main effect of maternal care. TBSS 2x2 ANOVA results showing main effect of maternal care (maltreated vs. control) on FA: (A) cluster 1, right internal capsule, maltreated < control, (B) cluster 2, right somatosensory WM, maltreated < control, (C) cluster 3, right medial occipital WM, maltreated > control, (D) cluster 4, left middle temporal-occipital WM, maltreated < control, (E) cluster 5, left cerebellar WM, maltreated < control, and (F) cluster 6, right cerebellar WM, maltreated < control. Affected regions are displayed in red overlaid on the mean FA image with the mean FA skeleton in green.

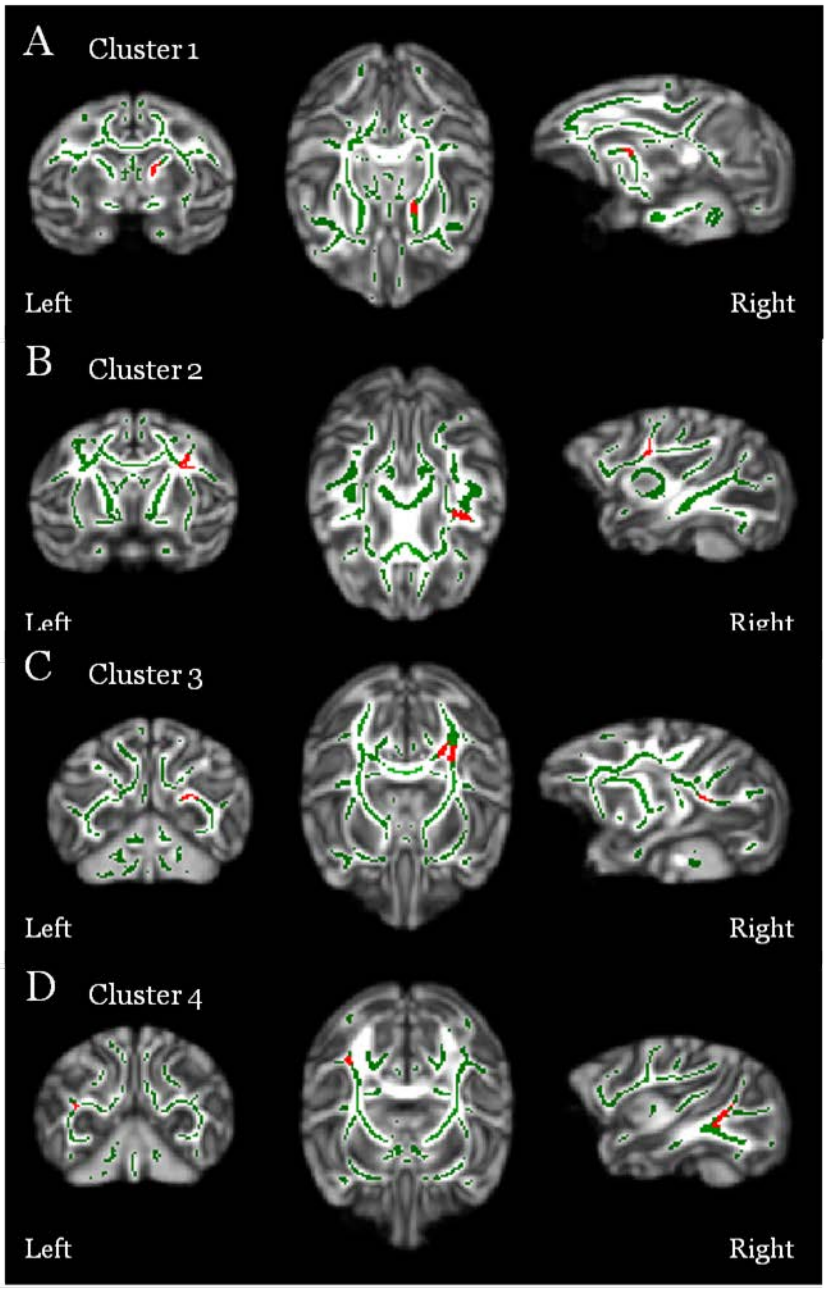


Figure 4.18 (cont.)

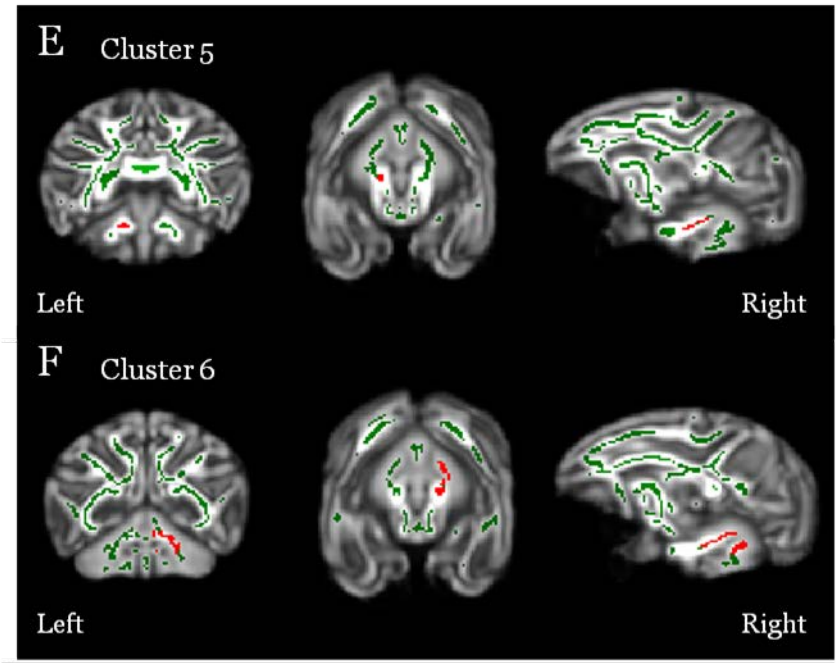


Figure 4. 19

Results of the DTI analysis at 2 weeks: maternal care by sex interaction effect. TBSS 2x2 ANOVA results showing a maternal care by sex interaction effect on FA in cluster 7, left cerebellar WM (maltreated females had lower FA than control females, while maltreated males had higher FA than control males). Affected regions are displayed in red overlaid on the mean FA image with the mean FA skeleton in green.

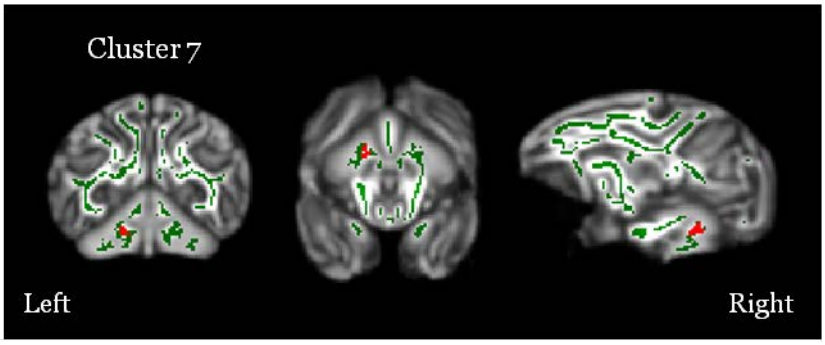


Figure 4.20

Results of the DTI analysis at 3 months: main effect of maternal care. TBSS 2x2 ANOVA results showing main effect of maternal care (maltreated vs. control) on FA: (A) cluster 8, left premotor WM, maltreated > control, (B) cluster 9, right primary motor WM, maltreated > control, (C) cluster 10, left internal capsule, maltreated > control, (D) cluster 11, right internal capsule, maltreated > control. Affected regions are displayed in red overlaid on the mean FA image with the mean FA skeleton in green.

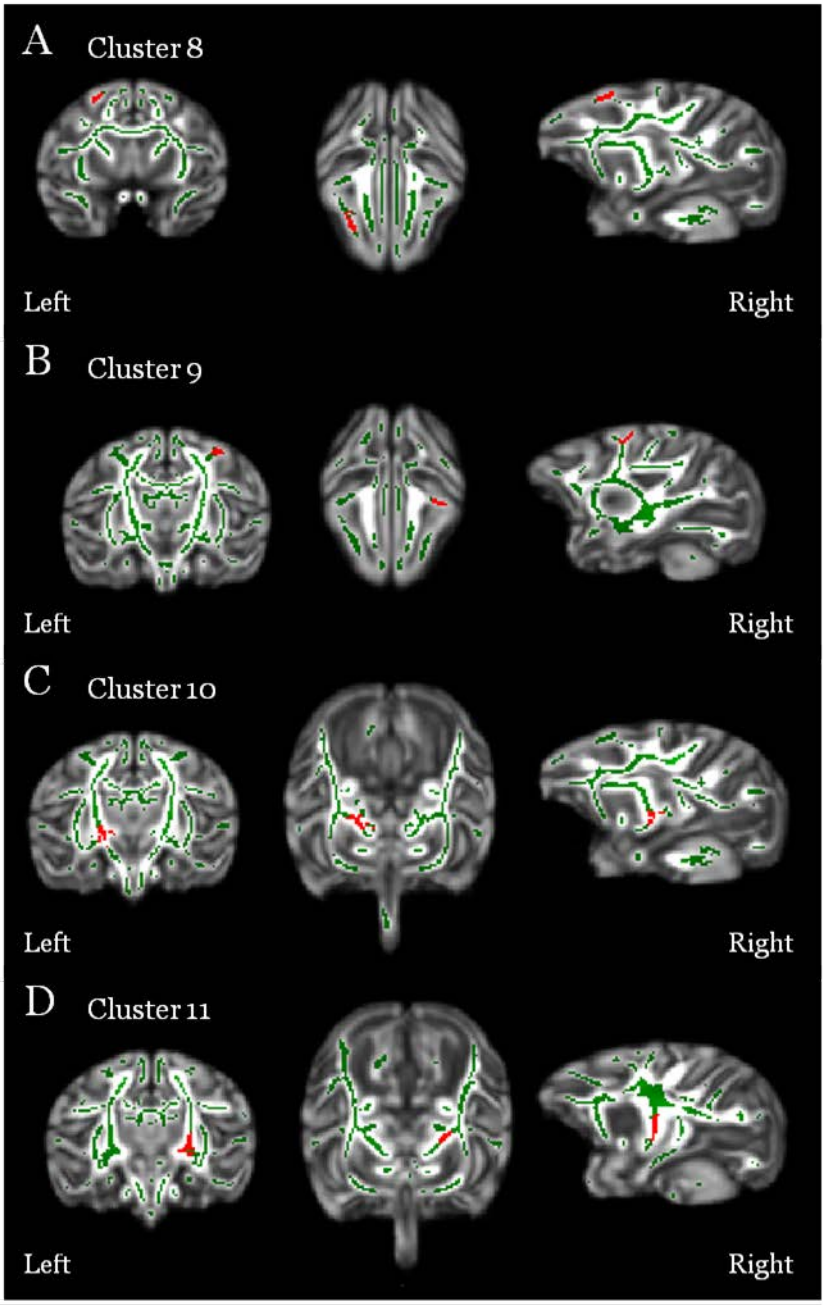


Figure 4.21

Results of the DTI analysis at 6 months: main effect of maternal care. TBSS 2x2 ANOVA results showing main effect of maternal care (maltreated vs. control) on FA: (A) cluster 12, genu of the corpus callosum, maltreated < control, (B) cluster 13, left prefrontal WM, maltreated < control, (C) cluster 14, left middle temporal-occipital WM, maltreated < control. Affected regions are displayed in red overlaid on the mean FA image with the mean FA skeleton in green.

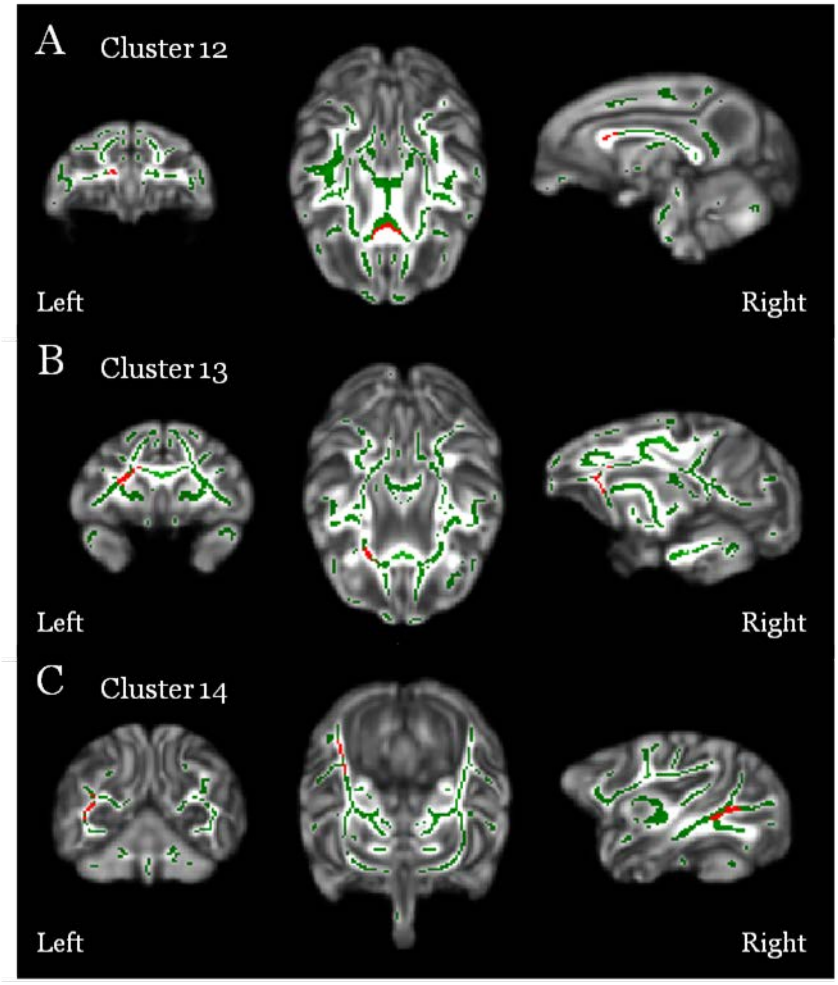


Figure 4.22

Results of the DTI analysis at 6 months: main effect of sex. TBSS 2x2 ANOVA results showing main effect of sex (male vs. female) on FA: (A) cluster 15, left internal capsule, female > male, (B) cluster 16, genu and anterior and middle portions of the midbody of the corpus callosum, male > female. Affected regions are displayed in red overlaid on the mean FA image with the mean FA skeleton in green.

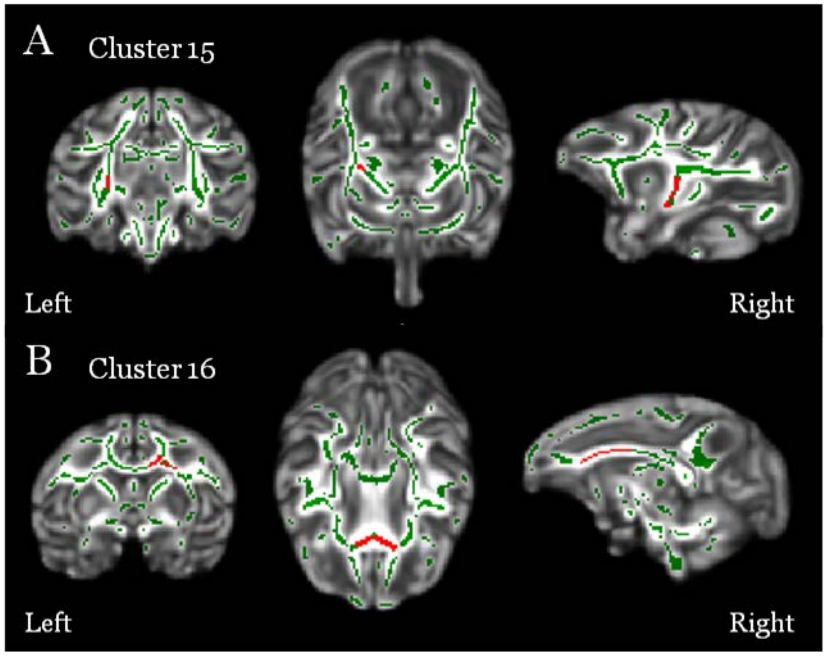
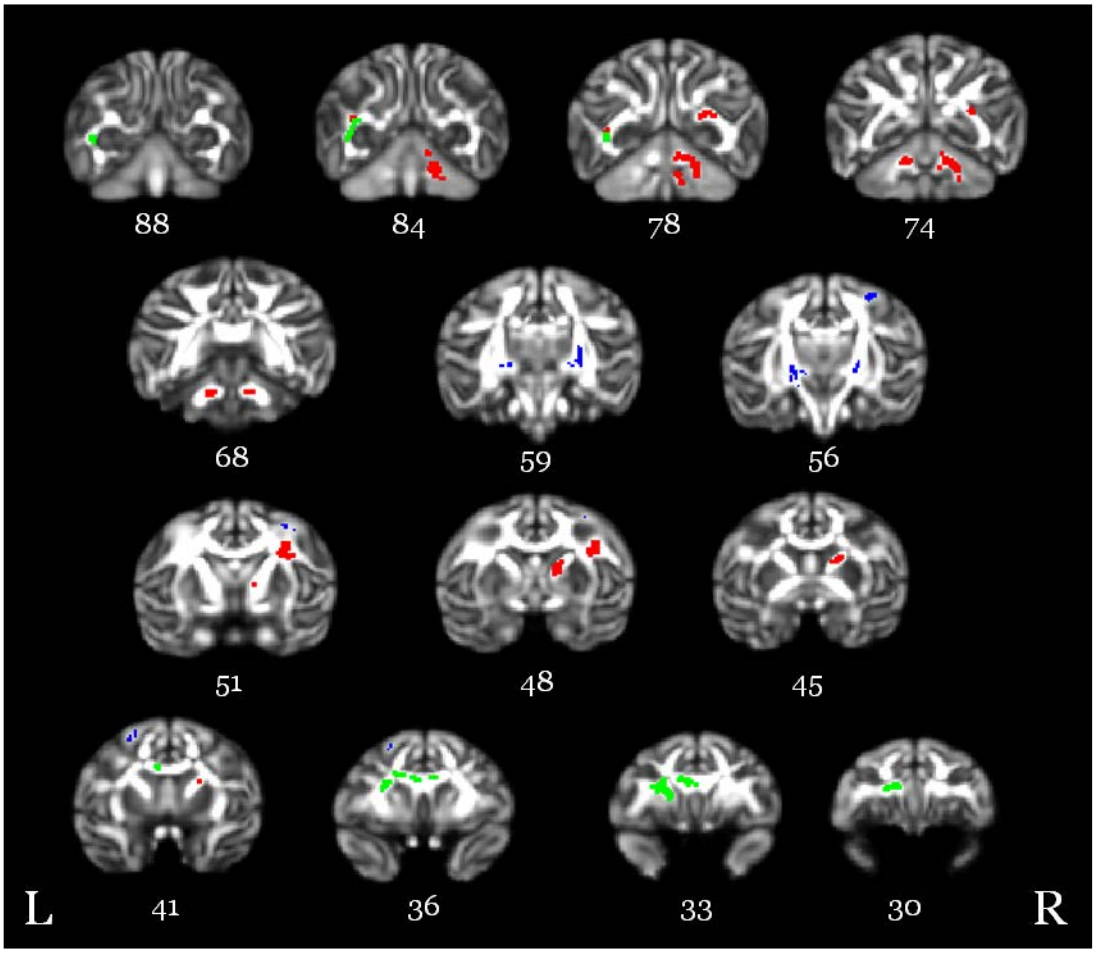


Figure 4.23

Spatiotemporal pattern of the effects of maternal care on FA. Red= clusters where maternal care effects were detected at 2 weeks; Blue= clusters where maternal care effects were detected at 3 months; Green= clusters where maternal care effects were detected at 6 months. Results are displayed on the 3 months mean FA image. The number below each image indicates the coronal slice pictured, with slice number decreasing from caudal to rostral slices.



Chapter 5 : Discussion, conclusions, and future directions

5.1 Summary of results

5.1.1 Effects of social subordination stress in the juvenile period

Social status differences were found in medial prefrontal (mPFC) white matter (WM) and cortico-thalamic tracts, with subordinates showing higher WM structural integrity (measured as fractional anisotropy, FA) than dominant animals. Serotonin transporter (5HTT) genotype-related differences were detected in the posterior limb of the internal capsule, where s-variants had higher FA than l/l animals. Status-by-5HTT genotype interaction effects were found in (1) external capsule (middle longitudinal fasciculus), (2) parietal WM and (3) short-range prefrontal cortex (PFC) tracts, with opposite effects in dominant and subordinate animals. In most regions showing FA differences, opposite differences were detected in radial diffusivity (RD), but none in axial diffusivity (AD), suggesting differences in tract integrity likely involve differences in myelin. Affected tracts connect prefrontal, sensory processing, motor and association regions. Differences in these tracts were associated with increased emotional reactivity in subordinates, particularly with higher submissive and fear behaviors.

5.1.2 Effects of infant maltreatment in adolescence

Significant reductions in WM structural integrity (measured as FA) in corpus callosum (CC), occipital white matter, external medullary lamina (EML), as well as in the brainstem of adolescent rhesus monkeys that experienced maternal infant maltreatment.. In most regions showing FA reductions, opposite effects were detected in radial diffusivity (RD), without changes in axial diffusivity (AD), suggesting that the alterations in tract integrity likely involve reduced myelin. An association with elevated cortisol plasma levels during the first month of life was detected in almost all the regions

showing reduced WM integrity in the maltreated infants. Reduced FA in occipital WM was also associated with increased aggression towards others in the social group in maltreated animals, an effect that remained after controlling for plasma cortisol during month one of life. These findings highlight the long-term impact of infant maltreatment on brain WM structural integrity, particularly in tracts involved in visual processing, emotional regulation, and somatosensory and motor integration. They also suggest a relationship between elevations in stress hormones detected in maltreated animals during infancy and long-term brain WM structural effects.

5.1.3 Effects of infant maltreatment in infancy

Maltreated infants had increased cortisol accumulation in hair over the first 6 months of life, the postnatal period when they received physical abuse and maternal rejection. No group differences were detected in physical growth. Behavioral alterations included increased emotional reactivity in the social group, but decreased reactivity in response to threat, as measured during the Human Intruder (HI) paradigm. Alterations in FA were detected at 2 weeks, 3 months and 6 months. At 2 weeks decreases in FA in maltreated infants were detected in cerebellar, frontal, and middle temporal-occipital WM, as well as in the internal capsule (IC). Maltreated infants also had higher FA than controls in occipital WM at 2 weeks. At 3 months increases in FA were detected in frontal WM and the IC. At 6 months decreases in FA were detected in the corpus callosum (CC), and prefrontal and middle temporal-occipital WM. Thus alterations in WM were detected in tracts involved in visual processing, emotional regulation, and somatosensory and motor integration. These results provide evidence that infant maltreatment is related to chronically elevated cortisol levels, alterations in socioemotional behavioral development, and alterations in the development of brain WM.

5.2 Integration of findings

The overarching goals of this dissertation were: (1) compare alterations in brain WM at different stages of development in order to address the hypothesis that WM microstructure in prefrontal-amygdala circuits will show alterations in development and in both models, (2) address the hypothesis that alterations in WM microstructure in other regions, such as tracts connecting association cortices will also be present, however the regions affected and even the directionality of the effects will differ as a function of the specific experience due to differences in age of exposure to, type and duration of the stressor (i.e. entire lifetime for social subordination versus during the first 6 postnatal months for infant maltreatment), (3) examine how WM microstructure relates to socioemotional behavior in these two models. Stress affected WM at all ages studied, but in different regions and directions for each model, thus the hypothesis of similar effects in prefrontal-amygdala circuits was not supported. Sensorimotor WM was affected by both chronic social stress and infant maltreatment, suggesting that *early* exposure to elevated glucocorticoids (GCs) may be a common mechanism in these alterations. The lack of an association with cortisol with the effects on brain WM in the social subordination model, which was contrasted by the association with cortisol and brain WM integrity in infant maltreatment, could be due to the sampling age for cortisol (concurrently in prepuberty in the social subordination study and in infancy in the study of the long term effects of infant maltreatment), and thus it is still possible that early elevations in cortisol (not measured in the social subordination model, and again, possibly buffered by maternal care) could be predictive of later alterations in brain WM in the social subordination model. This possibility requires further investigation using a longitudinal experimental design.

The hypothesis that different WM tracts (e.g. sensory association tracts) would be differentially effected in these models because of intrinsic differences in sensory stimuli and developmental timing involved in social subordination stress versus infant maltreatment was supported by the differences in regional effects on WM integrity (i.e. the IC and CC). These differences could be due to timing and chronicity of the stress (social subordination stress is a life-long stressor but prior to weaning infants likely experience maternal buffering, while infant maltreatment is experienced during the first 3-6 months of life). Keeping this difference between these models in mind, it is interesting that in the social subordination model submissive behaviors positively correlated with FA in regions where subordinates had significantly higher FA than dominants, many of which involve sensorimotor processing. This contrasts to the lack of correlation detected between sensorimotor regions and aggression in adolescents with histories of infant maltreatment, although these results may not be contradictory. Submissive behaviors are extremely adaptive for a subordinate, while aggression may not be for maltreated adolescents. Therefore the absence of a correlation between FA in the sensorimotor regions identified as being different in maltreated infants (decreased as compared to controls) and *maladaptive* behavior (i.e. aggression) is consistent with previous reports of increased FA supporting *adaptive* behaviors and learning (Paus 2010). Further analyses of the longitudinal data presented in Chapter 4 are necessary to determine how this association unfolds, and whether other behaviors, presumably adaptive, are supported by the alterations in FA detected in maltreated infants.

A consistent finding across the series of studies in both models is the spatiotemporal pattern of the effects of stress on the developing brain. In general, the regions shown to have alterations in WM microstructure were those regions in which myelination was occurring as a function of normative development. For example, in the longitudinal study, at 2 weeks alterations were found in the cerebellum, but not in the

PFC, while during the juvenile period alterations were found in the PFC. The studies in adolescent animals also support this idea, with alterations in the anterior CC and regions with protracted development being detected. This begs the question then: what is happening in regions that show alterations at one age and none at later ages? One possibility is that there is some form of catch-up in these regions in animals exposed to early life stress (ELS). Another possibility that remains to be tested is that ELS-related differences in behavior drive the alterations in the brain, thus observed alterations in WM microstructure could reflect more proximal effects of behaviors. In other words these differences in FA may support specific behaviors in response to the social environment. Although FA has been shown to directly affect the speed of neuronal information transfer (Lang and Rosenbluth 2003) and therefore can affect behavior, the opposite could also be true, and in fact behavior has been shown to alter FA (Takeuchi, Sekiguchi et al. 2010; Scholz, Klein et al. 2009; Johansen-Berg 2010). I am unaware of any studies that have experimentally manipulated WM and measured changes in behavior, but the efficacy of deep brain stimulation in treating depression (Haber and Brucker 2009) provides some indirect evidence to support this possibility.

5.3 Conclusions and future directions

The series of studies presented herein provides evidence that chronic social stress and the ELS of infant maltreatment affect primate brain development in an experience-dependent way, and that these effects are also related to the timing of the experience. It is difficult to draw specific over-arching conclusions due to the specific experimental designs employed in each study. The differences in these studies, although complimentary in many ways, still leave many questions unanswered: How do social subordination stress alterations in brain WM and behavior first emerge and how do they

unfold? If increased levels of GCs don't predict alterations in brain WM integrity in the social subordination model, through what other mechanisms (e.g. alterations in central CRH systems or inflammatory pathways?), might social subordination act to affect these neural systems? Does social subordination stress interact with infant maltreatment to affect brain WM in a cumulative way, i.e. would a maltreated subordinate show the alterations detected in subordinate animals and those detected in maltreated infants, or would a new pattern emerge? Are elevations of stress hormones early in development more predictive of outcomes than concurrent hormone levels? To directly compare the effects of social subordination and maltreatment across development future studies will need to incorporate a cross-fostering design with longitudinal sampling from birth in control and maltreated animals with large enough sample sizes to investigate the effects of both social status and sex, in addition to maltreatment. Future studies can use the strengths of each of these individual studies to disentangle the effects of specific early experiences, the mechanisms through which they exert their effects (e.g. glucocorticoids, inflammatory signals. etc.), and the interactions between sex and heritable factors on the outcomes of early experience.

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