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Caroline Fu April 13, 2015

Effects of Maternal Care on Amygdala, Prefrontal Cortex, and Hippocampal Development in Infant Rhesus Monkeys

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Abstract

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Childhood maltreatment is a devastating experience with significant emotional and neurobiological consequences. It is prevalent not only in humans, but also in non-human primates, including rhesus monkeys. Non-human primate models serve as excellent models for studying effects of maltreatment, as they allow experimental manipulations of subjects that cannot be performed in humans. The rhesus monkey, due to its highly complex social structure and strong and long-lasting mother-infant bonds, is an animal model of high translational value for human experience. This study seeks to investigate the effects of infant maltreatment on structural development of the infant brain, focusing primarily on the amygdala, prefrontal cortex (PFC), and hippocampus because of their critical roles on the regulation of emotional and stress responses. Another goal of the study is to examine whether exposure to elevated levels of the stress hormone cortisol induced by the maltreatment experience predicts the structural impact on those cortico-limbic regions. This study utilizes a cross-fostering experimental design, with random assignment to experimental group, where some offspring were cross-fostered to control mothers and others to maltreating mothers, to control for the potential effects of genetic and/or epigenetic factors transmitted by biological mothers. Brain structural development was studied at 6 and 12 months (infancy and transition to the juvenile period), including intracranial volume (defined as total white matter + total gray matter + total ventricular and subarachnoid cerebrospinal fluid-CSF), total white matter, gray matter, and CSF volumes, as well as amygdalar, PFC, and hippocampal volumes. We found decreased amygdalar and PFC gray matter and CSF volumes as a result of maltreatment and positive correlations between amygdalar

volume and PFC gray matter and early cortisol levels. However, we found no effects on the hippocampus. These findings suggest that maltreatment-related neurodevelopmental effects emerge during infancy but that the impact (presence, directionality, magnitude) is different depending on the brain region, potentially due to their different patterns of development and time needed to respond to stress, supporting the critical role of timing of the experience and of the measurement of the effects of stress on neural volumes.

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Table of Contents

Table of Figures

Introduction

Childhood maltreatment, including abuse and neglect, is a prevalent and devastating early adverse experience, with tremendous clinical and economical impact in our society. Not only can abuse and neglect result in physical injury, but it can also negatively impact the child's emotional health. Children who have been abused have increased risk for anxiety and depressive disorders as well as aggressive behaviors (Cohen et al. 2001, Manly et al. 2001, Dodge et al. 1997, Price & Glad 2003). While this link is well known, the biological mechanisms involved, as well as the developmental course of events, are not understood, so this is an area that requires further research. Such detailed study in humans would involve prospective and frequent observations early in life and possible manipulation of subjects. As this is not feasible or ethical, humans are not the ideal subject candidates for this type of study.

However, infant maltreatment is not just a human problem; it has been reported in nonhuman primates as well, both in captivity and in the wild (Brent et al., 2002; Maestripieri, 1998). Many of its behavioral, physiological and neurological outcomes have been reported in recent studies using nonhuman primates (Sanchez, 2006).

The rhesus macaque (*Macaca mulatta*) is an especially effective model for such experiments due to the species' complex social interactions and strong mother-offspring bonds that, in the case of females, can last a lifetime. The social network that rhesus macaques reside in includes a strong hierarchical structure based on their matrilineal background (i.e. each family line has a specific social status within the whole troop, and infants inherit their ranks from their mothers). Males frequently migrate out of their natal group when they reach reproductive maturity and form bachelor groups that move from troop to troop, establishing the females as the leaders of the groups. These matrilineal-based troops can contain anywhere from a few dozen to

hundreds of animals. As such, family relationships and strong alliances are particularly essential to rhesus macaques, as they determine the social status of the animals in the troop (Suomi, 2005).

Like in humans, maternal care is critical for infant rhesus monkeys. During the first month of life, the infants are in close proximity to their mothers, who provide the infants with food, comfort, and protection. Even in subsequent months, the mother is vigilant and limits the time her infant spends with non-relatives (Suomi, 2005). Although the intensity and duration of mother-infant interaction decrease after the first month after birth, they remain fairly high for the first year of life and even into the young's juvenile years (which span approximately from 1 to 4 years of ages) (Suomi, 2005; Sanchez, 2006). As such, the quality of mother-child interaction is essential to proper development of a rhesus monkey.

While most mothers provide excellent care to their offspring, a few others show impaired caregiving, including various forms of abuse and neglect. Physical abuse is an active form of infant maltreatment, operationalized as violent behaviors exhibited by the mother that cause pain and distress in the infant, such as the dragging, hitting, or tossing of the infant, whereas other passive forms of maltreatment, closer to the neglect construct in humans, involve maternal rejection and neglect of the infant. In fact, mothers frequently exhibit co-morbidity of both abuse and rejection/neglect behaviors (Maestripieri, 1998; Sanchez, 2006). Infant maltreatment happens very early in life in macaques (within the first six months postpartum). Physically abusive behaviors are often only exhibited during the first three months of the infant's life, after which the infant is often strong enough to resist or run away. However, neglectful behaviors are more pervasive, as they tend to be prevalent throughout the first six months of the infant's life (McCormack et al., 2006; Sanchez, 2006), which is equivalent to 2 years in humans.

The maltreatment of infants has been shown to be a stressful experience and have adverse effects on the main stress neuroendocrine system, the hypothalamic-pituitary-adrenal (HPA) axis (Sanchez, 2006; Howell et al., 2013). In response to stress, the hypothalamus releases corticotropin releasing factor (CRF), which triggers the release of adrenocorticotropic hormone (ACTH) from the pituitary, which in turn provokes the release of cortisol from the adrenal gland. Studies by our lab have shown that at one month of age, rhesus macaques that experienced maternal abuse have higher plasma levels of the stress hormone cortisol than infants raised by competent mothers. As the infants get older, although the HPA axis activity can normalize (baseline cortisol levels are not high at later ages, once the phase of intense maternal abuse and rejection is over), but there are long-term consequences of sustained activation of stress systems early in life. For example, it can impact the way the HPA axis itself develops. Thus, at six months of age, abused infants have blunted ACTH secretion in the pituitary in response to CRF, and therefore lower cortisol secretion by the adrenal than normal infants. Although counterintuitive, this finding suggests that CRF levels in the hypothalamus must have been high earlier on in the infant's life as a result of the stress of experiencing maltreatment, causing a down-regulation in the number of CRF receptors in the pituitary in response to the chronically high CRF levels in maltreated infants (Sanchez et al, 2010). Ongoing studies, including this one, are focused on understanding the long-term impact of these early life stress-induced increases in stress hormones for brain development.

A test was done in our lab on 20 rhesus macaques born in 2002 (10 maltreated, 10 control) that showed that maternal abuse also hindered aspects of behavioral development over the first six months of life. Thus, abused macaques also showed more emotional reactivity and fearful behaviors, including tantrums and screams, in addition to the elevated levels of the stress

hormone cortisol as described above (McCormack et al., 2009; McCormack et al., 2006). Additionally, our lab has shown infant maltreatment also results in long-term effects on brain structural development during adolescence, including decreases in white matter structural integrity, which is associated with increased aggressive behavior in adolescents as well as alterations in structure and size of limbic regions such as the amygdala, which are also associated with higher emotional reactivity of maltreated animals (Howell et al., 2014). However, further investigation is still needed to understand the emergence and developmental trajectory of these alterations, as well as the causes and mechanisms of neural and behavioral alterations detected during adolescence stemming from early life stress (ELS).

Therefore, the goal of this project is to investigate the unfolding of infant maltreatment effects on brain development of rhesus macaques as an animal model for effects on human children, focusing specifically on the infant and juvenile transition periods (i.e. rhesus infants were studied at 6 and 12 months of age, equivalent to human toddlers and preschool children). Specifically, I aimed to examine the effects of ELS on structural development of brain circuits involved in emotional and stress regulation (i.e. amygdala, prefrontal cortex (PFC), and hippocampus).

Primate brain development involves an increase in total brain volume driven mostly by increases in white matter (WM) volume throughout the first four years of life in macaques (from birth through adolescence), with the fastest growth happening within the first four months (Malkova et al., 2006). In contrast, gray matter (GM) volume decreases after about 10 months of age (corresponding to late infancy) (Liu et al., 2015). During early childhood, humans also exhibit increases in total brain, total WM, total GM, and total lateral ventricle volume. However, starting at adolescence, this trend reverses for total brain and total GM volume while continuing

for total WM and total lateral ventricle volume (Giedd & Rapoport, 2010). In addition to the diversity of growth patterns for different brain tissue types (WM, GM, CSF), different brain structures also follow different developmental trajectories and growth rates which could make them more susceptible to stress at different times (Lupien et al., 2009; Tottenham & Sheridan, 2010).

The specific focus of this thesis is to examine the emergence of developmental effects of ELS on amygdalar, PFC, and hippocampal regional volumes. The amygdala is involved in emotional response and coping with potentially dangerous situations (Tottenham & Sheridan, 2010). The PFC is involved in emotional and stress regulation, inhibitory control behavior, executive function, attention, and complex thinking (Arnsten, 2009). The hippocampus is important for memory formation and retention, as well as for HPA axis response inhibition (Tottenham & Sheridan, 2010). They are all highly sensitivity to stress, (Bremner, 2002, 2003, 2006; Dannlowski et al. 2012, Tiecher et al 2012; van Harmelen et al. 2010, Tottenham & Hare et al., 2010, Tottenham & Sheridan, 2010; Hanson et al. 2014), and they are also critical in the regulation of emotional and stress reactivity, which are additional behavioral and physiological parallel measures in our ongoing studies, and they are all regions that are still developing and sensitive to early environment and social experiences in young primates (Hanson et al., 2012; Tottenham & Sheridan, 2010).

The amygdala has a stimulatory role on HPA axis activity, leading to increased CRF release from the hypothalamus, and therefore, higher cortisol production. In addition, stress often stimulates the amygdala to grow at a faster rate and causes increased formation of dendritic connections within the amygdala (Tottenham & Sheridan, 2010; Vyas et al., 2006) Stress has the opposite effect on the hippocampus and the PFC. Likewise, whereas the amygdala stimulates

HPA axis activity, the hippocampus inhibits it through mineralocorticoid receptors and glucocorticoid receptors, which bind glucocorticoids (e.g. cortisol) to mediate HPA axis negative feedback. Prenatal stress has been shown to cause a decrease in levels of these receptors (Lupien et al., 2009). Additionally, chronic stress is known to cause death of hippocampal neurons, resulting in smaller hippocampus volume (Hanson et al., 2015) and impaired HPA function (Lupien et al., 2009; Tottenham & Sheridan, 2010). Cortisol and stress have also been linked to smaller PFC volumes (Carrion et al., 2010; Hanson et al., 2012). Specifically, chronic stress-induced glutamate excitotoxicity and catecholamine pathways (i.e. increased Protein Kinase C and cyclic AMP signaling as a result to increased catecholamine secretion) as well as cortisol elevations have been shown to cause damage on PFC dendritic fields, spines, and synapses (Arnsten, 2009; Popoli et al., 2011).

Potential confounding effects of genetic and epigenetic factors transmitted by the mother were controlled for in this study using a powerful cross-fostering experimental design with random assignment to experimental group including biological mother as a covariate in the statistical models. In addition, the Sanchez lab has already collected parallel measures of HPA axis function to examine developmental stress activation, via analysis of hair cortisol accumulation from birth through 6 months of age in the same animals I have studied. Therefore, I also sought to examine the associations between levels of stress hormones during early infancy and the development of emotional corticolimbic circuits by studying correlations between hair cortisol accumulation during pregnancy (to examine the impact of prenatal cortisol exposure on brain development) or during the first 6 months of postnatal life on the volumetric measures.

Given all the evidence presented thus far, it is hypothesized that ELS in the form of infant maltreatment will impact brain development, in particular corticolimbic circuits, resulting in

reduced volume of the PFC and hippocampus and increased volume of the amygdala, brain areas associated with the regulation of emotional responses (Bremner, 2002, 2003, 2006; Dannlowski et al. 2012, Tiecher et al 2012; van Harmelen et al. 2010, Tottenham, Hare et al., 2010; Hanson et al. 2014). In addition, it is hypothesized that infant maltreatment will result in exposure to elevated levels of the stress hormone cortisol during infancy (measured as hair cortisol accumulation from birth to 6 months of age). As a result, I also hypothesized that the elevated cortisol will be correlated with the alterations in amygdalar, PFC, and hippocampal structure.

Methods

Subjects and Housing

Forty-two rhesus monkeys (*Macaca mulatta*, 23 male and 19 female, were studied for long-term neurodevelopmental effects of maternal care at 6 and 12 months of age (late infancy and transition to the juvenile periods, respectively), as part of a bigger longitudinal study from birth through adolescence ongoing in the Sanchez lab. The subjects lived in complex social groups housed in outdoor compounds at the Yerkes National Primate Research Center (YNPRC) Field Station of Emory University, where they were also provided access to indoor, climate-controlled living areas. The social groups consisted of 2-3 adult males and 30-100 adult females with their sub-adult offspring. Animals were fed twice daily with fresh fruit, vegetables, and monkey chow, and water supply was always present. All studies complied with the NIH Guide

for the Care and Use of Laboratory Animals and the Emory University Institutional Animal Care and Use Committee (IACUC).

Cross-fostering design

We used a cross-fostering experimental design with random assignment of the infant at birth to either a control or a maltreating mother in which 12 control animals were fostered to maltreating mothers and 10 animals born to maltreating mothers were fostered to control mothers at birth. Another 10 control animals were fostered to control mothers, and 10 maltreated animals were fostered to maltreating mothers. Thus, no infants were raised by their biological mothers to control for potential confounding effects of heritable factors on developmental outcomes. The study consisted of 42 different foster mother-infant pairs with no siblings included to rule out genetic similarities (i.e no mother was used twice in the study). Social rank (high, medium, low) was also counterbalanced across all the experimental groups to control for its potential confounding effects on our measures. Table 1 shows details about the subjects used in the study. Infant maltreatment was defined as two co-morbid hallmark behaviors exhibited by the maltreating mothers: (1) physical abuse (which is operationalized as violent behaviors exhibited by the mother and that provoke pain and distress in the infant such as dragging, crushing, throwing, stepping or sitting on, or rough grooming of the subjects by the mothers) as well as (2) maternal rejection of the infant early in life (i.e. pushing the infant away or blocking it from making contact), following previously published criteria (Maestripieri et al., 2005; McCormack et al., 2006; Sanchez, 2006). Additionally, other members of the Sanchez lab collected data of the study subjects from birth through the latest ages studied and found no differences between the control and maltreated animals' body weight, height, or head circumference, and no study subject suffered from nutritional deficits (Howell et al., 2013).

Structural MR Brain Image Acquisition

Magnetic Resonance Imaging (MRI) scans of the subjects' brains were collected using a 3T Siemens scanner and an 8-channel phase array knee coil located in the Yerkes MRI Center. Both T1 and T2-weighted structural scans were gathered at the same scanning session from each subject at 6 and 12 months of age. The T1-weighted MR scan was acquired using a 3-dimensional (3D) magnetization-prepared rapid gradient-echo (3D-MPRAGE) parallel imaging sequence (TR/TE=3000/3.31ms, voxel-size=0.6mm^3, isotropic, 6 averages). A T2-weighted scan was collected in the same direction as the T1 (TR/TE=7,900/125ms, voxel size=0.5x0.5x1.0mm^3, 10 averages) to help with anatomical identification of white matter (WM), gray matter (GM) and cerebrospinal fluid (CSF) borders, and delineation of ROIs (Knickmeyer et al., 2010; Rapisarda et al., 1983). The subjects were scanned supine under isoflurane anesthesia. A custom-made head holder with ear bars and a mouth piece was used to secure and prevent movement of the head in order to avoid motion artifacts. A vitamin E capsule was placed to the right temple to identify the right brain hemisphere. Animals were intubated, administered dextrose/NaCl (I.V.) for hydration, placed over an MRI-compatible heating pad to maintain temperature, and monitored for their physiological measures during the scans. After each subject was scanned and had completely recovered from anesthesia, it was returned to its social group (Reding et al., in prep).

MRI Data Processing, Analysis and Region of Interest (ROI) Volume Computation

MR structural images were processed and analyzed using an in-house automatic, atlas-based segmentation program (AutoSeg, version 3.0.2), which is an open-source pipeline developed by our collaborators at the Neuro Image Research and Analysis Laboratories of University of North Carolina (Wang et al., 2014). AutoSeg was used to (1) automatically

segment brain tissue classes (WM, GM, CSF), (2) generate parcellations of cortical lobes (specifically for this study: the PFC), and subcortical structures (Amygdala and hippocampus) to compute their respective volumes in the rhesus macaque, following methods previously described (Howell et al., 2014; Knickmeyer et al., 2010; Wang et al., 2014). Briefly, in a first step, after the T1-and T2-MRI scans were collected, the brain images of 48 animals, including all 42 of the experimental subjects studied here, were aligned and "averaged" to create an overall T1-and T2-MRI age-specific template/atlas for each age (6 months and 12 months) using deformable registration tools in the Advanced Normalization Tools (ANTs) software. After the 6 and 12 months T1 and T2 atlases were created, they were used to obtain atlas probabilistic tissue maps (WM, GM, CSF, no-brain) and manually generated cortical lobar parcellations and subcortical region identifications that were later applied to each individual. For this we used AutoSeg 3.0.2 to process each of the subjects' T1 and T2-weighted images by performing inhomogeneity correction, and registration to the common image space (age-specific atlas/template: either 6 months or 12 months). An automatic tissue segmentation program called Atlas-Based Classification (ABC) was then used to automatically classify areas in each subject's images into brain tissue (WM, GM, CSF) or non-brain tissue (e.g. skull, vessels, muscle) and to remove non-brain tissue, such as skull, by warping the atlas tissue priors into the subject using ANTs affine and fluid registration of the atlas to each subject. Cortical lobar parcellations (PFC) and subcortical parcellations (amygdala, hippocampus) were also automatically generated. following a similar process and published procedures (Knickmeyer et al., 2010; Short et al., 2010; Wang et al., 2014). Figure 1 shows some of examples of the generated atlases and resulting tissue segmentations and parcellations. Volumes of total WM, GM, CSF, right and left PFC, right and left amygdala and right and left hippocampus were then automatically computed.

Total Intracranial volume (ICV: defined as total WM+ total GM+ total ventricular and subarachnoid CSF volumes) was arithmetically calculated (note that this was not calculated as the total volume inside the cranium).

Neuroanatomical definition of Regions of Interest (ROI) in the atlas:

The amygdalar boundary was marked rostrally by the anterior limit of the periamygdaloid cortex, posteriorly by the hippocampus, ventrally by CSF, ventrolaterally by WM (Amaral & Bassett, 1989; Price et al., 1987; Reding et al., in preparation) and when the latter was not visible due to low contrast, the rhinal fissure defined the ventromedial border. The PFC border was marked anteriorly and superiorly by CSF, posteriorly and inferiorly by the Sylvian fissure and then the arcuate sulcus (which also serves as the superior boundary posteriorly), and medially by the interhemispheric fissure. The hippocampus boundaries are as described in Rosene and Hoesen, 1987 and marked (1) superiorly by the lateral ventricle and temporal horn, except at the subiculum, where it is marked by WM; (2) WM separating the hippocampus from the entorhinal cortex also marks the inferior boundary; (3) the anterior border is defined by the lateral ventricle and temporal horn as well as the amygdala, and (4) the posterior border is defined by the lateral ventricle and WM, and medially by CSF (Knickmeyer et al., 2010).

For this study the automatic tissue class segmentations (WM, GM, CSF) from each individual brain were manually edited in the ITK Snap software by three raters blind to experimental group to ensure accurate neuroanatomical delineation. In order to avoid confounding effects of rater segmentation bias on our measures, in addition to being blind to group assignment, each rater was assigned a list of subjects counterbalanced by experimental group (foster mom, biological mom), sex (male, female), social rank (high, medium, low), and age (e.g. subject RMe14 at 6 months was manually edited by a different rater than RMe14 at 12

months). In addition, an expert independent observer (Dr. Mar Sanchez) made qualitative measures of the manual edits to ensure consistency across raters.

Hair Cortisol Samples

Cortisol is secreted by the adrenal gland into the systemic blood circulation, where it travels through the bloodstream and is known to accumulate in hair. Hair samples were collected by shaving the back of the neck of the animals at birth (postnatal day 2) and again at 6 months in order to measure chronic cortisol exposure throughout pregnancy and infancy, respectively. The hair was stored at -80 degrees C until assay, when it was washed twice using isopropanol for 3 minutes at a time. The samples were then dried and pounded into powder form using a Retsch ball mill. Methanol was used to extract the cortisol via microcentrifuge, and an EIA kit was used to measure cortisol amounts (in pg/mg hair units) following previously published protocols (Davenport et al., 2006). The 2006 study by Davenport and colleagues showed that this method was effective at measuring cortisol accumulation over an extended period of time (weeks or months) and it was validated against previous methods and tissue contents (i.e. hair cortisol concentration is correlated with cortisol concentration from saliva samples and blood). Measurement of hair cortisol concentrations is therefore a validated and effective method to measure effects of chronic stress-induced glucocorticoid elevations (Davenport et al., 2006).

Statistical Analysis

Before examining the effects of maternal care (foster mom group) on our volumetric measures, we first performed a statistical analysis to examine and control for the potential effect of heritable factors (biological mother groups: Control versus Maltreating) using Repeated Measures (RM) ANOVA (fixed factor: biological mother group; repeated measures: age and hemisphere). When a significant effect of biological mother was detected (i.e. Control versus

Maltreating biological mother), this factor was included in the subsequent experimental statistical models as a covariate for the effects of foster maternal care.

Total brain measures: intracranial volume (ICV), total white matter (WM), total gray matter (GM), and total cerebrospinal fluid (CSF) were analyzed using a RM ANOVA with foster mom group (control or maltreated) and sex (male or female) as fixed factors and age (6 months, 12 months) as the repeated measure. PFC GM, WM, and CSF volumes, as well as the amygdala and hippocampus, were also analyzed using RM ANOVA with foster mom group and sex as the fixed factors and age and hemisphere (right or left) as repeated measures. When no laterality (hemisphere) effect was detected (i.e. no main effect of hemisphere or any foster mom by hemisphere interaction), both hemispheres were combined for analyses. Due to the potential confounding effect of total ICV on amygdala, PFC, and hippocampal volumes, these measures were analyzed both uncorrected (i.e. raw volumetric values) and corrected by ICV (i.e. uncorrected volume divided by total ICV), as previously published (Howell et al., 2014).

Pearson's correlation was used to examine the relationship between volumetric measures and early exposure to the stress hormone cortisol, measured as prenatal hair cortisol accumulation (hair collected on postnatal day 2) and hair cortisol accumulation between birth and 6 months of life (when maternal abuse and rejection rates were the highest).

Statistical analyses were performed using SPSS (version 22.0). Significant p values were set at $p < 0.05$.

Results

Total Brain, White Matter, Gray Matter, and CSF Volumes

Preliminary analyses were performed for each volume to identify potential effects of biological mother group (Control versus Maltreating). No significant effects of biological mother group or interaction effects with age were found for total brain volume ($F_{1,40} = 0.106$, $p = 0.747$; age by biological mom interaction, $F_{1,40} = 0.039$, $p = 0.845$), WM ($F_{1,40} = 0.352$, $p = 0.556$; age by biological mom interaction, $F_{1,40} = 0.132$, $p = 0.718$), GM ($F_{1,40} = 0.009$, $p = 0.923$; age by biological mom interaction, $F_{1,40} = 0.005$, $p = 0.941$), or for CSF ($F_{1,40} = 0.234$, $p = 0.631$; age by biological mom interaction, $F_{1,40} = 0.347$, $p = 0.559$). Therefore, biological mother was not included as a covariate in further analyses of these measures.

Intracranial volume (ICV)

Significant main effects of age ($F_{1,38} = 9.218$, $p = 0.004$, $\eta^2 = 0.195$) and sex ($F_{1,38} = 14.256$, $p = 0.001$, $\eta^2 = 0.273$) were found on total ICV (defined as total WM + total GM + total CSF (both in ventricles and in subarachnoid space)), with greater volumes at 6 than at 12 months and in males than in females (Figure 2). No main effect of foster mom was found on total ICV ($F_{1,38} = 0.904$, $p = 0.348$, $\eta^2 = 0.023$), nor foster mom by age ($F_{1,38} = 0.657$, $p = 0.423$, $\eta^2 = 0.017$), foster mom by sex ($F_{1,38} = 0.165$, $p = 0.687$, $\eta^2 = 0.004$), age by sex ($F_{1,38} = 1.305$, $p = 0.260$, $\eta^2 = 0.033$), or foster mom by age by sex ($F_{1,38} = 0.255$, $p = 0.617$, $\eta^2 = 0.007$) interaction effects.

White matter (WM)

Significant effects of age ($F_{1,38} = 178.295$, $p < 0.001$, $\eta^2 = 0.824$) and sex ($F_{1,38} = 17.432$, $p < 0.001$, $\eta^2 = 0.314$) were found on total WM volume, with higher volumes at 12 than at 6 months and in males than in females (Figure 3). No main effect of foster mom was found on total WM ($F_{1,38} = 0.747$, $p = 0.393$, $\eta^2 = 0.019$). Additionally, no interaction effects were found for foster mom by age ($F_{1,38} = 3.720$, $p = 0.061$, $\eta^2 = 0.089$), foster mom by sex ($F_{1,38} = 0.130$, $p = 0.720$,

η^2= 0.003), age by sex ($F_{1,38}$ = 0.202, p = 0.655, η^2= 0.005), or foster mom by age by sex ($F_{1,38}$ = 0.292, p = 0.592, η^2= 0.008).

Gray matter (GM)

Total GM volume exhibited significant effects of sex ($F_{1,38}$ = 10.498, p = 0.002, η^2 = 0.216) with higher volumes in males than females (Figure 4). No main effects of foster mom ($F_{1,38}$ = 0.555, p = 0.461, η^2= 0.014) or age ($F_{1,38}$ = 3.487, p = 0.07, η^2= 0.084) were found, although there was a trend towards a decrease with age. Furthermore, no interaction effects were found between foster mom by age ($F_{1,38}$ = 2.776, p = 0.104, η^2= 0.084), foster mom by sex ($F_{1,38}$ = 1.05, p = 0.312, η^2= 0.027), age by sex ($F_{1,38}$ = 0.493, p = 0.487, η^2=0.013), or foster mom by age by sex ($F_{1,38}$ = 0.006, p = 0.937, η^2 < 0.001).

CSF

A main effect of age ($F_{1,38}$ = 80.895, p < 0.001, η^2= 0.68) and sex ($F_{1,38}$ = 7.485, p = 0.009, η^2= 0.165) was found on total CSF volume, with higher volumes at 6 than at 12 months and in males than females (Figure 5). An interaction effect for foster mom by age interaction was also detected ($F_{1,38}$ = 6.322, p = 0.016, η^2= 0.143); visual inspection of the graph (figure 5, top) indicates that the effect was driven by smaller CSF volumes in maltreated subjects than in controls at 6 months. No other significant effects were found for foster mom ($F_{1,38}$ = 1.53, p = 0.224, η^2= 0.039), foster mom by sex ($F_{1,38}$ = 0.211, p = 0.648, η^2= 0.006), age by sex ($F_{1,38}$ = 1.901, p = 0.176, η^2= 0.048), or foster mom by age by sex ($F_{1,38}$ = 0.258, p = 0.614, η^2= 0.007).

<u>Amygdala</u>

No significant effects of biological mom group (Control, Maltreating) were found on amygdalar volume, so biological mom was not used as a covariate in further analyses. The specific results of those statistical tests are as follows: *uncorrected*: biological mom $F_{1,40}$ =

0.345, p = 0.56); biological mom by age ($F_{1,40}$ = 0.003, p = 0.958); biological mom by hemisphere ($F_{1,40}$ = 0.2, p = 0.657); biological mom by age by hemisphere ($F_{1,40}$ = 2.117, p = 0.153); *ICV-corrected:* right- biological mom ($F_{1,40}$ = 0.007, p = 0.933); biological mom by hemisphere ($F_{1,40}$ = 0.044, p = 0.835); left- biological mom ($F_{1,40}$ = 0.107, p = 0.745); biological mom by hemisphere ($F_{1,40}$ = 1.745, p = 0.194).

Uncorrected volumes

As aforementioned, regions of interest (ROI's: amygdala, PFC, hippocampus) were analyzed using RM ANOVA with foster mom and sex as fixed factors and age and hemisphere as repeated measures; if no laterality (hemisphere) effect was detected (i.e. main effect of hemisphere or any hemisphere by foster mom interaction), both hemispheres were combined for analysis. Due to the potential confounding effect of total ICV on amygdala, PFC, and hippocampal volumes, these measures were analyzed both as uncorrected volumes as well as corrected for ICV, as previously published (Howell et al., 2014).

Main effects of age ($F_{1,38}$ = 324.805, p <0.001 , η^2= 0.895), sex ($F_{1,38}$ = 10.878, p = 0.002, η^2= 0.223) and hemisphere ($F_{1,38}$ = 22.142, p <0.001, η^2= 0.368) were detected. Greater volumes were present at 12 months than at 6 months, in males than in females, and in the right hemisphere than in the left hemisphere. There was also found to be interaction effects for foster mom by hemisphere ($F_{1,38}$ = 7.49, p = 0.009, η^2= 0.165), age by hemisphere ($F_{1,38}$ = 7.675, p = 0.009, η^2= 0.168), and foster mom by age by hemisphere ($F_{1,38}$ = 9.503, p = 0.004, η^2= 0.2). As shown in figure 6, the foster mom interaction effects seem to be driven by smaller right amygdala volumes in maltreated animals at the older age. No main effect of foster mom was found ($F_{1,38}$ = 0.928, p = 0.341, η^2= 0.024). Additionally, no interaction effects between foster mom and age ($F_{1,38}$ = 0.728, p = 0.399, η^2= 0.019), foster mom and sex ($F_{1,38}$ = 0.529, p = 0.472,

η^2= 0.014), age and sex ($F_{1,38}$ = 0.614, p = 0.438, η^2= 0.016), hemisphere and sex ($F_{1,38}$ = 0.027, p = 0.869, η^2= 0.001), foster mom and hemisphere and sex ($F_{1,38}$ = 3.204, p = 0.081, η^2= 0.078), age and hemisphere and sex ($F_{1,38}$ = 2.677, p = 0.11, η^2= 0.066), or foster mom and age and hemisphere by sex ($F_{1,38}$ = 1.534, p = 0.223, η^2= 0.039) were found.

ICV-corrected volumes

Significant main effects of age ($F_{1,38}$ = 344.017, p < 0.001, η^2= 0.901) and of hemisphere ($F_{1,38}$ = 21.594, p < 0.001, η^2= 0.362) were found, with greater volumes at 12 than at 6 months and in the right hemisphere than in the left. Additionally, significant interaction effects between foster mom and hemisphere ($F_{1,38}$ = 6.207, p = 0.017, η^2= 0.14), age by hemisphere ($F_{1,38}$ = 7.931, p = 0.008, η^2= 0.173), and foster mom by age by hemisphere ($F_{1,38}$ = 9.165, p = 0.004, η^2= 0.194) were found. No figure is provided for these findings to avoid redundancy due to similar directionality of effects with those reported above for the amygdala uncorrected values. No other main effects were found for foster mom ($F_{1,38}$ = 0.004, p = 0.953, η^2< 0.001) or sex ($F_{1,38}$ = 0.025, p = 0.876, η^2= 0.001) or interaction effects for foster mom by age ($F_{1,38}$ = 1.729, p = 0.196, η^2= 0.044), foster mom by sex ($F_{1,38}$ = 1.37, p = 0.249, η^2= 0.035), age by sex ($F_{1,38}$ = 0.828, p = 0.369, η^2= 0.021), hemisphere by sex ($F_{1,38}$ = 0.124, p = 0.727, η^2= 0.003), foster mom by age by sex ($F_{1,38}$ = 1.769, p = 0.191, η^2= 0.044), foster mom by hemisphere by sex ($F_{1,38}$ = 3.159, p = 0.084, η^2= 0.077), age by hemisphere by sex ($F_{1,38}$ = 2.218, p = 0.145, η^2= 0.055), or foster mom by age by hemisphere by sex ($F_{1,38}$ = 1.165, p = 0.287, η^2= 0.03).

Prefrontal Cortex (PFC)

No significant effects of biological mother group (Control versus Maltreating) were found on PFC volume, so biological mom was not used as a covariate in further analyses of these measures. The specific results of those statistical tests were as follows: *uncorrected*: WM-

biological mom ($F_{1,40} = 0.12$, $p = 0.731$); biological mom by age interaction ($F_{1,40} = 1.174$, $p = 0.285$); biological mom by hemisphere interaction ($F_{1,40} = 0.029$, $p = 0.865$); biological mom by age by hemisphere interaction ($F_{1,40} = 0.081$, $p = 0.777$) GM- biological mom ($F_{1,40} = 0.001$, $p = 0.976$); biological mom by age interaction ($F_{1,40} = 0.818$, $p = 0.371$); biological mom by hemisphere interaction ($F_{1,40} = 0.249$, $p = 0.62$); biological mom by age by hemisphere interaction ($F_{1,40} = 0.102$, $p = 0.751$); CSF- biological mom ($F_{1,40} = 0.134$, $p = 0.716$); biological mom by age interaction ($F_{1,40} = 0.174$, $p = 0.679$); biological mom by hemisphere interaction ($F_{1,40} = 0.62$, $p = 0.436$); biological mom by age by hemisphere interaction ($F_{1,40} = 0.367$, $p = 0.548$); *ICV-corrected:* right, WM-biological mom ($F_{1,40} = 0.741$, $p = 0.394$); biological mom by age interaction ($F_{1,40} = 0.743$, $p = 0.394$); left, WM-biological mom ($F_{1,40} = 1.038$, $p = 0.315$), biological mom by age interaction ($F_{1,40} = 1.711$, $p = 0.198$); right, GM-biological mom interaction ($F_{1,40} = 0.421$, $p = 0.52$); biological mom by age interaction ($F_{1,40} = 0.492$, $p = 0.487$); left, GM-biological mom ($F_{1,40} = 0.265$, $p = 0.609$); biological mom by age interaction ($F_{1,40} = 0.467$, $p = 0.498$); right, CSF- biological mom ($F_{1,40} = 0.076$, $p = 0.784$); biological mom by age interaction ($F_{1,40} = 0.169$, $p = 0.683$); left, CSF- biological mom ($F_{1,40} = 0.001$, $p = 0.977$); biological mom by age interaction ($F_{1,40} = 0.016$, $p = 0.9$).

Uncorrected volumes

PFC WM

Because no significant laterality effects were found on this volumetric measure (i.e., no main hemisphere effect ($F_{1,38} < 0.001$, $p = 0.987$, $\eta^2 < 0.001$) or foster mom by hemisphere ($F_{1,38} = 0.052$, $p = 0.822$, $\eta^2 = 0.001$), foster mom by hemisphere by sex ($F_{1,38} = 0.025$, $p = 0.875$, $\eta^2 = 0.001$), foster mom by age by hemisphere ($F_{1,38} = 1.728$, $p = 0.197$, $\eta^2 = 0.043$), or foster mom

by age by hemisphere by sex ($F_{1,38} = 2.264$, $p = 0.141$, $\eta^2 = 0.056$) interaction effects), the right and left hemispheres were combined for analysis. Main age ($F_{1,38} = 88.813$, $p < 0.001$, $\eta^2 = 0.7$) and sex ($F_{1,38} = 12.513$, $p = 0.001$, $\eta^2 = 0.248$) effects were found. Greater volumes were found at 12 months than at 6 months and in males than in females (Figure 7). No main effect of foster mom was found ($F_{1,38} = 0.814$, $p = 0.373$, $\eta^2 = 0.021$). Additionally, no interaction effects were found (for foster mom by age ($F_{1,38} = 0.068$, $p = 0.796$, $\eta^2 = 0.002$), foster mom by sex ($F_{1,38} = 0.987$, $p = 0.327$, $\eta^2 = 0.025$), age by sex ($F_{1,38} = 0.917$, $p = 0.344$, $\eta^2 = 0.024$), or foster mom by age by sex ($F_{1,38} = 0.224$, $p = 0.638$, $\eta^2 = 0.006$)). Main age ($F_{1,38} = 88.813$, $p < 0.001$, $\eta^2 = 0.7$) and sex ($F_{1,38} = 12.513$, $p = 0.001$, $\eta^2 = 0.248$) effects were found. Greater volumes were found at 12 months than at 6 months and in males than in females (Figure 7). No main effect of foster mom was found ($F_{1,38} = 0.814$, $p = 0.373$, $\eta^2 = 0.021$). Additionally, no interaction effects were found (for foster mom by age ($F_{1,38} = 0.068$, $p = 0.796$, $\eta^2 = 0.002$), foster mom by sex ($F_{1,38} = 0.987$, $p = 0.327$, $\eta^2 = 0.025$), age by sex ($F_{1,38} = 0.917$, $p = 0.344$, $\eta^2 = 0.024$), or foster mom by age by sex ($F_{1,38} = 0.224$, $p = 0.638$, $\eta^2 = 0.006$)).

PFC GM

Main effects were found for sex ($F_{1,38} = 6.754$, $p = 0.013$, $\eta^2 = 0.151$) and hemisphere ($F_{1,38} = 5.522$, $p = 0.024$, $\eta^2 = 0.127$), with males showing greater volumes than females, and the right being bigger than the left. No main effects of foster mom ($F_{1,38} = 1.448$, $p = 0.236$, $\eta^2 = 0.037$) or age ($F_{1,38} = 4.112$, $p = 0.05$, $\eta^2 = 0.098$) were found, and no interaction effects were found, either (for foster mom by age ($F_{1,38} = 0.549$, $p = 0.463$, $\eta^2 = 0.014$), foster mom by sex ($F_{1,38} = 0.001$, $p = 0.97$, $\eta^2 < 0.001$), foster mom by hemisphere ($F_{1,38} = 0.366$, $p = 0.549$, $\eta^2 = 0.01$), age by sex ($F_{1,38} = 2.823$, $p = 0.101$, $\eta^2 = 0.069$), age by hemisphere ($F_{1,38} = 3.231$, $p = 0.08$, $\eta^2 = 0.078$), hemisphere by sex ($F_{1,38} = 0.796$, $p = 0.378$, $\eta^2 = 0.021$), foster mom by age by

sex ($F_{1,38}$ = 2.37, p = 0.132, η^2= 0.069), foster mom by hemisphere by sex ($F_{1,38}$ = 0.027, p = 0.871, η^2= 0.001), foster mom by age by hemisphere ($F_{1,38}$ < 0.001 p = 0.987, η^2< 0.001), age by hemisphere by sex ($F_{1,38}$ = 0.013, p = .909, η^2< 0.001), or foster mom by age by hemisphere by sex ($F_{1,38}$ = 0.196, p = 0.66, η^2= 0.005)).

PFC CSF

Main effects of age ($F_{1,38}$ = 95.362, p < 0.001, η^2= 0.715) and hemisphere ($F_{1,38}$ = 27.755, p < 0.001, η^2= 0.422) were found on CSF volume of the PFC, with greater volumes at 6 than at 12 months and in the right hemisphere than in the left. Significant interaction effects of foster mom by hemisphere by sex ($F_{1,38}$ = 5.869, p = 0.02, η^2= 0.134) and age by hemisphere by sex ($F_{1,38}$ = 5.853, p = 0.02, η^2=0.133) were also found. As shown in Figure 9 (plotted for the ICV-corrected values, with similar significant effects), the foster mom interaction seems to be driven by the lowest PFC CSF volume shown by maltreated females at 6 months in comparison to the biggest volume shown by control males. No main effects of foster mom ($F_{1,38}$ = 1.238, p = 0.273, η^2= 0.032) or sex ($F_{1,38}$ = 1.81, p = 0.187, η^2= 0.045) were found, and no interaction effects for foster mom by age ($F_{1,38}$ = 3.805, p = 0.059, η^2= 0.091), foster mom by sex ($F_{1,38}$ = 0.092, p = 0.764, η^2= 0.002), hemisphere by foster mom ($F_{1,38}$ = 1.539, p = 0.222, η^2= 0.039), age by sex ($F_{1,38}$ = 3.207, p = 0.081, η^2= 0.078), age by hemisphere ($F_{1,38}$ = 0.14, p = 0.711, η^2= 0.004), hemisphere by sex ($F_{1,38}$ = 0.538, p = 0.468, η^2= 0.014), foster mom by age by sex ($F_{1,38}$ = 0.028, p = 0.867, η^2= 0.001), foster mom by age by hemisphere ($F_{1,38}$ = 0.243, p = 0.625, η^2= 0.006), or foster mom by age by hemisphere by sex ($F_{1,38}$ = 0.898, p = 0.349, η^2= 0.023) were found.

ICV-corrected volumes

PFC WM

Because no significant laterality effects were found on this volumetric measure (i.e., no main hemisphere effect ($F_{1,38} = 0.029$, $p = 0.826$, $\eta^2 = 0.001$) or foster mom by hemisphere ($F_{1,38} = 0.034$, $p = 0.854$, $\eta^2 = 0.001$), foster mom by hemisphere by sex ($F_{1,38} = 0.132$, $p = 0.718$, $\eta^2 = 0.003$), foster mom by age by hemisphere ($F_{1,38} = 2.225$, $p = 0.144$, $\eta^2 = 0.055$), or foster mom by age by hemisphere by sex ($F_{1,38} = 2.437$, $p = 0.127$, $\eta^2 = 0.06$) interaction effects), the right and left hemispheres were combined for analysis. Main effects of age ($F_{1,38} = 139.967$, $p < 0.001$, $\eta^2 = 0.786$) and sex ($F_{1,38} = 5.411$, $p = 0.025$, $\eta^2 = 0.125$) were found, with greater volume at 12 months than at 6 months and in males than in females. No main effects of foster mom ($F_{1,38} = 0.513$, $p = 0.478$, $\eta^2 = 0.013$) or interaction effects were found (for foster mom by age ($F_{1,38} = 0.003$, $p = 0.958$, $\eta^2 < 0.001$), foster mom by sex ($F_{1,38} = 3.306$, $p = 0.077$, $\eta^2 = 0.08$), age by sex ($F_{1,38} = 1.158$, $p = 0.289$, $\eta^2 = 0.03$), or foster mom by age by sex ($F_{1,38} = 0.351$, $p = 0.557$, $\eta^2 = 0.009$)).

PFC GM

Main effects of age ($F_{1,38} = 14.791$, $p < 0.001$, $\eta^2 = 0.28$) and hemisphere ($F_{1,38} = 5.228$, $p = 0.028$, $\eta^2 = 0.121$) were discovered, with larger volumes at 12 months than at 6 months and in the right hemisphere than in the left. An interaction effect foster mom by age by sex was also found ($F_{1,38} = 5.199$, $p = 0.028$, $\eta^2 = 0.12$) and is shown in Figure 8. This effect seems to be driven by smaller PFC GM volumes in maltreated females at 6 months, which catches up with other groups at the later age. No main effects of foster mom ($F_{1,38} = 0.423$, $p = 0.519$, $\eta^2 = 0.011$) or sex ($F_{1,38} = 0.06$, $p = 0.808$, $\eta^2 = 0.002$) were found, and no additional interaction effects were found for foster mom by age ($F_{1,38} = 0.169$, $p = 0.684$, $\eta^2 = 0.004$), foster mom by sex ($F_{1,38} = 0.266$, $p = 0.609$, $\eta^2 = 0.007$), foster mom by hemisphere ($F_{1,38} = 0.451$, $p = 0.506$, $\eta^2 = 0.012$), age by sex ($F_{1,38} = 3.429$, $p = 0.072$, $\eta^2 = 0.083$), age by hemisphere ($F_{1,38} = 3.678$, $p = 0.063$, $\eta^2 =$

0.088), hemisphere by sex ($F_{1,38}$ = 0.501, p = 0.483, η^2= 0.013), foster mom by hemisphere by sex ($F_{1,38}$ = 0.009, p = 0.926, η^2< 0.001), foster mom by age by hemisphere ($F_{1,38}$ < 0.001, p = 0.989, η^2< 0.001), age by hemisphere by sex ($F_{1,38}$ = 0.015, p = 0.905, η^2< 0.001), or foster mom by age by hemisphere by sex ($F_{1,38}$ = 0.137, p = 0.714, η^2= 0.004).

PFC CSF

Main effects of age ($F_{1,38}$ = 96.333, p < 0.001, η^2= 0.717) and hemisphere ($F_{1,38}$ = 27.129, p < 0.001, η^2= 0.417) were found. Greater volumes were found at 6 months than at 12 months and in the right hemisphere than in the left hemisphere. Three way interaction effects were found with foster mom by hemisphere by sex ($F_{1,38}$ = 5.532, p = 0.024, η^2= 0.127; graphed in Figure 9) and age by hemisphere by sex ($F_{1,38}$ = 5.916, p = 0.02, η^2= 0.135). Figure 9 shows that the foster mom interaction effects seem to be driven by the lowest PFC CSF volumes shown by maltreated females at 6 months in comparison to the biggest volumes shown by control males. No main effects of foster mom ($F_{1,38}$ = 0.298, p = 0.588, η^2= 0.008) or sex ($F_{1,38}$ = 0.273, p = 0.605, η^2= 0.007) were found. Also, no interaction effects were found between foster mom by age ($F_{1,38}$ = 2.566, p = 0.117, η^2= 0.063), foster mom by sex ($F_{1,38}$ = 0.289, p = 0.594, η^2= 0.008), foster mom by hemisphere ($F_{1,38}$ = 1.647, p = 0.207, η^2= 0.042), age by sex ($F_{1,38}$ = 1.648, p = 0.207, η^2= 0.042), age by hemisphere ($F_{1,38}$ = 0.040, p = 0.842, η^2= 0.001), hemisphere by sex ($F_{1,38}$ = 0.212, p = 0.648, η^2= 0.006), foster mom by age by sex ($F_{1,38}$ = 0.007, p = 0.935, η^2< 0.001), foster mom by age by hemisphere ($F_{1,38}$ = 0.344, p = 0.561, η^2= 0.009), or foster mom by age by hemisphere by sex ($F_{1,38}$ = 0.965, p = 0.332, η^2= 0.025)).

Hippocampus

Because biological mother was found to have a significant effect on hippocampal volume, this factor was included as a covariate in the statistical models when analyzing the main

and interaction effects of foster mom on this ROI. These are the specific details of the statistical analysis of potential confounding effects of biological mom: (1) for the *uncorrected* hippocampal volume, there was a biological mom by age by hemisphere interaction effect ($F_{1,40} = 4.15$, $p = 0.04$); no main effect of biological mom ($F_{1,40} = 0.697$, $p = 0.409$) or biological mom by age ($F_{1,40} = 0.627$, $p = 0.433$); hemisphere by biological mom ($F_{1,40} = 0.007$, $p = 0.934$) were detected; (2) *ICV-corrected*: right-biological mom ($F_{1,40} = 0.32$, $p = 0.575$); biological mom by age interaction ($F_{1,40} = 0.868$, $p = 0.357$) ; left-biological mom ($F_{1,40} = 0.264$, $p = 0.61$); biological mom by age interaction ($F_{1,40} = 0.096$, $p = 0.758$).

Uncorrected volumes

Significant main effects were found for age ($F_{1,37} = 69.089$, $p < 0.001$, $\eta^2 = 0.651$; graphed in figure 10), sex ($F_{1,37} = 12.548$, $p = 0.001$, $\eta^2 = 0.253$) and hemisphere ($F_{1,37} = 7.473$, $p = 0.01$, $\eta^2 = 0.168$). Greater volumes were found in males than in females, at 12 months than at 6 months, and in the right hemisphere than in the left hemisphere. An interaction effect of age by hemisphere was also found ($F_{1,37} = 6.996$, $p = 0.012$, $\eta^2 = 0.159$). No main effect of foster mom ($F_{1,37} = 2.491$, $p = 0.123$, $\eta^2 = 0.063$) or foster mom by age ($F_{1,37} = 0.001$, $p = 0.981$, $\eta^2 < 0.001$), foster mom by sex ($F_{1,37} = 0.179$, $p = 0.675$, $\eta^2 = 0.005$), foster mom by hemisphere ($F_{1,37} = 3.147$, $p = 0.084$, $\eta^2 = 0.078$), age by sex ($F_{1,37} = 0.702$, $p = 0.407$, $\eta^2 = 0.019$), hemisphere by sex ($F_{1,37} = 0.004$, $p = 0.953$, $\eta^2 < 0.001$), hemisphere by foster mom by sex ($F_{1,37} = 0.478$, $p = 0.494$, $\eta^2 = 0.013$), foster mom by age by hemisphere ($F_{1,37} = 0.638$, $p = 0.429$, $\eta^2 = 0.017$), age by hemisphere by sex ($F_{1,37} = 0.113$, $p = 0.739$, $\eta^2 = 0.003$), or foster mom by age by hemisphere by sex ($F_{1,37} = 0.506$, $p = 0.481$, $\eta^2 = 0.013$) interaction effects.

ICV-corrected volumes

Main effects were discovered for age ($F_{1,37} = 75.512$, $p < 0.001$, $\eta^2 = 0.671$) and for hemisphere ($F_{1,37} = 7.981$, $p = 0.008$, $\eta^2 = 0.177$), with greater volumes at 12 months than at 6 months and in the right hemisphere than in the left. There was also an interaction effect between age by hemisphere ($F_{1,37} = 5.915$, $p = 0.02$, $\eta^2 = 0.138$). There were no main effects of foster mom ($F_{1,37} = 0.483$, $p = 0.491$, $\eta^2 = 0.013$) and sex ($F_{1,37} = 0.469$, $p = 0.498$, $\eta^2 = 0.013$) or foster mom by age ($F_{1,37} = 0.246$, $p = 0.623$, $\eta^2 = 0.007$), foster mom by sex ($F_{1,37} = 0.523$, $p = 0.474$, $\eta^2 = 0.014$), foster mom by hemisphere ($F_{1,37} = 3.850$, $p = 0.057$, $\eta^2 = 0.094$), age by sex ($F_{1,37} = 0.014$, $p = 0.907$, $\eta^2 < 0.001$), hemisphere by sex ($F_{1,37} = 0.199$, $p = 0.658$, $\eta^2 = 0.005$), foster mom by age by sex ($F_{1,37} = 0.658$, $p = 0.423$, $\eta^2 = 0.017$), foster mom by age by hemisphere ($F_{1,37} = 0.51$, $p = 0.48$, $\eta^2 = 0.014$), foster mom by hemisphere by sex ($F_{1,37} = 0.663$, $p = 0.421$, $\eta^2 = 0.018$), age by hemisphere by sex ($F_{1,37} = 0.076$, $p = 0.785$, $\eta^2 = 0.002$), or foster mom by age by hemisphere by sex ($F_{1,37} = 0.459$, $p = 0.502$, $\eta^2 = 0.012$) interaction effects.

Hair Cortisol Data

Prior to this particular study, the Sanchez lab analyzed hair cortisol concentrations of the subjects both two days after birth and at six months. Hair cortisol levels were not significantly different between control and maltreated infants at 2 days of age, ruling out potential effects of prenatal stress on our brain measures. However, at 6 months, the maltreated group did exhibit significantly higher cortisol accumulation compared with the control group (Sanchez et al., in prep).

Correlations Between Brain Structural Measures and Cortisol

There were no significant correlations between hair cortisol concentrations at 2 days or at 6 months and total ICV, total GM, total GM, or total CSF values at either 6 months or 12 months (Table 2).

For uncorrected volumes, significant positive correlations were found between the cortisol levels at two days of age and the GM volumes of the left PFC ($r = 0.307$, $p = 0.048$) and right PFC ($r = 0.316$, $p = 0.042$), both at 12 months of age (Table 3). No other correlations were found for uncorrected volumes.

For ICV-corrected volumes, a significant correlation was found between hair cortisol at 6 months and right amygdala volumes at 6 months ($r = 0.371$, $p = 0.022$). Significant correlations were also found between hair cortisol at 2 days and right ($r = 0.36$, $p = 0.019$) and left ($r = 0.365$, $p = 0.018$) PFC GM at 12 months (Table 4).

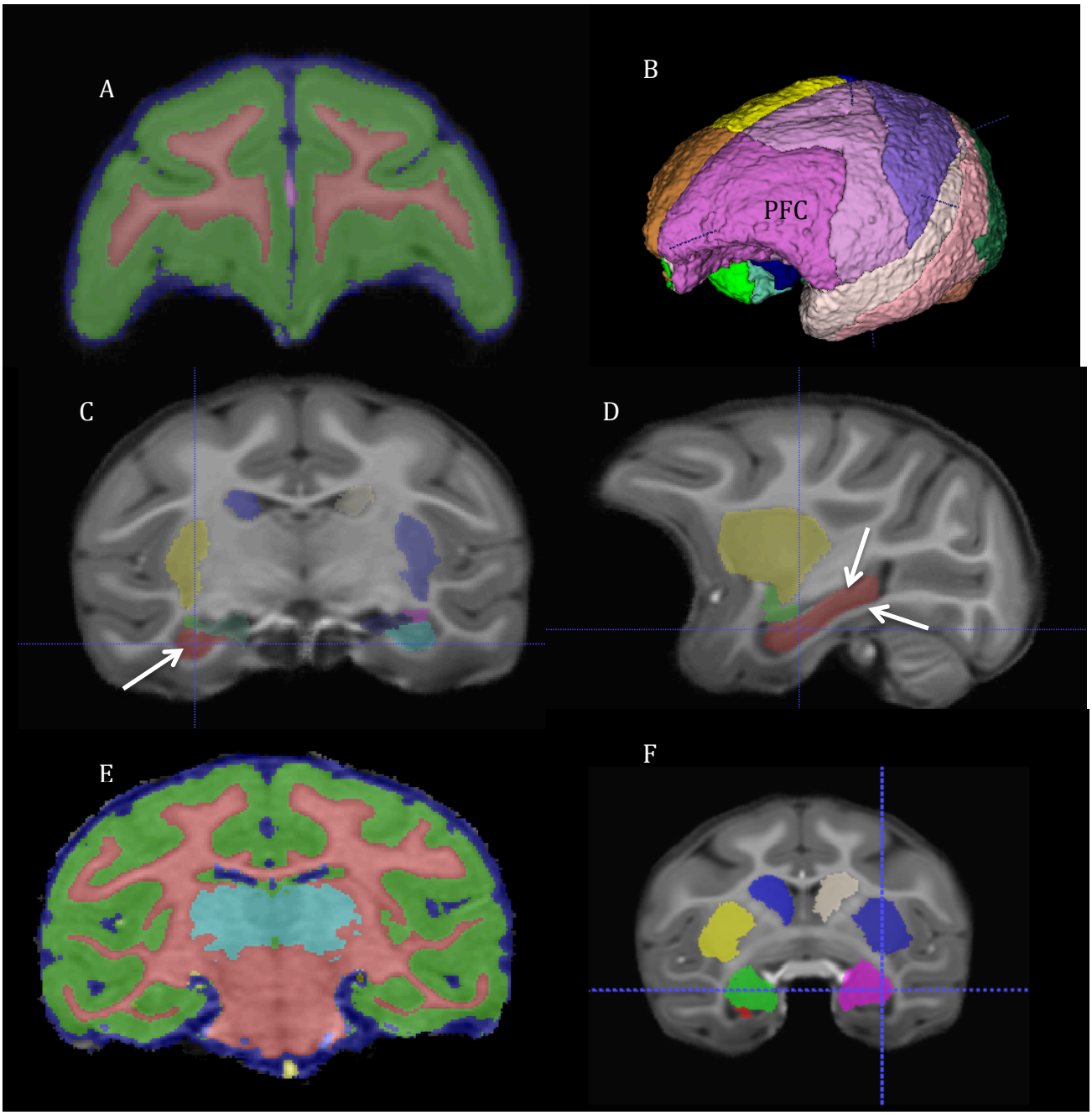

Figure 1. Representative images of the structural MRI infant/juvenile atlases, automatic tissue segmentation and parcellation processes using the AutoSeg software. (A): Prefrontal cortex (PFC) segmented into white matter (red), gray matter (green), and CSF (blue). (B): A 3-D image rendering of the brain, showing the cortical parcellations with the PFC segmented in hot pink and light brown. (C) and (D): The hippocampus is shown in red in both the coronal view (C) and the sagittal view (D). (E): Coronal view of the brain, showing the level of the temporal lobe that includes the hippocampus, segmented into white matter (red), gray matter (green), and CSF (blue). (F): Coronal view of the left (pink) and right (green) amygdala.

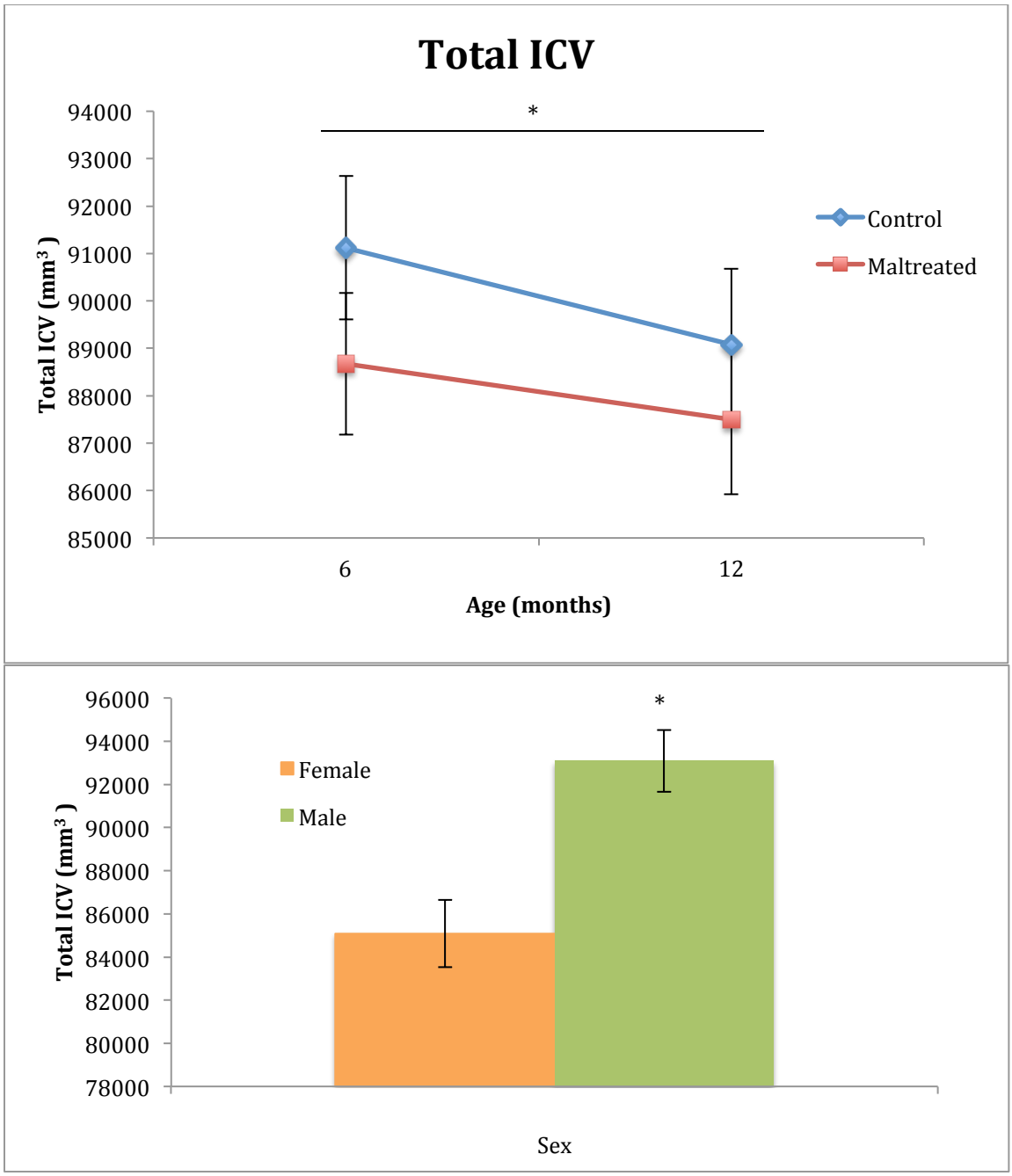

Figure 2. Analysis of total ICV. The top graph shows the main effect of age on ICV ($F_{1,38}$ = 9.218, p = 0.004 – represented by *, η^2= 0.195), with larger volumes at 6 than at 12 months; no main or interaction effects were detected for foster mom. The bottom graph depicts the main effect of sex on ICV ($F_{1,38}$ = 14.256, p = 0.001- represented by *, η^2 = 0.273), with bigger volumes in males than females.

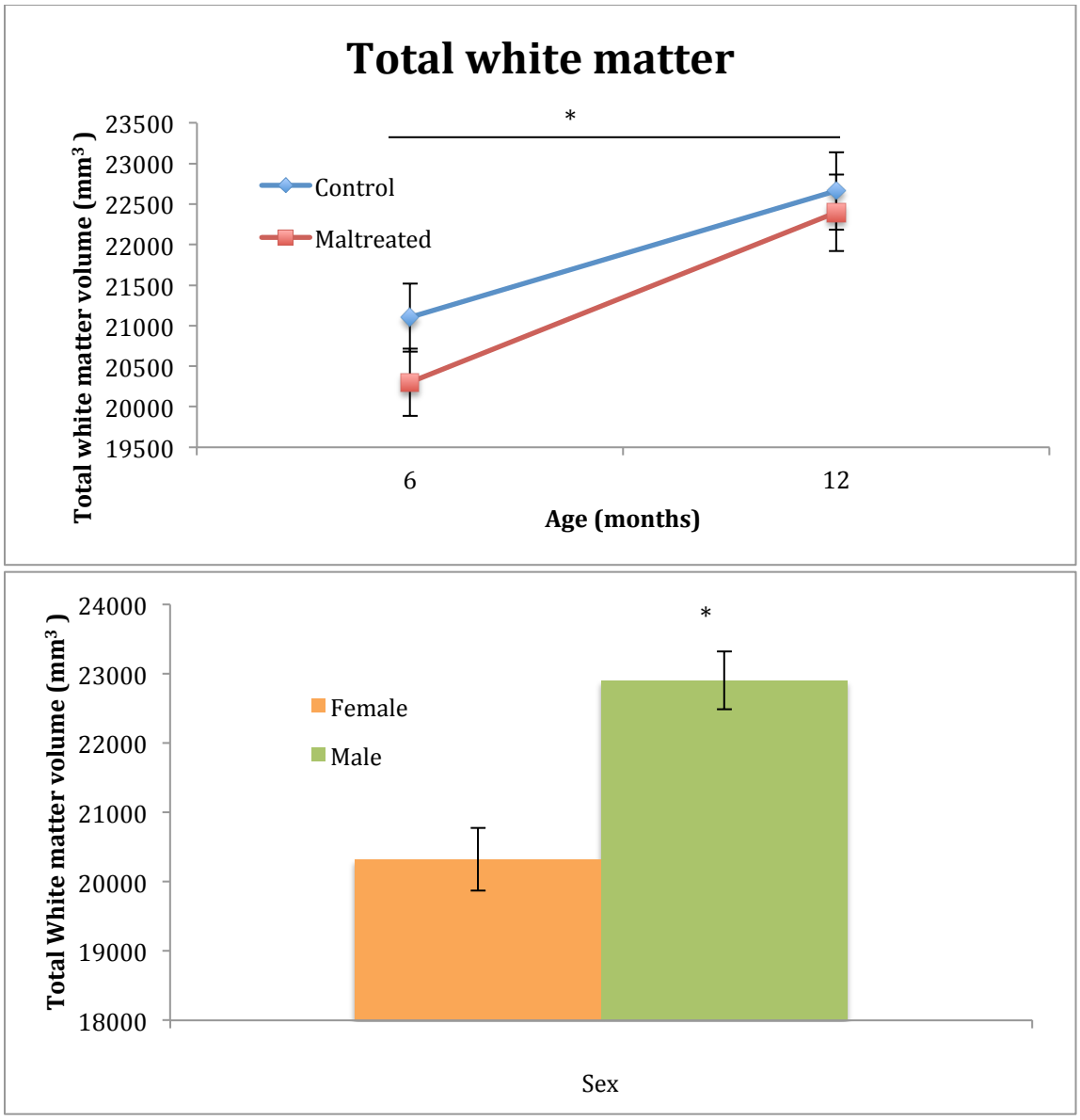

Figure 3. Analysis of total white matter volume. The top graph shows the main effect of age ($F_{1,38} = 178.295$, $p < 0.001$- represented by *, $\eta^2=0.824$) on white matter volume, with greater volumes at 12 than at 6 months; no main or interaction effects were found with foster mom. The bottom graph shows the main effect of sex on white matter volume ($F_{1,38} = 17.432$, $p < 0.001$, $\eta^2 = 0.314$), with larger volumes in males than in females.

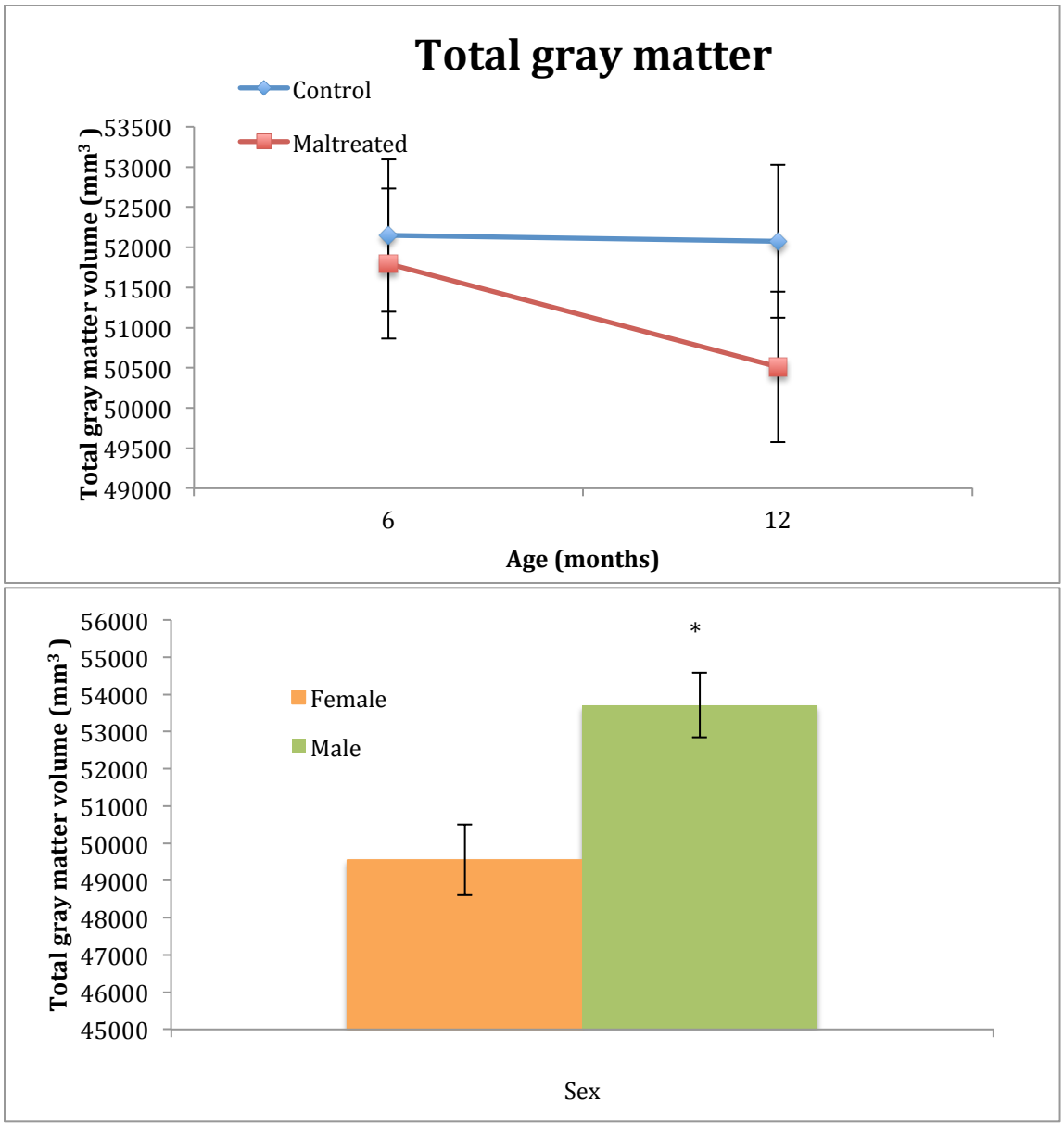

Figure 4. Analysis of total gray matter volume. There was no main effect of either foster mom or age, but there was a trend towards lower volumes at 12 months compared to 6 months ($F_{1,38}$ = 3.487, p = 0.07, η^2= 0.084). The bottom graph shows the main effect of sex on gray matter volume ($F_{1,38}$ = 10.498, p = 0.002- represented by *, η^2 = 0.216), which was bigger in males than in females.

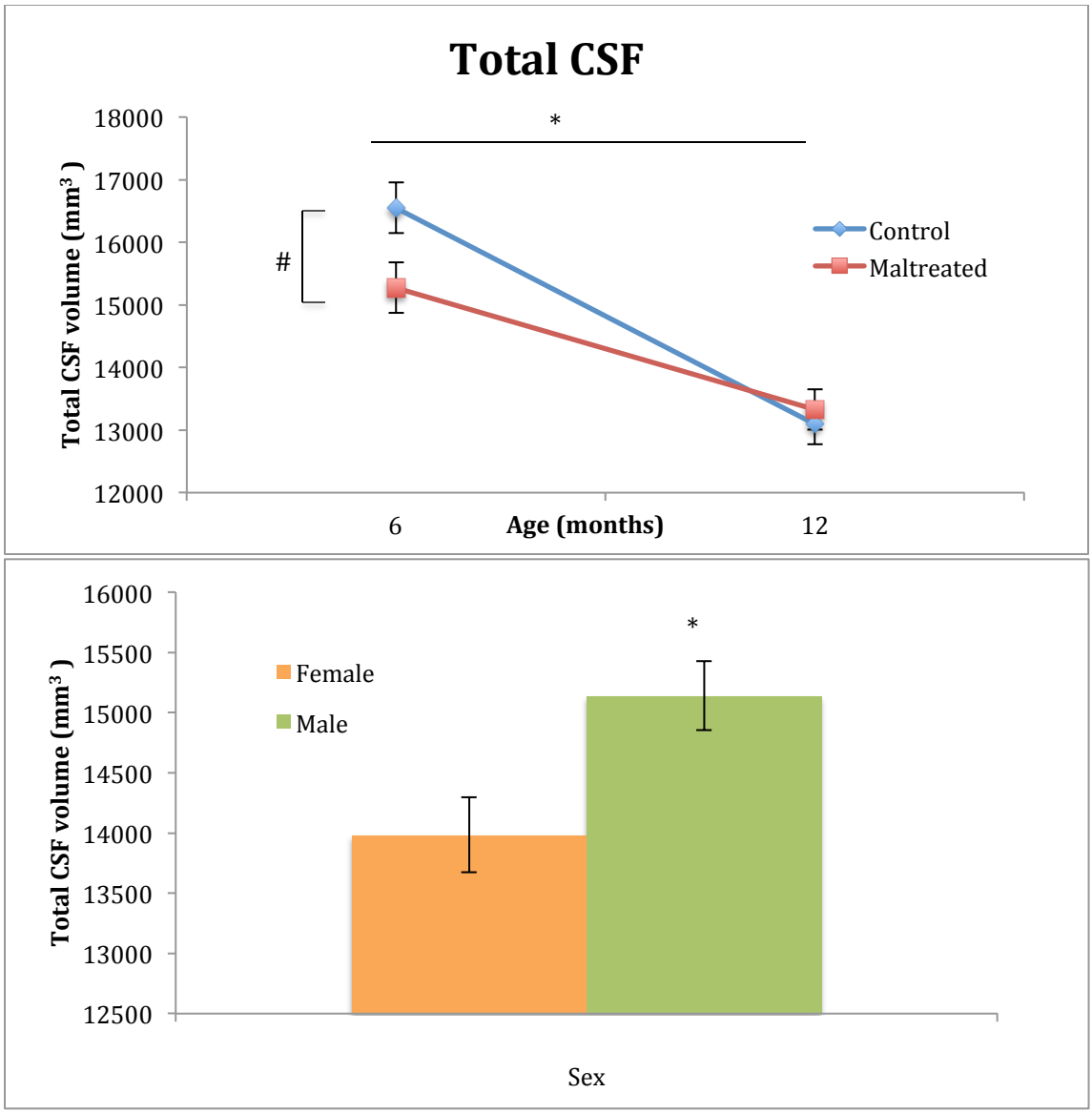

Figure 5. Analysis of total CSF volume. The top graph illustrates the main age effect ($F_{1,38}$ = 80.895, $p < 0.001$- represented by *, $\eta^2 = 0.68$). There was an interaction effect present between total CSF volume and foster mom by age ($F_{1,38} = 6.322$, $p = 0.016$- represented by #, $\eta^2 = 0.143$); although post-hoc analyses were not performed, visual inspection of the graph suggests that the effect was driven by smaller CSF volumes in maltreated subjects than in controls at the younger age. The bottom graph shows the main effect of sex on CSF volume ($F_{1,38} = 7.485$, $p = 0.009$- represented by *, $\eta^2 = 0.165$), which was bigger in males than in females.

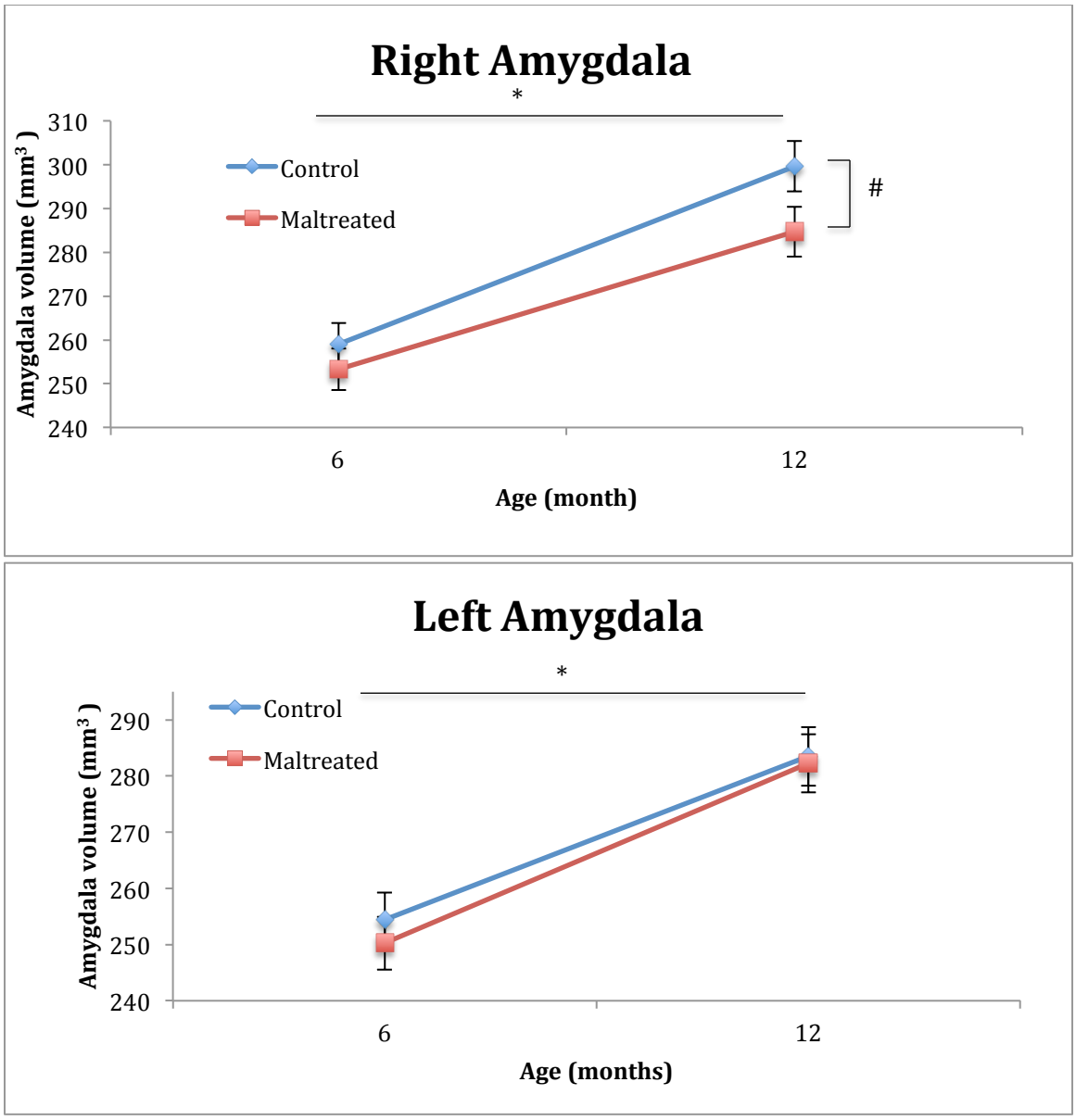

Figure 6. Analysis of uncorrected amygdalar volume. The right hemisphere data is shown on top, and left hemisphere on the bottom. Main effects of age ($F_{1,38}$ = 324.805, p <0.001- represented by *, η^2= 0.895) and hemisphere ($F_{1,38}$ = 22.142, p <0.001, η^2= 0.368) were found, with bigger volumes at 12 than 6 months and in the right than in the left hemisphere. A significant foster mom by age by hemisphere interaction effect is also shown ($F_{1,38}$ = 9.503, p = 0.004 – represented by #, η^2= 0.2), which seems to be driven by smaller amygdalar volumes in the right hemispheres of maltreated animals at the older age.

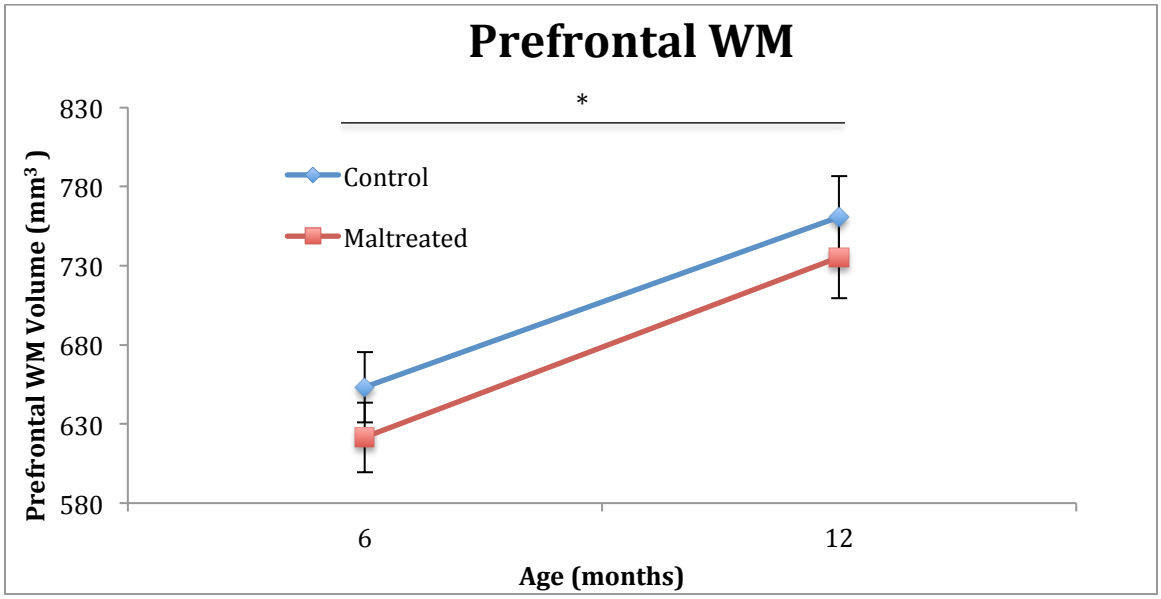

Figure 7. Analysis of uncorrected PFC white matter volume. Main effect of age on PFC WM ($F_{1,38} = 88.813$, $p < 0.001$ – represented by *, $\eta^2 = 0.7$) is shown, with PFC WM volume increasing from 6 to 12 months of age. No effects of foster mom or sex were detected. Since no interaction effect was found between foster mom and hemisphere, the hemispheres were combined for display in this figure.

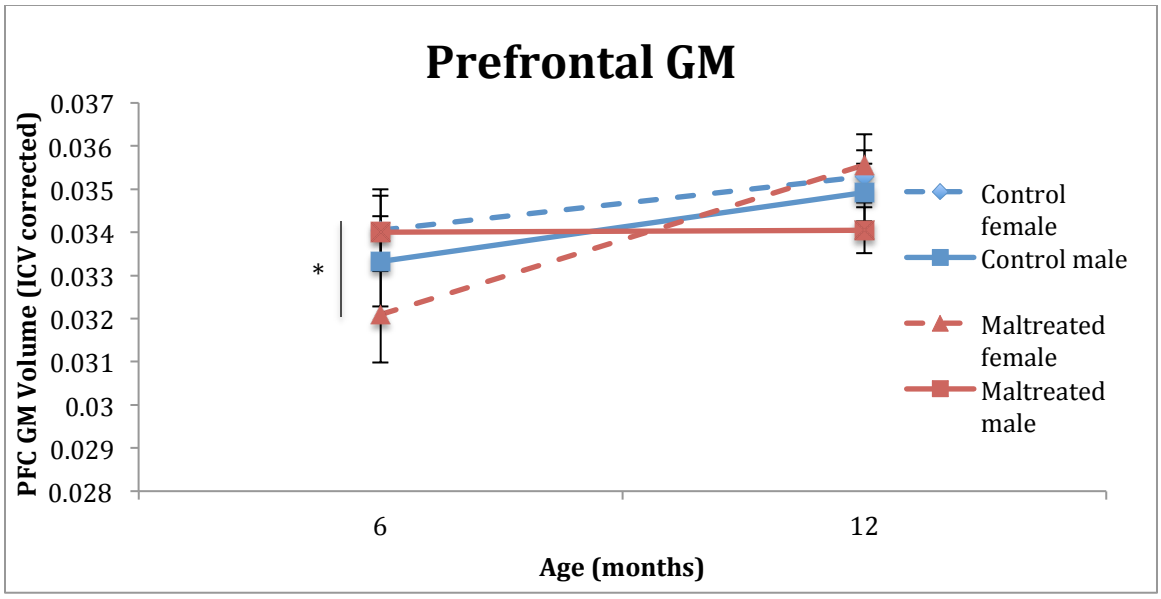

Figure 8. Analysis of ICV-corrected prefrontal GM volume. This graph shows the foster mom by age by sex interaction effect found ($F_{1,38} = 5.199$, $p = 0.028$ – represented by *, $\eta^2 = 0.12$), which seems to be driven by a lower volume in maltreated females at the younger age.

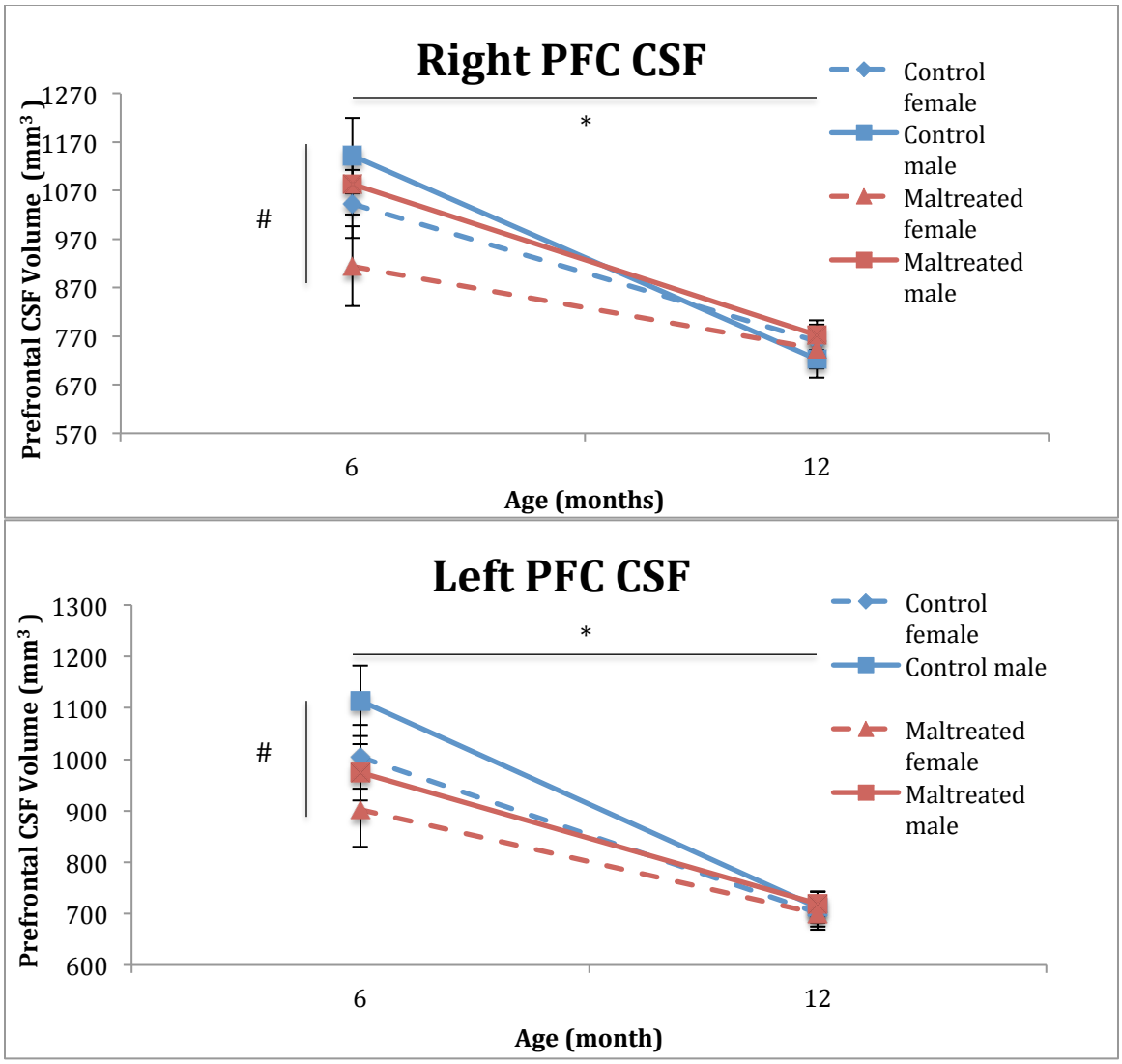

Figure 9. Analysis of uncorrected PFC CSF volume. Main effects of age ($F_{1,38} = 95.362$, $p < 0.001$ – represented by *, $\eta^2 = 0.715$) and hemisphere ($F_{1,38} = 27.755$, $p < 0.001$, $\eta^2 = 0.422$) were found, with higher volumes at 6 months than 12 months and in the right hemisphere than in the left. The significant interaction effect of foster mom by hemisphere by sex ($F_{1,38} = 5.869$, $p = 0.02$ – represented by #, $\eta^2 = 0.134$) is also depicted, which seems to be driven by the lowest volume in maltreated females and the highest volume in control males at the younger age.

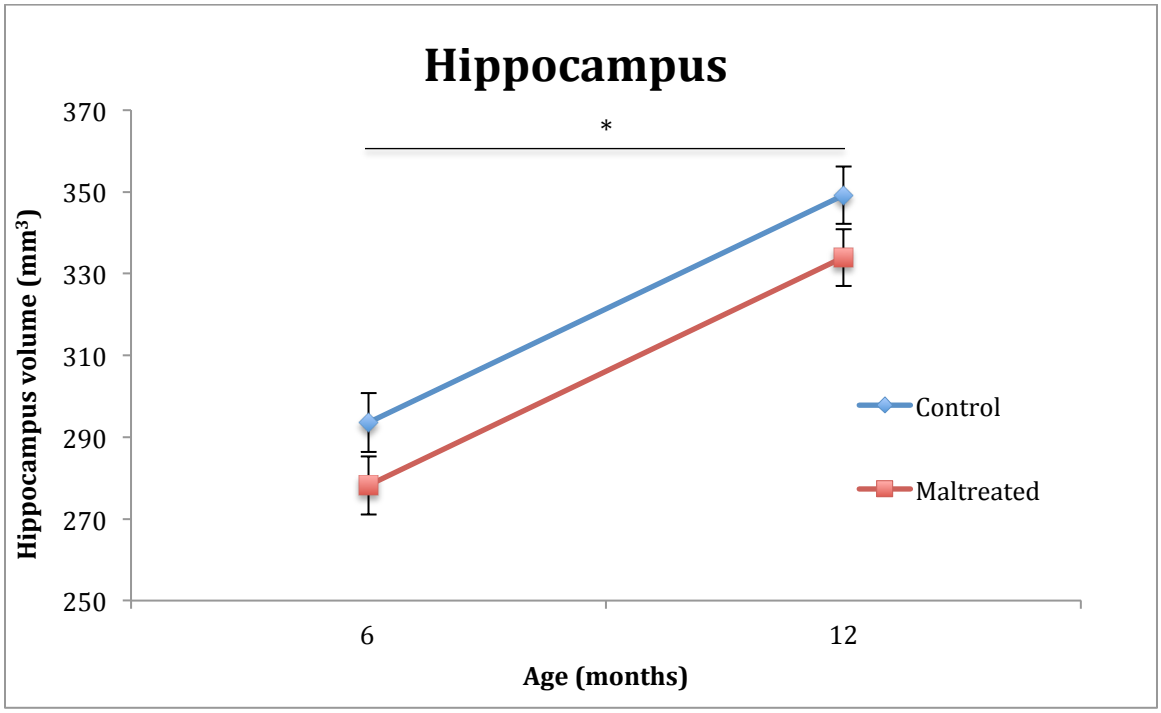

Figure 10. Analysis of uncorrected hippocampus volumes. The effect of age is depicted ($F_{1,37}$ = 69.089, p <0.001 – represented by *, η^2 = 0.651), with greater volumes at 12 than at 6 months. No significant effect of foster mom was found. Although a main effect of hemisphere ($F_{1,37}$ = 7.473, p = 0.01, η^2 = 0.168) was detected, since no foster mom by hemisphere interactions were found, the hemispheres were combined for display in this figure.

Table 1: Information about Study Subjects

Foster mom group	Biological mom group	Code Name	Gender	Cohort	Location (social group)	Totals
Control n = 20	Maltreating (i.e. animals born to a maltreating female crossfostered to a control foster mom	Bf13	Female	2009	A2	6 female 4 male
		Hm13	Male	2009	BC2A	
		Me14	Male	2010	C2	
		Nc14	Female	2010	A2	
		On14	Female	2011	A1	
		Ph15	Male	2012	C2	
		Sa15	Female	2012	T3	
		Sb14	Male	2010	A1	
		Th13	Female	2009	C2	
		Uf14	Female	2010	A1	
	Control (i.e. animals born to a control female crossfostered to a control mom)	Bo14	Male	2011	C2	5 female 5 male
		Fo14	Male	2011	A1	
		Je13	Female	2009	C2	
		Ne14	Male	2010	A2	
		Nt14	Male	2011	C2	
		St14	Female	2011	A1	
		Uu14	Female	2011	A1	
		Wm14	Female	2011	C2	
		Ym14	Male	2011	A1	
		Zu14	Female	2011	C2	
Maltreated n = 22	Control (i.e. animals born to a control female crossfostered to a maltreating mom)	Ch13	Female	2009	A2	3 female 9 male
		Df13	Male	2009	C2	
		Df15	Male	2012	C2	
		Fv13	Male	2010	C2	
		Im13	Female	2009	A2	
		Lc14	Male	2010	C2	
		Nn14	Male	2011	A2	
		Py13	Male	2010	C2	
		Rm13	Male	2009	A2	
		Ta15	Male	2012	S6	
		Ub14	Female	2010	A2	
		Vf14	Male	2010	A2	
	Maltreating (i.e. animals born to a maltreating female crossfostered to a maltreating mom)	Ct15	Female	2013	A3	5 female 5 male
		Dw13	Male	2010	A1	
		Ew13	Male	2010	C2	
		Kr14	Male	2011	A1	
		Pv13	Male	2010	A1	
		Rv13	Female	2010	C2	
		Ul13	Female	2009	A1	
		Ws14	Female	2011	A2	
		Ws15	Female	2013	A1	
		Yl13	Male	2009	C2	

Table 2: Pearson's Correlation Between Total Brain Volumes and Hair Cortisol Levels

	Hair cortisol at 2 days (pg/mg hair)	Hair cortisol at 6 months (pg/mg hair)
Total ICV 6 months	r = 0.17 p = 0.281	r = -0.088 p = 0.581
Total WM 6 months	r = 0.094 p = 0.555	r = -0.105 p = 0.508
Total GM 6 months	r = 0.237 p = 0.132	r = -0.066 p = 0.677
Total CSF 6 months	r = 0.008 p = 0.96	r = -0.062 p = 0.696
Total ICV 12 months	r = 0.117 p = 0.461	r = -0.069 p = 0.666
Total WM 12 months	r = 0.061 p = 0.703	r = -0.059 p = 0.71
Total GM 12 months	r = 0.216 p = 0.17	r = -0.029 p = 0.857
Total CSF 12 months	r = -0.154 p = 0.33	r = -0.173 p = 0.274

Table 3: Pearson's Correlation Between Uncorrected Volumes and Hair Cortisol Levels

	Hair cortisol at 2 days (pg/mg hair)	Hair cortisol at 6 months (pg/mg hair)
Hemispheres separate		
Right amygdala 6 months	r = 0.219 p = 0.163	r = 0.063 p = 0.708
Left amygdala 6 months	r = 0.191 p = 0.226	r = 0.006 p = 0.971
Right PFC WM 6 months	r = 0.236 p = 0.132	r = -0.06 p = 0.72
Left PFC WM 6 months	r = 0.167 p = 0.289	r = -0.029 p = 0.861
Right PFC GM 6 months	r = 0.256 p = 0.102	r = -0.04 p = 0.81
Left PFC GM 6 months	r = 0.273 p = 0.08	r = -0.021 p = 0.899
Right PFC CSF 6 months	r = 0.056 p = 0.724	r = -0.033 p = 0.845
Left PFC CSF 6 months	r = 0.054 p = 0.735	r = -0.055 p = 0.745
Right hippocampus 6 months	r = 0.194 p = 0.218	r = 0.075 p = 0.656
Left hippocampus 6 months	r = 0.249 p = 0.112	r = 0.063 p = 0.706
Right amygdala 12 months	r = 0.164 p = 0.299	r = 0.166 p = 0.319
Left amygdala 12 months	r = 0.118 p = 0.457	r = 0.138 p = 0.408
Right PFC WM 12 months	r = 0.094 p = 0.554	r = -0.096 p = 0.565
Left PFC WM 12 months	r = 0.078 p = 0.622	r = -0.046 p = 0.785
Right PFC GM 12 months	r = 0.316* p = 0.042	r = -0.023 p = 0.889
Left PFC GM 12 months	r = 0.307* p = 0.048	r = 0.023 p = 0.89
Right PFC CSF 12 months	r = -0.079 p = 0.62	r = 0.067 p = 0.689
Left PFC CSF 12 months	r = -0.14 p = 0.375	r = -0.105 p = 0.529
Right hippocampus 12 months	r = 0.186 p = 0.238	r = 0.164 p = 0.325
Left hippocampus 12 months	r = 0.207 p = 0.189	r = 0.147 p = 0.379
Hemispheres combined		
PFC WM 6 months	r = 0.205 p = 0.193	r = -0.046 p = 0.785
PFC WM 12 months	r = 0.087 p = 0.583	r = -0.073 p = 0.663

* denotes significance at $p < 0.05$ level

Table 4: Pearson's Correlation Between ICV-Corrected Volumes and Hair Cortisol Levels

	Hair cortisol at 2 days (pg/mg hair)	Hair cortisol at 6 months (pg/mg hair)
Hemispheres separate		
Right amygdala 6 months	r = 0.115 p = 0.469	r = 0.371* p = 0.022
Left amygdala 6 months	r = 0.086 p = 0.589	r = 0.263 p = 0.111
Right PFC WM 6 months	r = 0.215 p = 0.172	r = 0.077 p = 0.646
Left PFC WM 6 months	r = 0.117 p = 0.462	r = 0.124 p = 0.457
Right PFC GM 6 months	r = 0.222 p = 0.157	r = 0.144 p = 0.389
Left PFC GM 6 months	r = 0.245 p = 0.118	r = 0.172 p =0.302
Right PFC CSF 6 months	r = 0.007 p =0.964	r = 0.055 p = 0.744
Left PFC CSF 6 months	r = 0.006 p =0.968	r = 0.031 p = 0.856
Right hippocampus 6 months	r = 0.121 p = 0.445	r = 0.286 p = 0.082
Left hippocampus 6 months	r = 0.178 p = 0.26	r = 0.242 p =0.143
Right amygdala 12 months	r = 0.069 p = 0.666	r = 0.283 p = 0.086
Left amygdala 12 months	r = 0.017 p =0.917	r = 0.252 p = 0.127
Right PFC WM 12 months	r = -0.189 p = 0.23	r = 0.008 p = 0.963
Left PFC WM 12 months	r = 0.001 p = 0.993	r = 0.003 p = 0.986
Right PFC GM 12 months	r = 0.36* p = 0.019	r = 0.086 p = 0.608
Left PFC GM 12 months	r = 0.365* p = 0.018	r = 0.177 p = 0.288
Right PFC CSF 12 months	r = -0.134 p = 0.398	r = 0.124 p = 0.46
Left PFC CSF 12 months	r = -0.261 p = 0.095	r = -0.051 p = 0.763
Right hippocampus 12 months	r = 0.116 p =0.464	r = 0.262 p =0.113
Left hippocampus 12 months	r = 0.131 p = 0.41	r = 0.233 p = 0.158
Hemispheres together		
PFC WM 6 months	r = 0.148 p = 0.348	r = 0.077 p = 0.645
PFC WM 12 months	r = 0.017 p = 0.913	r = -0.043 p = 0.8

* denotes significance at $p < 0.05$ level

Discussion

The aim of this study was to examine the effects of early maternal care on the structural development of brain, focusing primarily on the PFC, amygdala, and hippocampus in primates because of their critical role on the regulation of emotional and stress responses. For this, I studied the impact of adverse caregiving in the form of maltreatment on the structural changes of these brain regions in rhesus monkey infants from 6 to 12 months of age (equivalent to 2-4 years of age in humans) using structural MRI approaches. Because maltreatment is a stressful experience for the infants and results in increased release of the stress hormone cortisol (measured as increased hair cortisol accumulation during the first 6 months of life), I also examined the correlation between early cortisol levels and brain structural effects. I found decreases in amygdalar, PFC GM, and PFC CSF volumes in response to maltreatment, as well as positive correlations between amygdala and PFC GM and early cortisol levels. However, no such effects were found for the hippocampus. These results suggest that maltreatment-induced neurodevelopmental changes are indeed emerging during infancy and the juvenile period in these cortico-limbic structures critical for emotional and stress regulation, but the presence, directionality, and magnitude of the changes might vary with age, as previously suggested for humans (Tottenham & Sheridan, 2010).

In looking at the total brain of the subjects, we found a significant effect of age on ICV with lower volumes at 12 months than at 6 months. This is not consistent with previous reports in rhesus macaques, with the only two longitudinal structural MRI studies we are aware of showing significant increases in ICV across the first year of life, although particularly rapid during the first 4 months of life (Liu et al., 2015; Malkova et al., 2006). One of the important differences between our study and those two studies in infant macaques in ours is that we

included the subarachnoid CSF volume in the total measurements of CSF, for addition to the total WM and total GM volumes in the computation of ICV, while the other two studies only included WM, GM, and ventricular CSF. The ICV decrease seems to be driven by a reduction in total CSF volume in our study, which was the only tissue class exhibiting significant decreases with age, possibly compensating for the increase in WM. Given that we see a significant CSF decrease with age, this change may be the one driving the differences across studies. Interestingly, in humans, ICV (i.e. WM + GM + CSF, not total volume inside cranium) is reported to follow an inverted U shaped growth curve, with volumes increasing throughout childhood and peaking at age 10.5 in females and 14.5 in males and then decreasing thereafter (Giedd & Rapoport, 2010). It is possible that rhesus macaque ICV's follow a similar pattern, but with the peak and decrease occurring at an earlier (juvenile) age.

A critical difference in the experimental design between our study and those of Malkova et al. (2006) and Liu et al. (2015) is the way in which the animals were housed. In the Malkova et al. study, the subjects resided in a nursery until they were 2 months old and were caged individually with fairly limited contact with other animals until they were a year old, when they were moved to permanent social groups. In the Liu et al. study, the subjects lived in cages with their mothers until they were 6 months old and were then moved to new cages. In contrast, the subjects in our experiment resided in complex social groups from birth. The differences in rearing environments of the subjects can change the developmental trajectory of the brain. It is possible that being raised in social groups resulted in accelerated brain development and therefore an earlier peak in ICV compared to animals that were not raised in complex social groups. A more longitudinal study of the subjects from early infancy through adolescence would be needed to confirm this.

Our total brain WM data did show significant increases with age, which fits with previous studies reporting intense myelination throughout infant development, which continues at slower rates throughout childhood and adolescence in macaques and in humans (Giedd & Rapoport, 2010; Malkova et al., 2006; Knickmeyer et al., 2010). Also consistent with previous studies (Giedd & Rapoport, 2010; Gilmore et al., 2012; Liu et al., 2015), total GM volume showed a trend to decrease with age, though this change did not reach statistical significance. For example, Gilmore and colleagues (2012) described significant increases in GM volume during the first year of life in humans (equivalent to the first 3 months in rhesus), but a much smaller increase in GM volume the second year of life (Gilmore et al., 2012). A study done with older human children showed an inverted U shaped pattern of development of GM volume, with volumes peaking during childhood (at approximately 8 years in girls and 11 years in boys) and declining thereafter (Giedd & Rapoport, 2010). A study in rhesus macaques showed that the decline in GM volume occurred even earlier, during late infancy/early juvenile period, in rhesus macaques (Liu et al., 2015), consistent with our findings.

There were also significant effects of sex on ICV and total brain WM, GM, and CSF volumes. The overall higher brain volume in males than in females for all of these global volumetric measures is in line with previous reports in developing macaques (Franklin et al., 2000; Malkova et al., 2006) and humans (Giedd & Rapoport, 2010).

A study of normal brain development in macaques by Payne and colleagues in 2010 showed increasing amygdalar volumes throughout the first two years of life, with the most rapid rate of increase happening within the two weeks following birth. Males, but not females were found to have significant lateralization effects, with greater right amygdalae than left amygdalae (Payne et al., 2010). Studies of the human amygdala have also shown increasing volumes in

childhood, with the greatest rate of increase happening soon after birth, just like in rhesus monkeys. This development of human amygdalae has been shown to exhibit sexual dimorphism, with male amygdalae growing until adulthood but female amygdalae reaching its full size at 4 years of age, but these results are inconclusive. Human amygdalae have been shown to be more active during childhood and adolescence, when its functionality is still being fine-tuned, than during adulthood (Tottenham & Sheridan, 2010).

The quality of maternal care had a significant effect on amygdala development, although volume differences were only detected at the later age and in the right hemisphere. Infants that experienced maltreatment had smaller right amygdala volumes at 12 months than animals reared by competent mothers. Lateralization effects of early adverse experiences, including maltreatment (abuse, neglect) and poverty, on amygdala development, have also been demonstrated in previous research in children. For example, a study by Hanson and colleagues in 2015 showed smaller left amygdalae volumes in children exposed to early life stress, but did not report such findings for the right amygdala (Hanson et al., 2015). This result also supports the differences in growth patterns between hemispheres aforementioned in the Payne study (Payne et al., 2010). That the maltreated subjects exhibited lower amygdala volumes contradicts some previous reports, suggesting that early life stress in the form of adverse caregiving results in faster amygdala development and therefore increased size of the amygdala (Tottenham & Sheridan, 2010). However, the literature concerning the developmental effects of early stressful experiences on amygdala volume is not conclusive. Some studies have reported either no effects of those early adverse experiences on amygdala volume (Cohen et al., 2006; Howell et al., 2014) or smaller volumes in individuals of various ages as a result of early life stress (Hanson et al., 2015) or increased volumes (Tottenham et al., 2010). An interesting explanation for these

inconsistent findings in literature has been proposed, suggesting that the amygdala might actually undergo an initial increase in volume and activity level shortly after a stressful experience, but over time, this increased activity might result in high rates of amygdala cell death and structural damage (e.g. reduction in dendritic field, synapses), and therefore, reduced subsequent volume (Hanson et al., 2015; McEwen, 2005; Tottenham & Sheridan, 2010).

Some of the known biological mechanisms underlying stress-induced cellular damage include stress-induced excitotoxic damage and other effects of stress hormones on cell metabolism and structure. For example, higher levels of glucocorticoid release resulting from stress can lead to increased levels of glutamate release. At extremely high levels, this can result in damage to PFC and hippocampal dendrites while causing amygdalar dendrites to grow (Popoli et al., 2011). Additionally, while the amygdala is known to be sensitive to stress early in life, it is also possible that these volumetric changes are not detectable until later in childhood compared to very early in life (Howell et al., 2014). A combination of these factors might explain the directionality (i.e. smaller amygdala volumes in maltreated than in control subjects) and the timing (i.e. significant effects were not detected until 12 months of age) of the results.

The positive correlation found between corrected right amygdala volumes and cortisol accumulated in hair from birth until the same age would support this view that initial stress leads to increased amygdala volume and activity, followed by a shrinkage at later ages, as the maltreated infants showed significantly higher hair cortisol accumulation throughout the first 6 months of life, but not at birth, consistent with chronic stress-induced HPA axis activation (Sanchez, in preparation). This positive correlation is consistent with studies showing increased HPA axis activity simultaneous with increased amygdala activity (Tottenham & Sheridan, 2010).

Ongoing studies at earlier ages (2 weeks, 3 months) will provide a more complete picture of the developmental trajectories of the amygdala in both control and maltreated animals.

Normal development of the rhesus PFC involves an inverted U growth pattern, with both WM and GM volumes increasing until adolescence and decreasing thereafter (Knickmeyer et al., 2010). In the human PFC, GM also shows an inverted U growth pattern and is one of the last brain regions to experience its volumetric peak during adolescence. In contrast, WM levels continue to increase throughout childhood and adolescence (Giedd & Rapoport, 2010).

Effects of maternal care (i.e. foster mom) were also found on PFC GM through an interaction effect with age and sex, which seems to be driven by lower PFC GM volumes in maltreated females at 6 months. The smaller PFC GM volumes as a result of early adverse experience is consistent with previous reports that stress exposure results in reduced PFC GM volumes and decreased cognitive functions as a result (Arnsten, 2009; J. L. Hanson et al., 2012). Effects of the foster mom on PFC CSF was also found through an interaction effect between hemisphere and sex, showing increased left hemisphere volumes in control males and decreased right hemisphere volumes in maltreated females, both at 6 months. Lateralization effects of early life stress have been found in another study, with significant effects of the left frontal pole and certain parts of the right and left occipital lobe (Hanson et al., 2012). No effect of maternal care was found in the analysis of PFC WM. However, the PFC is known to develop well past early childhood (Hanson et al., 2012), so it is possible that the effects of stress on WM are not present or detectable until later on. The fact that the effects of maltreatment on PFC GM and CSF are present at 6 months but not at 12 months also support the possibility that the effects of the early life stress might be mitigated over time, since in this animal model of infant maltreatment, physical abuse is exhibited by the mother only during the first three months postpartum, and the

high rates of infant rejection during the first six months (McCormack et al., 2006; Sanchez, 2006) This is especially plausible considering the that the earlier ages constitute critical periods of environmental impacts due to intense developmental processes and plasticity of the primate infant brain (Hanson et al., 2012; Lupien et al., 2009). Ongoing longitudinal studies at later ages will be able to further investigate these hypotheses.

Unlike the association detected between exposure to elevated cortisol level during the first 6 months of life and amygdalar volume, we did not find correlations with PFC volumes (WM, GM, or CSF), suggesting that the mechanisms underlying the maltreatment effects on PFC are of a different nature. Instead, a significant positive correlation was found between hair cortisol concentrations at birth and left and right PFC GM at 12 months, which supports programming effects of prenatal glucocorticoids on brain development proposed in the literature, which states that cortisol from the mother can cross the placenta and activate the infant's HPA axis (Lupien et al., 2009).

This particularly study did not find any effects of maltreatment by the foster moms on hippocampus volume, nor correlations between hippocampal volume and hair cortisol levels. While this contradicts our hypothesis that it will be decreased, at least based on some findings from previous studies (Hanson et al., 2015; Tottenham & Sheridan, 2010), the literature in developing primates is inconsistent with lack of effects commonly reported. The lack of effects can be explained by the age at measurement and the overall developmental pattern of the hippocampus. The hippocampus exhibits slow development up until 2 years of age in humans (Gogtay et al., 2006; Tottenham et al., 2010; Knickmeyer et al., 2008) , which corresponds to 6 months of age in rhesus macaques, and its development continues well into adulthood. Normal hippocampal development has been shown to increase throughout the first two years of life with

the greatest growth rates happening within the first two weeks of life. Hemisphere effects, but not sex effects, have been shown in these analyses (Payne et al., 2010). Our study also found significantly higher hippocampal volumes at the older age and lateralization effect, but we found greater hippocampal volumes in the right rather than the left hemispheres, both uncorrected and corrected for ICV. We did find larger hippocampal volumes in males than in females, but this significance disappeared when the data were corrected for ICV.

While hippocampal volume decreases resulting from stress have been commonly observed in adults, some other studies in children have reported no significant effects of early life stress or adversity on hippocampal volume during development (De Bellis, 2001; Tottenham & Sheridan, 2010; Woon & Hedges, 2008). It has been proposed that stress may impact the amygdala earlier than it affects the hippocampus so that, while effects of stress on the amygdala are observed during childhood, effects on the hippocampus might remain virtually absent or undetectable until later in life, after adolescence (Tottenham & Sheridan, 2010). An alternative explanation is that hippocampal volumes simply might not change as much as amygdala volumes in response to early life stress (e.g. specific effects due to impact on neurogenesis processes; hippocampus is a much bigger structure than the amygdala) or the MRI techniques used to successfully detect significant changes in amygdala volume might not be sensitive enough to detect the changes in hippocampal volume (Tottenham & Sheridan, 2010). Therefore, at the relatively young ages of the subjects studied here, hippocampal volume changes and corresponding cortisol level correlations (Lupien et al., 2009) might not be as apparent as they would be at later ages.

A limitation of this study lies in the fact that results have only been analyzed over a short period of development (i.e. 6 and 12 months). We are currently working on analyzing additional

longitudinal data from the subjects at 2 weeks, 3 months, 18 months, and adolescence (5 years) to generate more complete analyses of both normative rhesus brain developmental trajectories since birth and the impact of infant maltreatment.

In summary, our findings show that maternal care affects primate structural brain development. In particular, using a powerful cross-fostering experimental design with random assignment of infants to control or maltreating mothers, animals that experienced infant maltreatment showed significant differences in amygdalar and PFC GM and CSF volumes during the infant-juvenile transition period, in comparison to animals reared by competent mothers; the differences in amygdalar volumes seemed to emerge at 12 months, while the effects on PFC GM and CSF were detected earlier (at 6 months) but seemed to disappear by 12 months. This could suggest that the most persistent structural effects are those observed in the amygdala. Significant correlations were also found between the amygdala and PFC volumes and levels of the stress hormone cortisol during infancy and pregnancy, respectively. Neither of those significant effects was found on the hippocampus. We studied animals at ages equivalent to toddlerhood and early childhood in humans, and it is known that effects of stress have noticeable impacts on the amygdala and the PFC before such effects are present or found in the hippocampus (Arnsten, 2009; Tottenham & Sheridan, 2010). Therefore, our findings are consistent with human developmental studies and support the view concerning the importance of the age at which the volumetric effects of stress are measured. We have shown that early life stress and adversity do indeed cause structural neural changes, but additional longitudinal studies would be needed to better understand the implications of the findings.

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