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Kaela Kuitchoua April 10, 2018

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

by

Kaela Kuitchoua

Mar Sanchez, Ph.D.
Adviser

Department of Neuroscience and Behavioral Biology

Mar Sanchez, Ph.D.

Adviser

Daniel Dilks, Ph.D.

Committee Member

Michael Treadway, Ph.D.

Committee Member

Kim Wallen, Ph.D.

Committee Member

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Kaela Kuitchoua

Dr. Mar Sanchez

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Abstract

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Childhood maltreatment is a major public health concern because it can lead to psychiatric illnesses and disorders, as well as neurobiological alterations. Childhood maltreatment is a phenomenon prevalent in both human and nonhuman primates. Due to the eedifficulty of studying childhood maltreatment in humans, this study used a rhesus monkey model to examine the developmental effects of child maltreatment on brain structure. Rhesus macaques in particular are an ideal model because of their similarities to humans in that they are highly social complex primates, and mother-infant bonds are critical for their development, making them a translational model for human infant development. The goal of this study was to further our knowledge on the effects of infant maltreatment on brain development by focusing on the impact on structural development of the hippocampus, amygdala, and prefrontal cortex (PFC), due to their critical role in stress and emotion regulation and their vulnerability to early life stress (ELS). In addition, this study examined whether exposure to elevated prenatal or postnatal cortisol levels, as well as rates of abuse and rejection experienced during infancy predicted structural changes in those cortico-limbic regions. This study used a powerful cross-fostering design with a random assignment of infants to either maltreating or competent mother at birth. The purpose of the design was to minimize the potential of confounding effects of heritability. Structural MRI techniques were used at 3, 6 and 12 months to examine the developmental effects of maltreatment from infancy to early juvenile period. This study found reduced total brain, PFC grey matter and hippocampal volumes in the maltreated group in

comparison to controls, but the differences emerged with increasing age. No effects of maternal care were found on the amygdala. These findings suggest that the ELS experience had a negative impact on neurodevelopment, but that the effects are region-specific and emerge with age.

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Introduction

Early life adversity/stress, including child maltreatment and social, emotional and sensory deprivation experienced by children reared in orphanages, is a well-documented risk factor for developmental psychopathology (Ehlert, 2013; Neigh et al., 2009; M. Teicher, H. et al., 2006). In particular, child maltreatment is a form of early life stress (ELS) linked to increased anxiety, depression, PTSD, substance abuse, cognitive and social deficits, aggression and criminal behavior leading to incarceration, as well as ADHD (Heim & Nemeroff, 2001; Bremner & Vermetten, 2001; Glaser, 2000; Gunnar & Vazquez, 2006: P. Cohen et al., 2001; Manly et al., 2001). Furthermore, the earlier the onset of child maltreatment, the more severe the negative outcomes, including anxiety and depression (Kaplow & Widom, 2007) and it is more prevalent in sections of the population exposed to poverty and in minorities (Maguire-Jack et al., 2015).

Although many studies have shown the link between ELS and psychopathology, the biological mechanisms are still not fully understood. One potential mechanism is that these early adverse experiences induce stress responses in the infant, which include the activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to increase release of cortisol, a highly catabolic stress hormone with broad effects on tissue gene expression, including in the brain, through binding to glucocorticoid and mineralocorticoid receptors –GR, MR- (Mar M. Sanchez, 2006). Thus, exposure to chronically high cortisol levels in early life due to repeated activations of the HPA axis could alter the development of brain circuits involved in emotional and stress regulation, such as the amygdala, hippocampus and prefrontal cortex (Mar M. Sanchez, 2006; Howell et al., 2013; Howell et al., 2016) and it is thought to be one of the biological mediators of increased risk for developmental psychopathology associated with early adversity (Doom & Gunnar, 2013). Stress activates the HPA axis by stimulating release of corticotropin releasing hormone (CRH) from the hypothalamus, which binds to receptors in the anterior pituitary,

causing the release of adrenocorticotropic hormone (ACTH). ACTH binds to receptors in cells in the adrenal cortex, stimulating the synthesis and release of cortisol, a stress hormone that mobilizes energy stores and activates catabolic processes. Cortisol has a long half-life in the blood and shuts down HPA axis activation through negative feedback by binding to GRs in the hypothalamus, anterior pituitary and suprahypothalamic structures, such as the hippocampus and prefrontal cortex. Therefore, and as mentioned above, the amygdala, hippocampus and prefrontal cortex play a very important role in emotion regulation and control of the stress response, including HPA axis activity (Herman et al., 2003; Herman et al., 2005; Ulrich-Lai & Herman, 2009). At the same time, these corticolimbic regions are sensitive to the effects of HPA axis stress activations through cortisol actions on GRs and MRs expressed in these regions (López et al., 1999; Patel et al., 2000; Sánchez et al., 2000).

Previous studies have shown that early adversity/ELS, including childhood maltreatment, leads to developmental psychopathology via impacts on the structural and functional development of brain regions such as the prefrontal cortex, amygdala and hippocampus (M. H. Teicher et al., 2003). Here, I am focusing on the impact of ELS on the development of prefrontal cortex (PFC), hippocampus, and amygdala, as they are critical regions for emotion and stress regulation. Additionally, their protracted development in comparison to other brain regions, given that these circuits are undergoing major growth, remodeling, and development, including synaptogenesis, synaptic pruning and remodeling and myelination, during childhood and adolescence, which makes them very vulnerable to ELS (Hanson et al., 2012; Tottenham & Sheridan, 2010). PFC not only regulates stress and emotion regulation, but is also important for executive function, goal-directed behavior, impulse control and reward (Funahashi & Andreau, 2013). Damage to the PFC typically results in impaired decision-making and executive function

skills, emotional and stress dysregulation, and substance abuse (Funahashi & Andreau, 2013; Kumaran et al., 2015). The amygdala is a limbic region with a critical role in detection of stimuli salience and threat, stress and emotional responses (LeDoux, 1993), and the formation of emotional memories. Amygdala dysfunction is found in many people with drug addiction, PTSD, and depression (LeDoux, 1993; Shin & Liberzon, 2010; Grotegerd et al., 2014). The hippocampus is another important brain region in the limbic system for learning and memory and it controls stress neuroendocrine function (Tottenham & Sheridan, 2010).

The primate hippocampus and PFC have high levels of GRs and MRs, and the amygdala moderate levels, providing a mechanism by which cortisol could have local effects on dendritic field and myelination (Sapolsky et al., 1985; McEwen, 1998; Sánchez et al., 2000). These local effects result in structural and volumetric changes in these regions (Arnsten, 2009) and subsequent impaired PFC, amygdala and hippocampal function, which is associated with stress-related disorders such as PTSD and depression (Nadel et al., 2007; Geuze et al., 2005). Chronic stress and cortisol elevations have been shown to reduce PFC volumes by shrinking dendritic fields, branching, spines, and synapses (Radley, 2012; Arnsten, 2009; Popoli et al., 2011). The hippocampal volume can also be reduced from chronic stress exposure (Lupien et al., 2009) through different mechanisms ranging from reductions in dendritic fields and synapses (Sapolsky et al., 1985; McEwen, 1998; Lupien et al., 2009) to even impacts on neurogenesis and neuronal death (Hanson et al., 2015). However, the effects of stress and glucocorticoids on the amygdala appear to have an opposite effect, that is, to result in increased amygdala volumes (Mitra et al., 2005; Tottenham et al., 2010). The amygdala plays a strong role in activating the HPA axis and sympathetic system during the stress response and it also mediates fear and anxiety responses, in part through CRH synthesis and projections to hypothalamus and brainstem. Some studies

suggest that the amygdala is stimulated when exposed to stress, and this leads to increased synaptic connectivity in the amygdala with threat-response systems (Tottenham & Sheridan, 2010; Vyas et al., 2006).

Despite the strong link between early adversity/stress and long-term structural alterations in PFC, hippocampal and amygdala, and developmental psychopathology in humans, the underlying neurobiological mechanisms and developmental emergence of the changes has been studied using animals models, which offer a unique opportunity to understand the basic neurobiological and developmental underpinnings of these disorders at a level not possible in humans (Kaufman et al., 2000). Interestingly, infant maltreatment is a form of ELS not only present in human populations, but in nonhuman primate species as well, both in the wild and in captivity (Brent et al., 2002; Maestripieri, 1998). Rhesus macaques (*Macaca mulatta*) in particular, have been more widely studied and are a highly translational model for humans due to unique advantages, including their complex, social relationships (Suomi, 2005) and neurobiological and neurodevelopmental similarities with humans (Machado & Bachevalier, 2002). Similarly to humans, maternal care is critical for rhesus macaque infant development. Infant maltreatment exhibited by the mothers occurs spontaneously in about 2-5% of the rhesus macaque population which includes physical abuse of the infant, very often co-morbid with rejection (Maestripieri, 1998; Howell et al., 2016). The first three months of life is a critical time for mother-infant bonding and when the infants spend the most time with their mother, and around three months of age the infants begin to wean (Suomi, 2005; Hinde & Spencer-Booth, 1967). It is during the first three months, when maternal caregiving is most intense and infants spend most of the time with the mother, when the highest rates of abuse and rejection occur. Infant maltreatment in macaques has been shown to be an stressful experience for infants,

leading to activations of the HPA axis, that result in elevated levels of the stress hormone cortisol, and negative developmental consequences, including higher anxiety and emotional reactivity that has been linked to decreased brain serotonin function and increased activity in pro-inflammatory pathways (Howell et al, 2016). In addition, using MRI neuroimaging approaches, Howell and colleagues reported that infant maltreatment is associated with increased amygdala volumes in adolescence, which was also associated with increased emotional reactivity (Howell et al, 2016). These studies provide the foundation for the questions in my thesis, which focuses on understanding the developmental emergence and trajectory of structural alterations not just in the amygdala, but also in the PFC and hippocampus in maltreated animals throughout the infant and juvenile periods.

Due to the limitations of prospective, longitudinal studies in child maltreatment in humans, we use this naturalistic, highly translational infant maltreatment model in rhesus macaques to examine the longitudinal effects of childhood maltreatment on the structural development of these critical regions for stress and emotional regulation.

The **goal** of my thesis is to understand the developmental alterations caused by ELS on primate brain structure during the infant and juvenile periods in macaques, and to examine potential biological mechanisms underlying those. Using an infant maltreatment model in rhesus macaques, I examined the structural, volumetric changes of the PFC, hippocampus, and amygdala longitudinally, at 3, 6 and 12 months of age, using structural magnetic resonance imaging (MRI) approaches. In order to disentangle the effects of postnatal experience from potential effects of heritable factors, this study used a cross-fostering design with random assignment of the infants at birth to either a competent mother or to a mother who consistently maltreated previous offspring. Additionally, hair cortisol accumulation was measured at birth

and 6 months old, to examine and compare the potential programming effects of prenatal versus postnatal cortisol exposure (i.e. the latter due to ELS-related HPA axis activations) on infant brain developmental measures. In addition to the examination of ELS-induced cortisol elevations as a potential predictor of alterations in neurodevelopmental trajectories of infants that experienced maltreatment by their mothers, I also examined the potential predictor role of maternal abuse and rejection rates experienced on regional brain growth.

Based on the information presented thus far, I **hypothesize** that the maltreated group will show smaller PFC and hippocampal volumes, but increased amygdala volumes compared to the control group. However, these differences will emerge with age, due to accumulated exposure to the ELS experience. In addition, I hypothesize that the changes in structure found in the maltreated animals, will be associated with elevated postnatal (but not prenatal), hair cortisol levels, as well as with higher abuse, and rejection rates.

Methods

Subjects and Housing

Fifteen rhesus monkeys (*Macaca mulatta)*, 8 Control (5 females, 3 males), 7 Maltreated (3 females, 4 males), were studied from 3 to 12 months of age as part of a bigger longitudinal project that examined the long-term neurodevelopmental effects of maternal care from birth through the juvenile, prepubertal period (through18 months of age). The subjects were housed in outdoor compounds at the Yerkes National Primate Research Center (YNPRC) Field Station of Emory University, where subjects also had access to indoor, climate-controlled living areas. All animals lived with their mothers and families in complex social groups which consisted of 2-3 adult males and 30-100 adult females with their offspring. Water was available *ad libitum*, and animals were fed twice daily with monkey chow, supplemented with vegetables, and fresh fruit. All studies complied with the Animal Welfare Act and the U.S. Department of Health and Human Services "Guide for the Care and Use of Laboratory Animals" and were approved by the Emory University Institutional Animal Care and Use Committee (IACUC).

Cross-fostering design and Collection of Maternal Care Data

In order to disentangle postnatal experience from potential confounds of heritable factors on developmental outcomes, a cross-fostering experimental design was used with random assignment of the infant at birth to either a control or maltreating mother following previously published protocols (Howell et al, 2017; Drury et al., 2017), in which eight infants were raised by competent, nurturing mothers (Control group), and the other seven were raised by maltreating mothers. No infants were raised by their biological mothers. No biological siblings were included to rule out genetic similarities, and 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. Infant maltreatment was defined as two co-morbid 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 infant by its mother) 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., 2006; McCormack et al., 2006; Mar M. Sanchez, 2006; Howell et al, 2017.; Drury et al., 2017).

Behavioral observations were collected in all mother-infant pairs from birth through 3 months of age to examine maternal care received by the infants, using established ethograms and procedures where the infant was the focal animal (Howell et al, 2017). Each observation lasted 30 minutes and was conducted on separate days, five times per week in the first month postpartum (20 observations), twice per week in the second month (8 observations), and once per week in the third month (4 observations), resulting in 32 total observations (a total of 16 hours) per mother-infant pair. Maternal rates of abuse and rejection were used for this study. The observation schedule was chosen based on previous published protocols documenting optimal time of observation based on the ability to capture early maternal care received by the infant (D. Maestripieri, 1998; Maestripieri & Carroll, 1998; Kai McCormack et al., 2009; K. McCormack et al., 2006) Additionally, data were collected from subjects from birth through the latest ages studied and there were 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 MRI Image Acquisition

Each subject was transported with its mother from the YNPRC Field Station to the Main Center either the day before or the morning of their scheduled scan. Magnetic Resonance Imaging (MRI) scans of the subjects' brains were collected at 3, 6 and 12 months of age using a 3T Siemens TRIO scanner (Siemens Med. Sol., Malvern, PA, USA) and an 8-channel phase array knee coil located in the Yerkes MRI Center. Both T1 and T2-weighted structural MRI scans were acquired during the same scanning session. The T1-weighted 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=0.6mm3, isotropic, 6 averages). A T2-weighted scan was collected in the same direction as the T1 (TR/TE=7,900/125ms, voxel size=0.5x0.5x1.0mm3, 10 averages) to help with anatomical identification of white matter (WM), gray matter (GM) and cerebrospinal fluid (CSF) borders, and delineation of the regions of interest –ROIs- (Rebecca C. Knickmeyer et al., 2010; Rapisarda et al., 1983). To limit motion artifacts, the subjects were scanned under isoflurane anesthesia (0.8-1%, inhalation, to effect) following injection with telazol (approx. 2-3mg/Kg BW, i.m.) and intubation, and placed on a custom-made head holder with ear bars and a mouth piece to secure and prevent movement of the head. A vitamin E capsule was placed to the right temple to identify the right brain hemisphere. Physiological parameters were monitored throughout the scan using an oximeter, ECG, rectal thermistor, and blood pressure monitor. An intravenous catheter was used to administer dextrose/NaCl (0.45%, I.V.) for hydration, and the animal was 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 mother and the pair returned to their social group on the following day.

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 3.3.2), 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: prefrontal cortex (PFC), frontal lobe as a negative control region), and subcortical structures (amygdala and hippocampus) to compute their respective volumes in the rhesus macaque, following methods previously described (Howell et al., 2014; R. C. Knickmeyer et al., 2010;Wang et al., 2014; Shi et al., 2016). In the first step, the brain images were corrected for signal intensity inhomogeneity through an N4-ITK bias field correction step. Next, the images were registered to age-specific, population-based, infant rhesus brain T1- and T2-MRI atlases using a reference space algorithm (Wang et al., 2014; Styner et al., 2007;Short et al., 2010, Shi et al., 2016).

The 3, 6 and 12 months UNC-Emory infant rhesus brain atlases were used to register the subjects' images to the atlas closest to their age, due to significant developmental changes in GM/WM signal contrast across ages (Shi et al., 2016). These infant atlases were generated from 48 animals scanned longitudinally, and then were aligned and "averaged" to create an overall T1-and T2-MRI age-specific template/atlas for each age (3, 6, and 12 months; Figure 1) using deformable registration tools in the Advanced Normalization Tools (ANTs) software. These 3, 6, and 12 months T1- and T2-MRI atlases 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 the tissue maps and parcellations,

we used AutoSeg 3.3.2 to process each of the subjects' T1 and T2-weighted images by performing inhomogeneity correction, and registration of the subject's image to the atlas image space (age-specific template: 3, 6, or 12 months) using BRAINSFit for rigid body and affine registration (Wang et al., 2014). An automatic tissue segmentation step was then applied using Atlas-Based Classification (ABC) for probabilistic tissue class classification of each subject's images into GM, WM or CSF brain tissue or non-brain tissue (e.g. skull, vessels, muscle, skull) and to remove non-brain tissue for analysis (called "skull-stripping"), by warping the atlas-specific probabilistic tissue priors into the subject twice, once before skull-stripping and once after (Wang et al., 2014). The skull-stripping step requires manual quality control and final editing. The next step is the cortical lobar (PFC, frontal lobe) and subcortical ROI parcellations (amygdala, hippocampus) during which AutoSeg uses ANTS registration (Wang et al., 2014) of the ROIs in the skull-stripped brain atlas image to the subject image. The tissue class segmentations generated (GM, WM, CSF) are also applied to the cortical parcellations to generate WM, GM, and CSF of each cortical region. Figure 2 shows some of examples of the skull-stripped, tissue segmentation and parcellation images. Volumes of right and left PFC cortex (GM, WM and CSF), right and left amygdala, right and left hippocampus, and right and left frontal cortex (GM, WM and CSF) were then automatically computed. Total Intracranial volume was arithmetically calculated (total GM + total WM + total CSF) as a measure of total brain volume.

For this study manual editing of the skull-stripped mask was done in ITKSnap software by two raters blind to the experimental group to ensure accurate neuroanatomical delineation of brain versus non-brain tissue. In order to avoid confounding effects of rater segmentation bias on our measures, cases were counterbalanced by experimental group, and sex and an independent

expert guided and monitored interrater reliability to ensure consistency of manual edits across raters.

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

The atlas ROIs were described in previous publications (Howell et al., 2014; R. C. Knickmeyer et al., 2010).The amygdala boundary was defined rostrally by the anterior limit of the periamygdaloid cortex, posteriorly by the hippocampus, ventrally by CSF, ventrolaterally by WM (Amaral & Bassett, 1989; Price, 1987) 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 & 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).

In order to show that the effects of maltreatment are region specific, a negative control region was added to the analysis. The frontal lobe was chosen as a negative control region because it is more mature at birth due to the motor cortex being very developed in infant rhesus monkeys (Sclafani et al., 2015). Additionally, the frontal lobe does not have as high a density of GRs as the prefrontal cortex (Sánchez et al., 2000), making it less sensitive to cortisol effects. The frontal lobe boundary was established anteriorly by the prefrontal trace, and posteriorly by the central sulcus. In anterior slices, the inferior boundary was the sylvian fissure. In more

posterior slices, a line drawn (in the coronal plane) across the central white matter between the central sulcus and the inferior extent of the corpus callosum constituted the inferior boundary (Knickmeyer et al., 2010).

Hair Cortisol Samples

Approximately one square inch of 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 cortisol accumulation into the growing hair to assess exposure to chronic stress-induced cortisol elevations either throughout pregnancy or during infancy, respectively. The hair was stored at -80 degrees C until assayed. Hair samples were processed and assayed using previously described protocols (Davenport et al., 2006; Meyer et al., 2014). Briefly, each sample was weighed, was washed twice in isopropanol to remove external contaminants, dried and grounded into a fine powder form using a Retsch ball mill, and then extracted with methanol overnight. The methanol was evaporated, the residue was redissolved in assay buffer, and then cortisol was measured (in pg/mg hair units) using the Salimetrics (Carlsbad, CA) enzyme immunoassay kit (cat. # 1-3002), according to manufacturer's directions. Intra- and inter-assay coefficients of variation were <10%. Two animals had only on time points, 18 had two time points, and 23 had three time points. The 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. plasma and salivary cortisol concentrations). 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

Intracranial volume (ICV) was analyzed as a total brain measure using a repeated measures analysis of variance (RM ANOVA) with foster mom group (control, maltreated) as fixed factor and age (3 months, 6 months, 12 months) as the repeated measure. Left and right PFC (total, as well as GM, WM, and CSF), hippocampus, amygdala and frontal cortex were also analyzed using RM ANOVA with foster mom group as the fixed factor and age (3, 6, 12 months) and laterality (right, left) as a repeated measures. In cases where a significant group by age interaction effect was detected, the nature of this interaction was examined by reporting whether a linear or quadratic component was detected. Due to the potential confounding effect of developmental changes in total ICV on amygdala, PFC, hippocampal, frontal lobe volumes, these measures were analyzed both uncorrected (i.e. raw volumetric values) and corrected by ICV (i.e. uncorrected volume divided by total ICV). Although the study is underpowered to examine the main and interaction effects of sex, a repeated measures analysis of covariance (RM ANCOVA) analyses was also ran adding sex as covariate in the statistical model.

Pearson correlations were used to examine whether maltreatment-related effects on volumetric measures were predicted by early exposure to stress-induced elevations in the stress hormone cortisol (measured as hair cortisol accumulation between birth and 6 months of life, when maternal abuse and rejection rates were the highest; and results were compared with the correlations with hair cortisol at birth –postnatal day 2-, as a measure of prenatal cortisol exposure), or to abuse and rejection rates received.

Statistical analyses were performed using SPSS (version 22.0). Significant p values were set at $p < 0.05$. Trends towards significance were reported as $0.1>p>0.05$. Effects sizes were also reported.

Results

Intracranial volume (ICV)

Significant main effects of age ($F_{2,13} = 77.53$, $p = 1.10x10^{-11}$, $\eta^2 = 0.86$) were found for total ICV (defined as total CSF+GM+WM+Blood vessels), with volume increasing with age (Figure 3). An age by group interaction effect was also detected ($F_{2,13} = 3.76$, $p = 0.04$, $\eta^2 = 0.22$), best fit to a linear interaction ($F_{2,13} = 5.84$, $p = 0.03$, $\eta^2 = 0.31$). However, post-hoc pairwise comparisons between the control and maltreated means at 12 months are not significantly different ($F_{2,13} = 0.01$, $p = 0.93$, $t = 0.95$). No main effects of group were detected ($F_{2,13} = 0.04$, $p = 0.85$, $\eta^2 = 2.88x10^{-3}$). Data in Figure 3 shows that ICV grew at a slower rate in the maltreated animals between 6 and 12 months.

When sex was added as a covariate, the significant main effect of age persisted ($F_{2,13} = 6.83$, $p = 4.48x10^{-3}$, $\eta^2 = 0.36$), but the age by group interaction disappeared ($F_{2,13} = 3.31$, $p = 0.05$, $\eta^2 = 0.22$) suggesting this interaction is driven by one of the sexes, as shown in Figure 4. No group effects ($F_{2,13} = 0.21$, $p = 0.66$, $\eta^2 = 0.02$), sex effects ($F_{2,13} = 1.74$, $p = 0.21$, $\eta^2 = 0.02$) or age by sex interaction effects ($F_{2,13} = 0.29$, $p = 0.75$, $\eta^2 = 0.02$) were detected.

Total WM, GM, and CSF volumes

A significant main effect of age was detected in total WM, GM, and CSF (WM: $F_{2,13} = 81.52$, $p = 6.29x10^{-12}$, $\eta^2 = 0.86$; GM: $F_{2,13} = 18.72$, $p = 9.19x10^{-06}$, $\eta^2 = 0.59$; CSF: $F_{2,13} = 46.76$, $p = 2.45x10^{-9}$, $\eta^2 = 0.78$), where volume appears to increase with age, as shown in Figure 5. In total GM, a significant age by group interaction effect was detected ($F_{2,13} = 6.24$, $p = 0.01$, $\eta^2 = 0.32$) with a linear interaction fit ($F_{2,13} = 8.49$, $p = 0.01$, $\eta^2 = 0.40$), however, post-hoc pairwise comparisons between the control and maltreated means at 12 months are not significantly

different ($F_{2,13} = 0.02$, $p = 0.88$, $t = 0.65$). Total WM and CSF did not have an age by group interaction effect (WM: $F_{2,13} = 2.54$, $p = 0.10$, $\eta^2 = 0.16$; CSF: $F_{2,13} = 0.46$, $p = 0.63$, $\eta^2 = 0.03$). None showed significant group effects (WM: $F_{2,13} = 0.30$, $p = 0.59$, $\eta^2 = 0.02$; GM: $F_{2,13} = 8.49$, $p = 0.01$, $\eta^2 = 0.40$; CSF: $F_{2,13} = 2.75\text{x}10^{-4}$, $p = 0.99$, $\eta^2 = 2.12\text{x}10^{-5}$).

When sex was added as a covariate a main effect of age persisted in total WM ($F_{2,13} = 6.78$, $p = 4.62\text{x}10^{-3}$, $\eta^2 = 0.36$), but not in total GM ($F_{2,13} = 1.96$, $p = 0.16$, $\eta^2 = 0.14$) or total CSF ($F_{2,13} = 2.62$, $p = 0.09$, $\eta^2 = 0.17$). In total GM, the significant age by group interaction effect also persisted (GM: $F_{2,13} = 5.15$, $p = 0.01$, $\eta^2 = 0.30$) with a linear interaction fit ($F_{2,13} = 7.07$, $p = 0.02$, $\eta^2 = 0.37$). Total WM and CSF did not have an age by group interaction effect (WM: $F_{2,13} = 2.23$, $p = 0.13$, $\eta^2 = 0.16$; CSF: $F_{2,13} = 0.46$, $p = 0.64$, $\eta^2 = 0.04$) or age by sex (WM: $F_{2,13} = 0.06$, $p = 0.06$, $\eta^2 = 0.93$; GM: $F_{2,13} = .89$, $p = 0.42$, $\eta^2 = 0.07$; CSF: $F_{2,13} = 0.33$, $p = 0.72$, $\eta^2 = 0.03$) interactions were detected. In addition, none showed significant group effects (WM: $F_{2,13} = 0.85$, $p = 0.38$, $\eta^2 = 0.07$; GM: $F_{2,13} = 0.05$, $p = 0.82$, $\eta^2 = 4.46\text{x}10^{-3}$; CSF: $F_{2,13} = 0.02$, $p = 0.90$, $\eta^2 = 1.29\text{x}10^{-3}$) or sex effects (WM: $F_{2,13} = 3.04$, $p = 0.11$, $\eta^2 = 0.20$; GM: $F_{2,13} = 1.24$, $p = 0.29$, $\eta^2 = 0.09$; CSF: $F_{2,13} = 0.31$, $p = 0.59$, $\eta^2 = 0.03$).

Hippocampal volume

In the hippocampus, a significant main effect of age was found ($F_{2,13} = 704.40$, $p = 2.27\text{x}10^{-23}$, $\eta^2 = 0.98$), showing that volumes increase with age (Figure 6). An age by group interaction was detected in the hippocampus ($F_{2,13} = 4.07$, $p = 0.03$, $\eta^2 = 0.24$) and the interaction fit a linear trend ($F_{2,13} = 5.31$, $p = 0.04$, $\eta^2 = 0.29$), suggesting that the hippocampus grew at a slower rate in the maltreated animals, but only between 6 and 12 months of age, as shown in Figure 6. Post-hoc pairwise comparisons show that the left hippocampal volumes of maltreated

animals are significantly smaller than in the control group at 6 months ($F_{2,13} = 7.17$, $p = 0.02$, $t = 1.48$). No main effects of group ($F_{2,13} = 2.17$, $p = 0.16$, $\eta^2 = 0.14$) or hemisphere ($F_{2,13} = 0.60$, $p = 0.45$, $\eta^2 = 0.04$),or hemisphere by group ($F_{2,13} = 0.16$, $p = 0.70$, $\eta^2 = 0.12$), hemisphere by age ($F_{2,13} = 2.85$, $p = 0.08$, $\eta^2 = 0.18$) or age by group by hemisphere interactions ($F_{2,13} = 2.48$, $p = 0.10$, $\eta^2 = 0.16$)

When sex was added as a covariate, the main effect of age ($F_{2,13} = 58.13$, $p = 6.30\text{x}10^{-10}$, $\eta^2 = 0.83$), and age by group interaction ($F_{2,13} = 4.20$, $p = 0.03$, $\eta^2 = 0.26$) persisted (Figure 7). There were no main effects of group ($F_{2,13} = 3.24$, $p = 0.10$, $\eta^2 = 0.21$), main effects of sex ($F_{2,13} = 2.17$, $p = 0.17$, $\eta^2 = 0.15$) or main effects of hemisphere ($F_{2,13} = 0.5$, $p = 0.83$, $\eta^2 = 3.86\text{x}10^{-3}$) detected. No age by sex ($F_{2,13} = 1.09$, $p = 0.35$, $\eta^2 = 0.08$), hemisphere by sex ($F_{2,13} = 0.23$, $p = 0.64$, $\eta^2 = 0.02$), hemisphere by group ($F_{2,13} = 0.22$, $p = 0.65$, $\eta^2 = 0.02$), age by hemisphere ($F_{2,13} = 0.76$, $p = 0.48$, $\eta^2 = 0.06$), age by hemisphere by sex ($F_{2,13} = 0.36$, $p = 0.70$, $\eta^2 = 0.03$), or age by hemisphere by group ($F_{2,13} = 2.34$, $p = 0.12$, $\eta^2 = 0.16$) interaction effects were detected.

Hippocampus (ICV-Corrected)

When hippocampal volumes were ICV-corrected, the main effect of age persisted ($F_{2,13} = 831.55$, $p = 2.72\text{x}10^{-24}$, $\eta^2 = 0.99$) showing region-specific hippocampal volumes increases with age, either independent of ICV growth or at higher rates than ICV (Figure 8). However the age by group interactions disappeared, suggesting that the interaction was driven by ICV or the group by age affect affected ICV at similar rates. No main effects of group ($F_{2,13} = 2.40$, $p = 0.15$, $\eta^2 = 0.16$), main effect of hemisphere ($F_{2,13} = 0.63$, $p = 0.44$, $\eta^2 = 0.05$) or group by age interaction effects ($F_{2,13} = 2.31$, $p = 0.12$, $\eta^2 = 0.15$), hemisphere by group ($F_{2,13} = 0.08$, $p = 0.78$, $\eta^2 = 6.23\text{x}10^{-3}$), and age by hemisphere ($F_{2,13} = 2.45$, $p = 0.11$, $\eta^2 = 0.16$) were detected.

Amygdala volume

In the amygdala, a significant main effect of age was detected, ($F_{2,13} = 269.11$, $p = 1.08x10^{-10}$, $\eta^2 = 0.98$) suggesting that volumes increase with age (Figure 9). A main effect of hemisphere was detected ($F_{2,13} = 176.73$, $p = 6.06x10^{-9}$, $\eta^2 = 0.93$) and a hemisphere by group interaction was detected, as well ($F_{2,13} = 10.26$, $p = 6.94x10^{-3}$, $\eta^2 = 0.44$). There were main effects of group ($F_{2,13} = 0.43$, $p = 0.52$, $\eta^2 = 0.03$), group by age interaction effects ($F_{2,13} = 1.62$, $p = 0.22$, $\eta^2 = 0.11$), or age by hemisphere interaction effects ($F_{2,13} = 0.31$, $p = 0.74$, $\eta^2 = 0.02$) .

When sex was added as a covariate, the main effect of age ($F_{2,13} = 56.76$, $p = 7.98x10^{-10}$, $\eta^2 = 0.83$), main effect of hemisphere ($F_{2,13} = 22.11$, $p = 5.13x10^{-4}$, $\eta^2 = 0.65$), and hemisphere by group interaction effect ($F_{2,13} = 10.19$, $p = 0.01$, $\eta^2 = 0.46$) persisted in the amygdala (Figure 10). There were no main effects of sex ($F_{2,13} = 0.34$, $p = 0.57$, $\eta^2 = 0.03$) or group ($F_{2,13} = 0.55$, $p = 0.47$, $\eta^2 = 0.04$). Additionally, no age by sex ($F_{2,13} = 1.32$, $p = 0.29$, $\eta^2 = 0.10$), age by group ($F_{2,13} = 1.43$, $p = 0.26$, $\eta^2 = 0.11$), hemisphere by sex ($F_{2,13} = 0.40$, $p = 0.54$, $\eta^2 = 0.03$), age by hemisphere ($F_{2,13} = 0.37$, $p = 0.69$, $\eta^2 = 0.03$), age by sex by hemisphere ($F_{2,13} = 0.40$, $p = 0.68$, $\eta^2 = 0.03$), or age by hemisphere by group ($F_{2,13} = 2.17$, $p = 0.14$, $\eta^2 = 0.15$) interaction effects were detected.

Amygdala (ICV-Corrected)

In the amygdala, a significant main effect of age was detected, ($F_{2,13} = 560.86$, $p = 4.13x10^{-22}$, $\eta^2 = 0.98$) showing that volumes increase with age, independent of ICV growth (Figure 11). The main effect of hemisphere ($F_{2,13} = 131.57$, $p = 3.58x10^{-8}$, $\eta^2 = 0.91$) as well as a hemisphere by group interaction effect persisted ($F_{2,13} = 6.76$, $p = 0.02$, $\eta^2 = 0.34$), suggesting the lateralization effects were independent of ICV growth. No main effects of group ($F_{2,13} = 0.13$, $p = 0.73$, $\eta^2 = 0.01$) were detected. Additionally, no age by hemisphere interaction effects ($F_{2,13} =$

0.93, p = 0.41, η^2 = 0.07), age by group by hemisphere interaction ($F_{2,13}$ = 2.83, p = 0.08, η^2 = 0.18) or group by age interaction effects ($F_{2,13}$ = 0.56, p = 0.58, η^2 = 0.04) were detected.

PFC volume (WM, GM, CSF)

Within the PFC there was a significant main effect of age in WM ($F_{2,13}$ = 21.79, p = $2.77x10^{-6}$, η^2 = .63), GM ($F_{2,13}$ = 21.56, p = $3.01x10^{-6}$, η^2 = 0.62), and CSF ($F_{2,13}$ = 20.23, p = $5.02x10^{-6}$, η^2 = 0.61). There was also a significant age by group interaction effect in PFC GM, as shown in Figure 12, ($F_{2,13}$ = 5.45, p = 0.01, η^2 = 0.30), and the interaction followed a linear trend (right: $F_{2,13}$ = 9.47, p = 0.01, η^2 = 0.42) (Figure 12). This interaction was not detected in PFC WM ($F_{2,13}$ = 1.75, p = 0.19, η^2 = 0.12) or PFC CSF ($F_{2,13}$ = 0.53, p = 0.60, η^2 = 0.04), show in Figure 13. No main effect of group is detected in WM ($F_{1,13}$ = $5.21x10^{-4}$, p = 0.98, η^2 = $4.00x10^{-5}$), GM ($F_{2,13}$ = $4.08x10^{-3}$, p = 0.95, η^2 = $3.13x10^{-4}$), and CSF ($F_{2,13}$ = 0.16, p = 0.69, η^2 = 0.01). In PFC WM main effect of hemisphere was not detected ($F_{1,13}$ = 2.87, p = 0.11, η^2 = 0.18) or in GM ($F_{2,13}$ = 0.36, p = 0.56, η^2 = 0.03), but was detected in CSF ($F_{2,13}$ = 7.80, p = 0.02, η^2 = 0.38), A hemisphere by group interaction was not detected WM ($F_{1,13}$ = 0.84, p = 0.38, η^2 = 0.06) GM ($F_{2,13}$ = 0.01, p = 0.92, η^2 = $8.41x10^{-4}$) CSF ($F_{2,13}$ = 0.04, p = 0.85, η^2 = $3.02x10^{-3}$)or age by hemisphere interaction WM ($F_{1,13}$ = 1.88, p = 0.17, η^2 = 0.13) and CSF ($F_{2,13}$ = 0.19, p = 0.83, η^2 = 0.01). There was a significant age by hemisphere interaction in GM ($F_{2,13}$ = 4.79, p = 0.02, η^2 = 0.27).

PFC volume (WM, GM, CSF – ICV Corrected)

When corrected with ICV, a significant main effect of age in PFC WM ($F_{2,13}$ = 9.42, p = $8.37x10^{-4}$, η^2 = 0.42), GM ($F_{2,13}$ = 38.62, p = $1.64x10^{-8}$, η^2 = 0.75), and CSF ($F_{2,13}$ = 14.71, p = $5.33x10^{-5}$, η^2 = 0.53). No main effects of group were detected in PFC WM ($F_{2,13}$ = 0.03, p = 0.85,

$\eta^2 = 2.85x10^{-3}$), GM ($F_{2,13} = 0.01$, $p = 0.92$, $\eta^2 = 8.49x10^{-4}$), and CSF ($F_{2,13} = 0.31$, $p = 0.59$, $\eta^2 = 0.02$).

In PFC WM, no main effects of hemisphere ($F_{2,13} = 2.94$, $p = 0.11$, $\eta^2 = 0.18$), age by group interaction effects ($F_{2,13} = 2.54$, $p = 0.10$, $\eta^2 = 0.16$), hemisphere by group interaction effects ($F_{2,13} = 0.76$, $p = 0.40$, $\eta^2 = 0.06$), age by hemisphere interaction effects ($F_{2,13} = 1.47$, $p = 0.25$, $\eta^2 = 0.10$), age by group by hemisphere interaction effects ($F_{2,13} = 0.48$, $p = 0.63$, $\eta^2 = 0.04$).

In PFC GM, an age by hemisphere interaction effect persisted ($F_{2,13} = 5.25$, $p = 0.01$, $\eta^2 = 0.28$). No main effects of hemisphere ($F_{2,13} = 0.19$, $p = 0.67$, $\eta^2 = 0.01$), age by group interaction effects ($F_{2,13} = 1.15$, $p = 0.33$, $\eta^2 = 0.08$), hemisphere by group interaction effects ($F_{2,13} = 0.04$, $p = 0.85$, $\eta^2 = 2.90x10^{-3}$), or age by group by hemisphere interaction effects ($F_{2,13} = 1.08$, $p = 0.36$, $\eta^2 = 0.08$) were detected.

In PFC CSF, a main effect of hemisphere was detected ($F_{2,13} = 7.35$, $p = 0.02$, $\eta^2 = 0.36$). No age by group interaction effects ($F_{2,13} = 0.29$, $p = 0.75$, $\eta^2 = 0.02$), hemisphere by group interaction effects ($F_{2,13} = 0.04$, $p = 0.84$, $\eta^2 = 3.21x10^{-3}$), age by hemisphere interaction effects ($F_{2,13} = 0.11$, $p = 0.90$, $\eta^2 = 0.01$), or age by group by hemisphere interaction effects ($F_{2,13} = 0.23$, $p = 0.80$, $\eta^2 = 0.02$) were detected.

PFC volume (Total)

In the PFC, a significant main effect of age ($F_{2,13} = 43.83$, $p = 4.69x10^{-9}$, $\eta^2 = 0.77$) was detected, showing that volumes increase with age (Figure 14). No group effects ($F_{2,13} = 4.96x10^{-4}$, $p = 0.98$, $\eta^2 = 3.81x10^{-5}$), hemisphere effects ($F_{2,13} = 0.28$, $p = 0.61$, $\eta^2 = 0.02$), hemisphere by group interactions ($F_{2,13} = 0.05$, $p = 0.82$, $\eta^2 = 3.99x10^{-3}$), age by hemisphere interactions ($F_{2,13} =$

1.29, p = 0.293, η^2 = 0.09), or age by group interactions were detected ($F_{2,13}$ = 2.58, p = 0.10, η^2 = 0.17).

When sex was added as a covariate, the main effect of age persisted ($F_{2,13}$ = 3.48, p = 0.047, η^2 = 0.23). No main effects of group ($F_{2,13}$ = 0.03 p = 0.87, $\eta^2 = 2.42x10^{-3}$), main effects of hemisphere ($F_{2,13}$ = 0.98, p = 0.34, η^2 = 0.08), or main effects of sex ($F_{2,13}$ = 0.96, p = 0.35, η^2 = 0.07) were detected. Additionally, no age by sex ($F_{2,13}$ = 0.11, p = 0.90, η^2 = 0.01), age by group ($F_{2,13}$ = 2.34, p = 0.12, η^2 = 0.16), hemisphere by sex ($F_{2,13}$ = 1.49, p = 0.25, η^2 = 0.11), hemisphere by group ($F_{2,13} = 1.43x10^{-4}$, p = 0.99, $\eta^2 = 1.19x10^{-5}$), age by hemisphere ($F_{2,13}$ = 0.63, p = 0.54, η^2 = 0.05), age by hemisphere by sex ($F_{2,13}$ = 1.02, p = 0.38, η^2 = 0.08), age by hemisphere by group ($F_{2,13}$ = 0.03, p = 0.97, $\eta^2 = 2.79x10^{-3}$).

PFC (ICV-Corrected)

In the ICV corrected PFC, a significant main effect of age was not detected (Figure 16), ($F_{2,13}$ = 2.40, p = 0.11, η^2 = 0.16). No main effects of group ($F_{2,13}$ = 0.09, p = 0.77, η^2 = 0.01) or hemisphere ($F_{2,13}$ = 0.35, p = 0.57, η^2 = 0.03). No age by group interaction effects ($F_{2,13}$ = 0.96, p = 0.40, η^2 = 0.07), hemisphere by group interaction effects ($F_{2,13}$ = 0.07, p = 0.80, η^2 = 0.01, age by hemisphere interaction effects ($F_{2,13}$ = 1.50, p = 0.24, η^2 = 0.10), or age by group by hemisphere interaction effects ($F_{2,13}$ = 0.15, p = 0.86, η^2 = 0.01) were detected.

Negative control cortical regions

Frontal Lobe volume

In the frontal lobe a significant main effect of age ($F_{2,13}$ = 106.17, $p = 3.09x10^{-13}$, η^2 = 0.89) was detected, as shown in Figure 17. A significant main effect of hemisphere ($F_{2,13}$ = 23.86, $p = 2.98x10^{-4}$, η^2 = 0.65) and an age by hemisphere interaction ($F_{2,13}$ = 4.31, p = 0.02, η^2 =

0.25) was detected. No group effects ($F_{2,13} = 0.01$, $p = 0.91$, $\eta^2 = 1.04x10^{-3}$), age by group interactions effects ($F_{2,13} = 2.73$, $p = 0.08$, $\eta^2 = 0.17$), or group by hemisphere effects ($F_{2,13} = 0.28$, $p = 0.61$, $\eta^2 = 0.02$) were detected.

Frontal Lobe (ICV-Corrected)

When volumes were ICV-corrected, The significant main effect of age ($F_{2,13} = 58.69$, $p = 2.49x10^{-10}$, $\eta^2 = 0.82$) and main effect of hemisphere ($F_{2,13} = 24.19$, $p = 2.81x10^{-4}$, $\eta^2 = 0.65$) persisted as shown in Figure 18, suggesting the growth and lateralization effects were independent of ICV. No group effects (right: $F_{2,13} = 0.03$, $p = 0.86$, $\eta^2 = 2.38x10^{-3}$), age by group interaction effects ($F_{2,13} = 0.99$, $p = 0.39$, $\eta^2 = 0.07$), hemisphere by group interaction effects ($F_{2,13} = 0.32$, $p = 0.58$, $\eta^2 = 0.02$), age by hemisphere interaction effect ($F_{2,13} = 3.29$, $p = 0.05$, $\eta^2 = 0.20$), or aby by group by hemisphere interaction effects ($F_{2,13} = 0.27$, $p = 0.76$, $\eta^2 = 0.02$), were detected.

Correlations between brain volumetric, cortisol, and maternal care measures.

Correlations were measured between cortisol and behavioral measures (abuse rates and rejection rates), means and standard errors shown in Table 1, and increase growth in ROI volumes with age (calculated as 12 month volume – 3 month volume). There were no significant correlations between abuse rates and total ICV, hippocampus, amygdala, PFC, or frontal lobe. A significant negative correlation was detected between rejection and volume growth of the right hippocampus ($r=-0.638$, $p=0.035$) and a trend for negative correlation in the left hippocampus ($r=-0.591$, $p=0.056$), suggesting that infants who experienced the most rejection showed smaller hippocampal volume growth (correlations are shown in Table 2).

Figures and Tables:

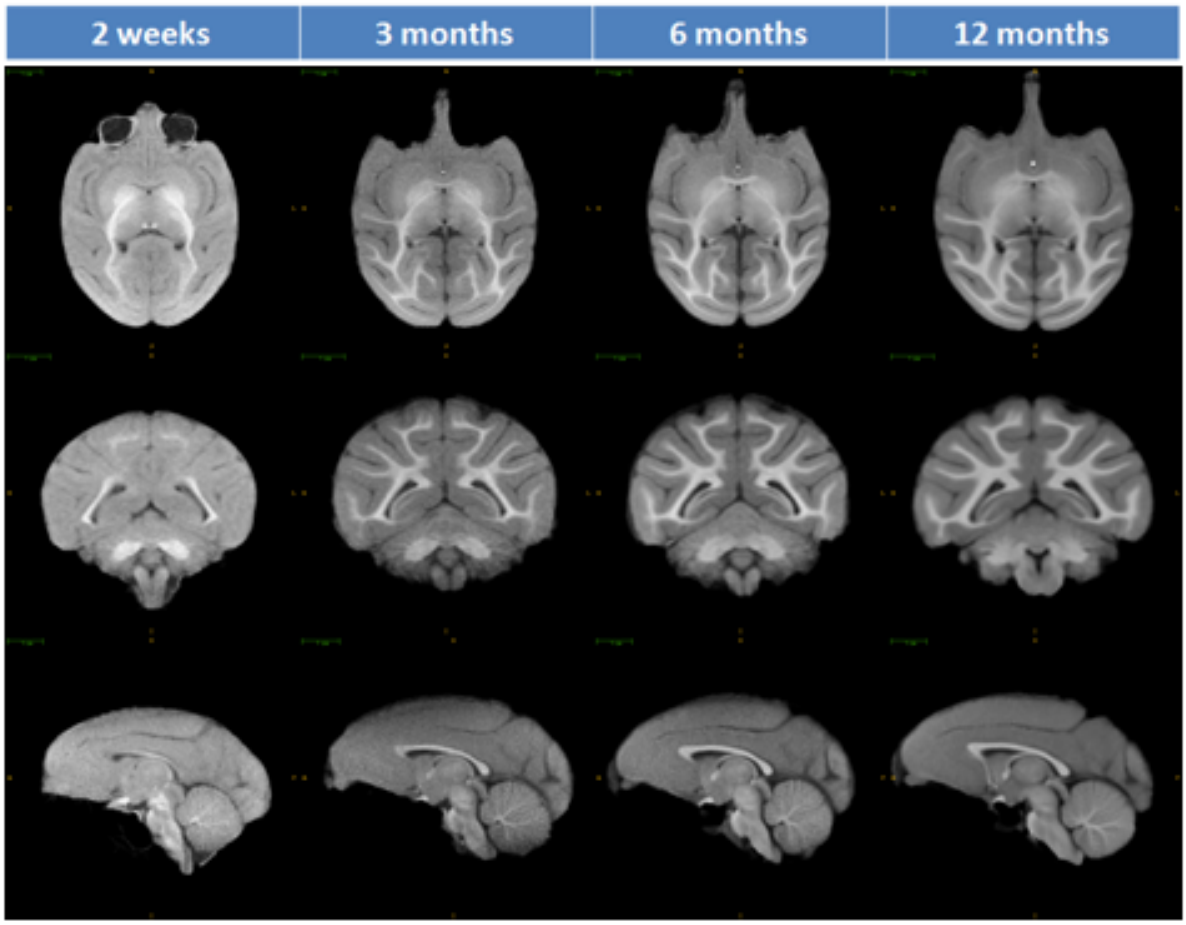

Figure 1. UNC-Emory infant rhesus structural MRI brain atlases

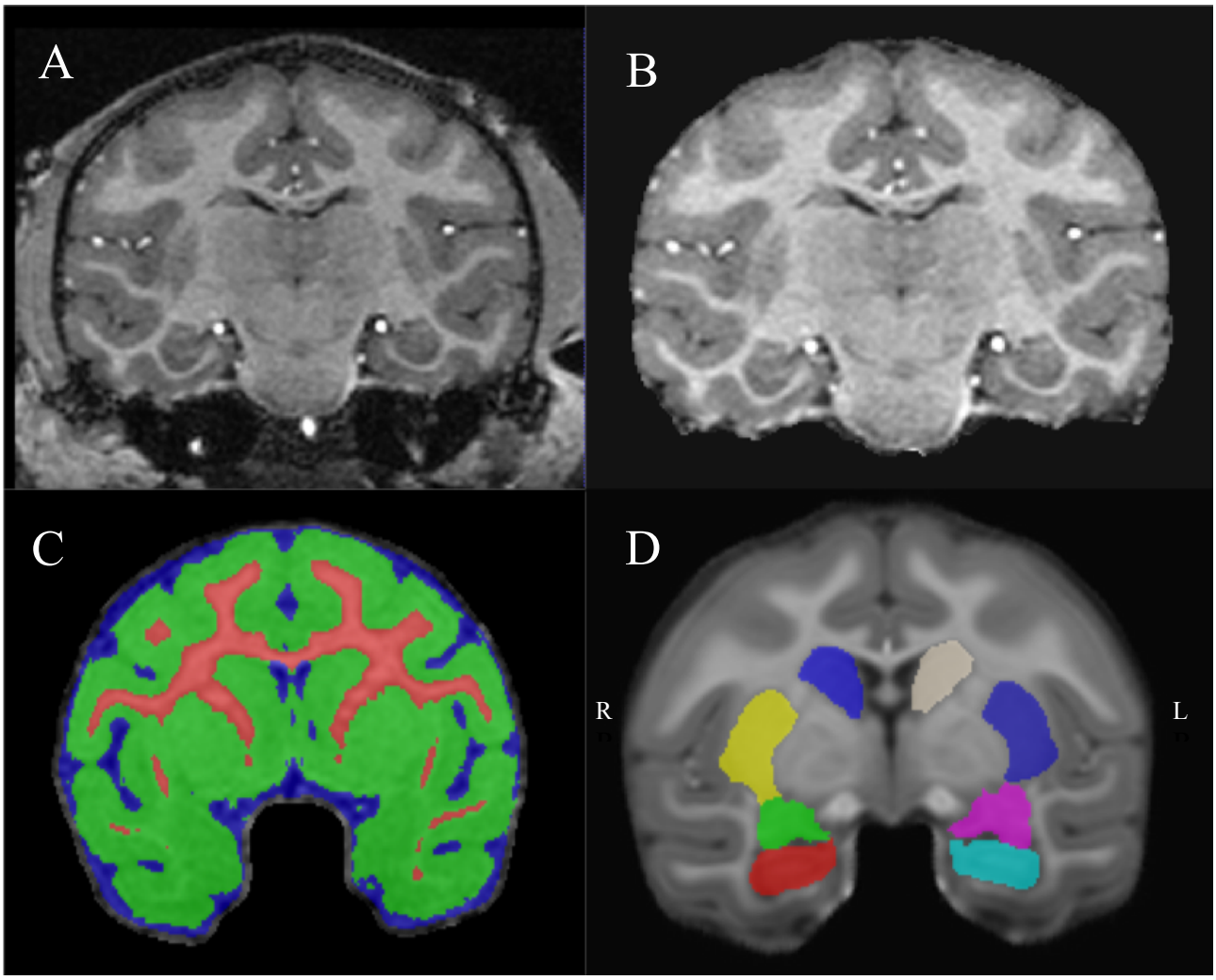

Figure 2. Representative images of skull-stripping, automatic tissue segmentation and subcortical ROI parcellation generated from AutoSeg. A) Coronal view of the brain in a T1-MRI non-skull stripped image. B) The T1 image as A, but skull-stripped. C) Coronal view of the brain, segmented into white matter (red), gray matter (green), and CSF (blue). D) Coronal view of subcortical parcellations: right hippocampus (red), right amygdala (green), right putamen (yellow), right caudate (dark blue), left caudate (beige), left putamen (violet), left amygdala (pink), and left hippocampus (light blue).

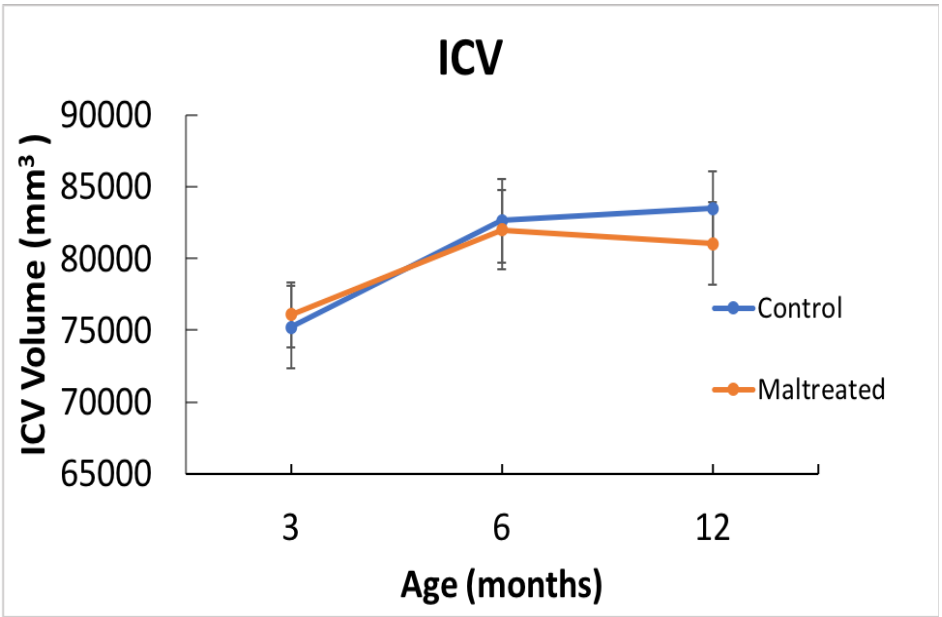

Figure 3. Developmental effects of infant maltreatment on total ICV. Total ICV increased with age ($F_{2,13} = 77.528$, $p = 1.10x10^{-11}$, $\eta^2 = 0.856$). A significant age by group interaction effect was detected ($F_{2,13} = 3.758$, $p = 0.042$, $\eta^2 = 0.224$) with a linear interaction fit ($F_{2,13} = 5.842$, $p = 0.031$, $\eta^2 = 0.310$). Post-hoc pairwise comparisons at 12 months did not detect statistically significant differences between the control and maltreated means ($F_{2,13} = 0.009$, $p = 0.925$), $t = 0.948$). No main effect of group was detected. Plots represent mean±standard error of the mean (SEM).

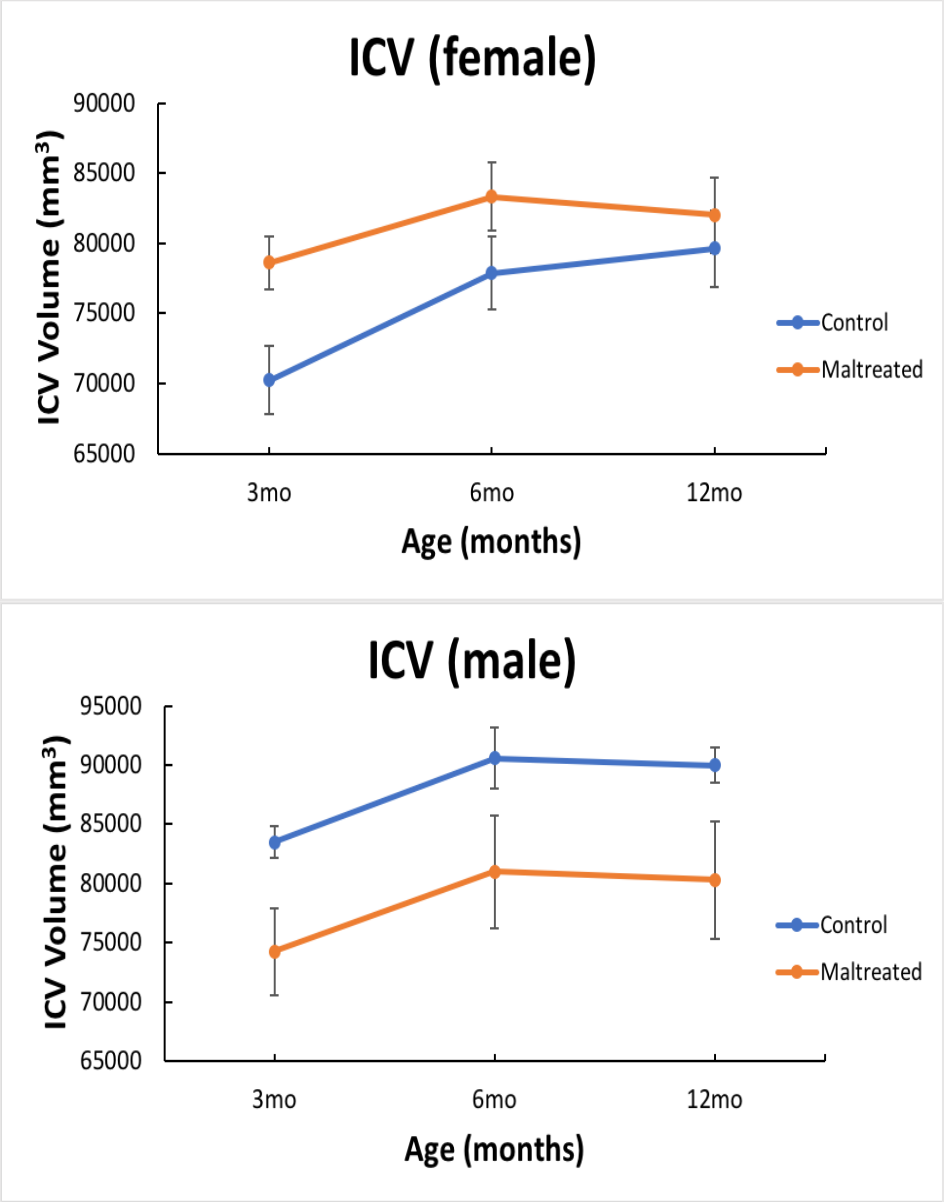

Figure 4. Developmental effects of infant maltreatment on total ICV, separated by sex. The graphs suggest that for females, the maltreated animals have larger ICVs than control females, but for males, the maltreated animals have smaller ICVs than controls. Plots represent mean± SEM.

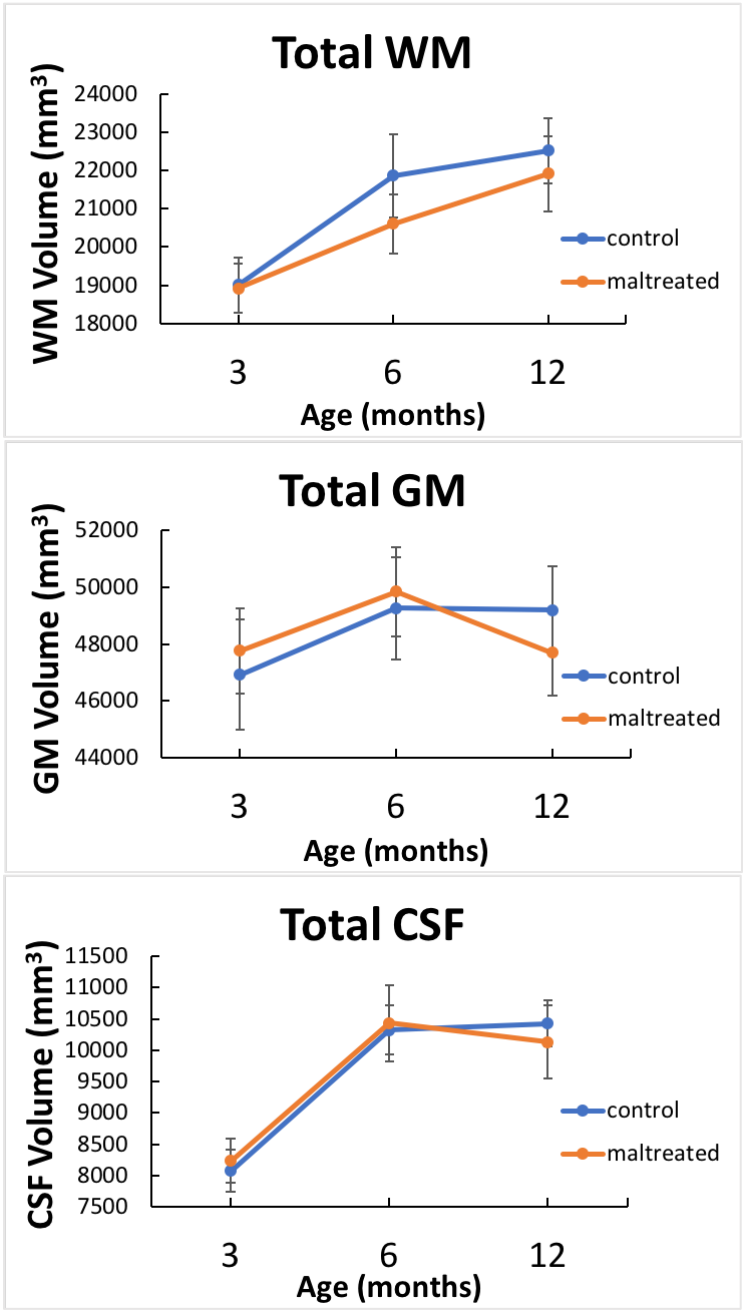

Figure 5. Developmental effects of infant maltreatment on total WM, GM, and CSF. Total WM, GM, and CSF increased with age (WM: $F_{2,13} = 81.523$, $p = 6.290 \times 10^{-12}$, $\eta^2 = 0.862$; GM: $F_{2,13} = 18.724$, $p = 9.186 \times 10^{-06}$, $\eta^2 = 0.590$; CSF: $F_{2,13} = 46.756$, $p = 2.445 \times 10^{-9}$, $\eta^2 = 0.782$). A significant age by group interaction effect was detected in total GM ($F_{2,13} = 6.239$, $p = 0.006$, $\eta^2 = 0.324$) with a linear interaction fit ($F_{2,13} = 8.487$, $p = 0.012$, $\eta^2 = 0.395$), however, post-hoc pairwise comparisons at 12 months did not detect statistically significant differences between the control and maltreated means ($F_{2,13} = 0.023$, $p = 0.881$, $t = 0.645$). Total WM and CSF do not have an age by group interaction effect, and none showed significant group effects. Plots represent mean±standard error of the mean (SEM).

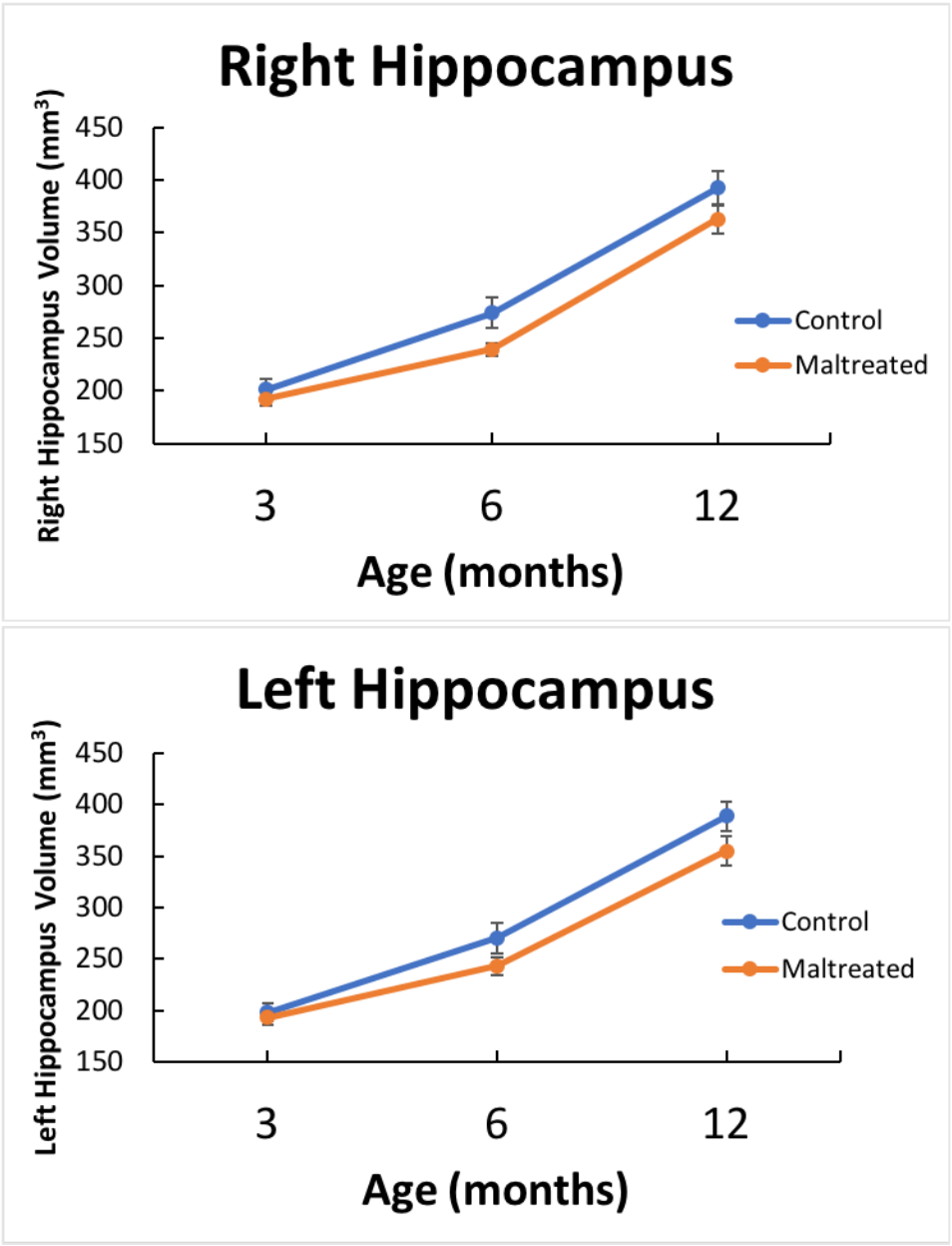

Figure 6. Developmental effects of infant maltreatment on total hippocampal volume. A significant main effects of age was detected ($F_{2,13} = 704.40$, $p = 2.27x10^{-23}$, $\eta^2 = 0.98$), with volume getting bigger as age increases. An age by group interaction ($F_{2,13} = 4.07$, $p = 0.03$, $\eta^2 = 0.24$), with a linear interaction fit ($F_{2,13} = 5.31$, $p = 0.02$, $\eta^2 = 0.29$) was detected in the hemisphere, suggesting the maltreated subjects' hippocampal volume increases at a slower rate compared to the controls. Post-hoc pairwise comparisons show that the control and maltreated left hippocampal volumes are significantly different from each other at 6 months ($F_{2,13} = 7.165$, $p = 0.019$, $t = 1.480$). No age and group interactions were detected in the right hemisphere and no group effects were detected in either hemisphere. Plots represent mean± SEM.

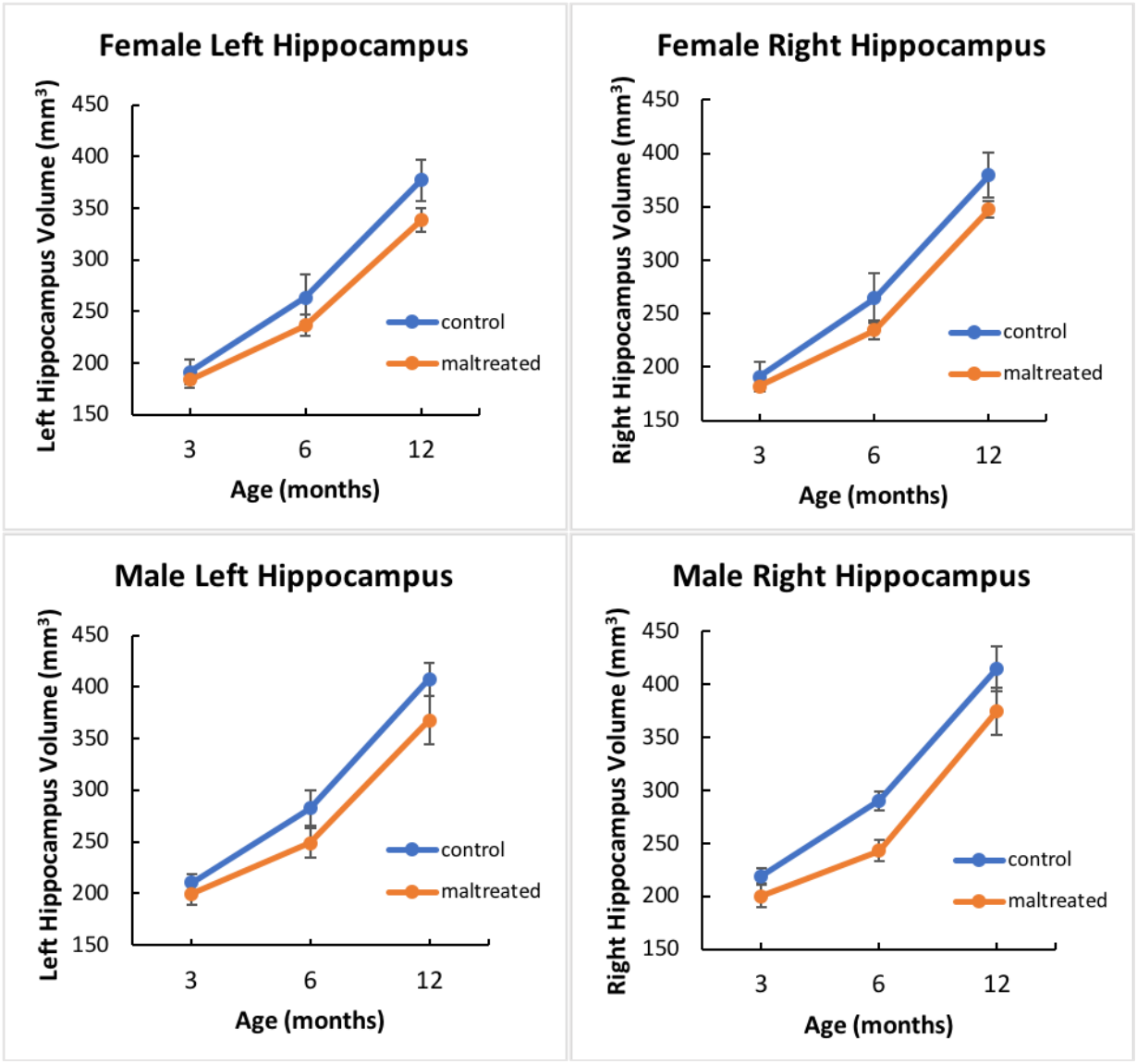

Figure 7. Developmental effects of infant maltreatment on hippocampal volume, separated by sex. Plots suggest that in both males and females show a similar trend of the maltreated group's hippocampal volumes growing at a slower rate than the controls. Plots represent mean± SEM.

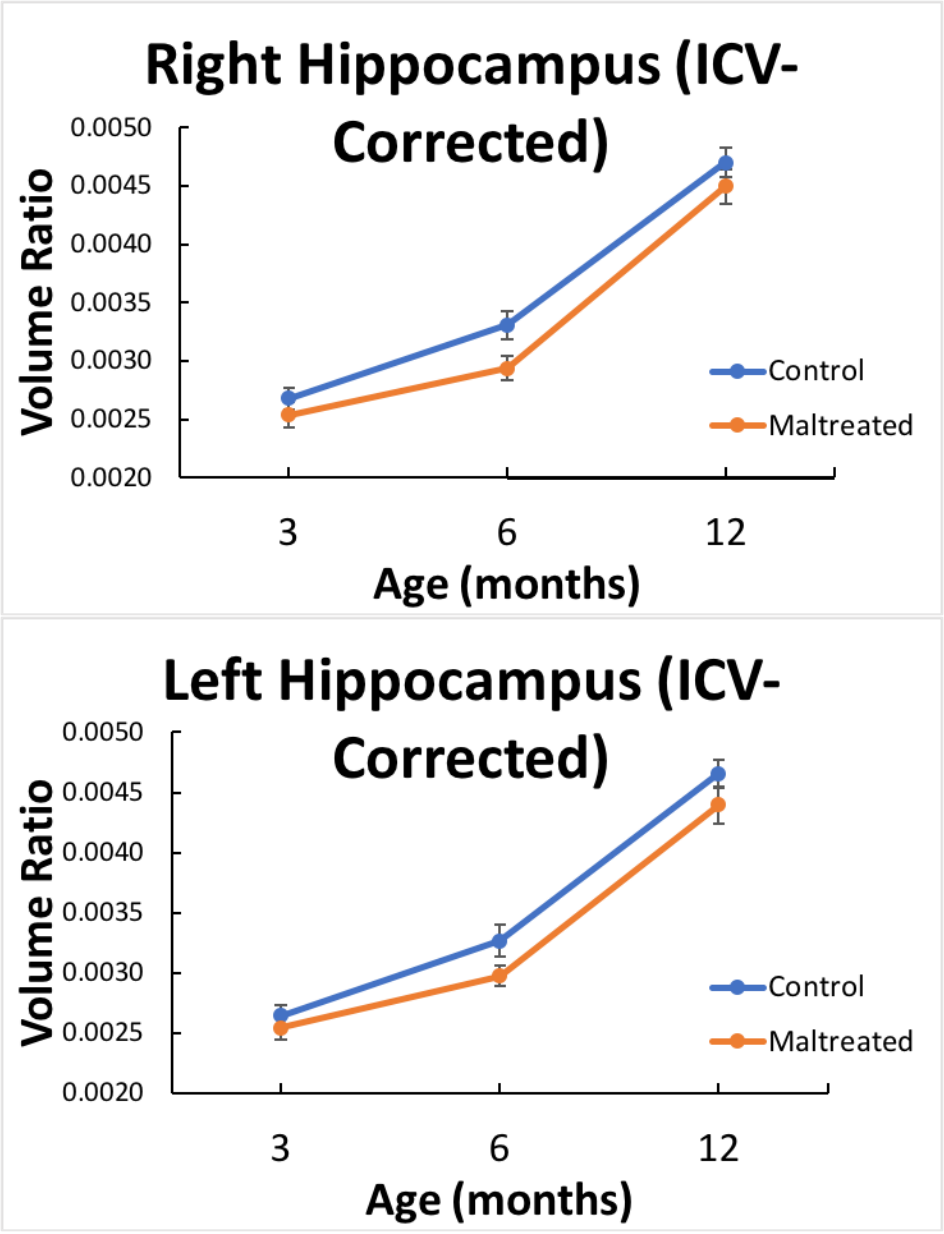

Figure 8. Developmental effects of infant maltreatment on ICV-Corrected hippocampal volumes. Main effects of age were detected in the ($F_{2,13}$ = 831.55, p = 2.72x10^{-24}, η^2 = 0.99), showing that volumes increase with age, independent of ICV growth. No main group or group by age interaction effects were detected. Plots represent volume ratios (hippocampal volume/ICV volumes) ±SEM.

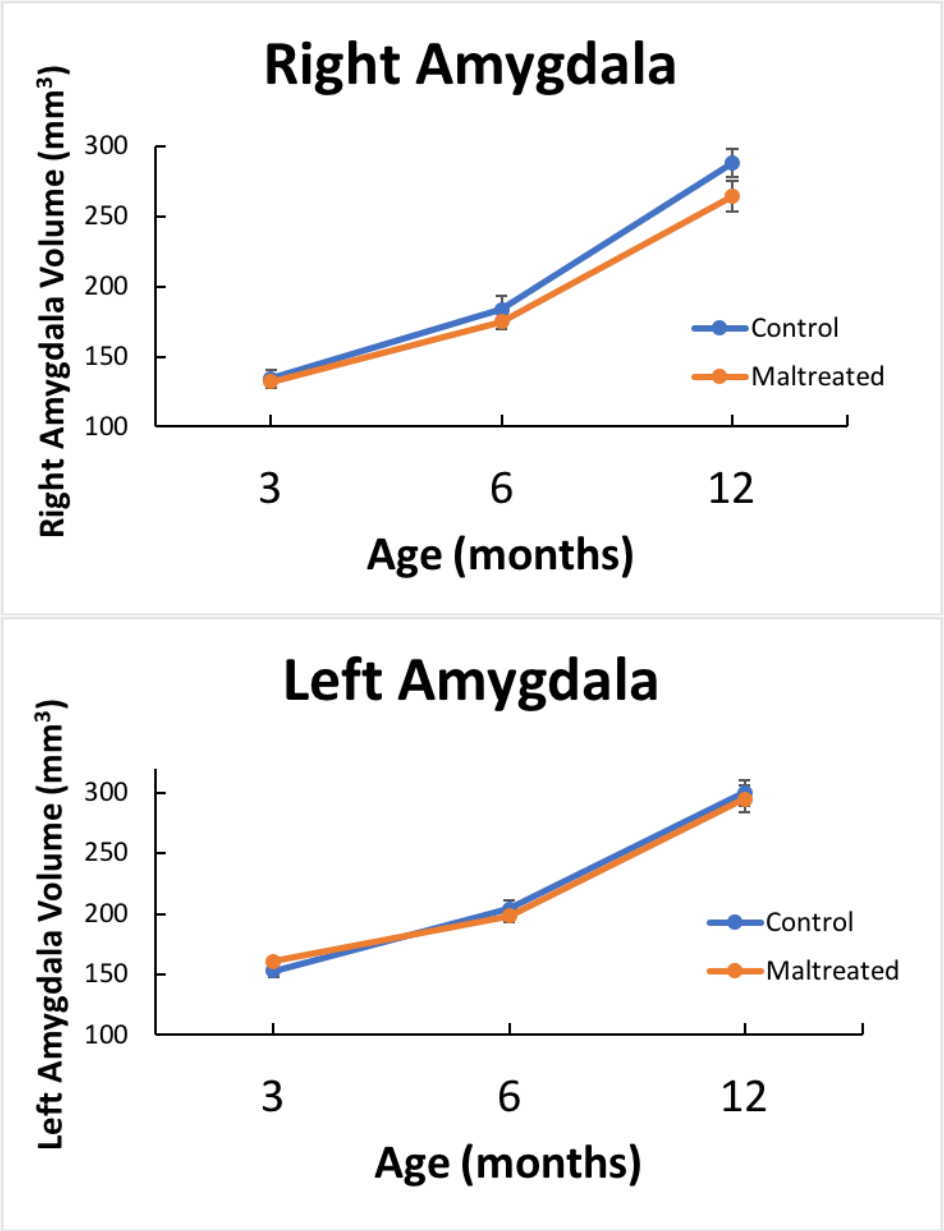

Figure 9. Developmental effects of infant maltreatment on Amygdala volume. In the amygdala, a significant main effect of age was detected, ($F_{2,13}$ = 269.112, p = 1.076x10^{-10}, η^2 = 0.978) suggesting that volumes increase with age. A main effect of hemisphere was detected ($F_{2,13}$ = 176.725, p = 6.06x10^{-9}, η^2 = 0.931) and a hemisphere by group interaction was detected ($F_{2,13}$ = 10.256, p = 0.007, η^2 = 0.441). Plots represent mean± SEM.

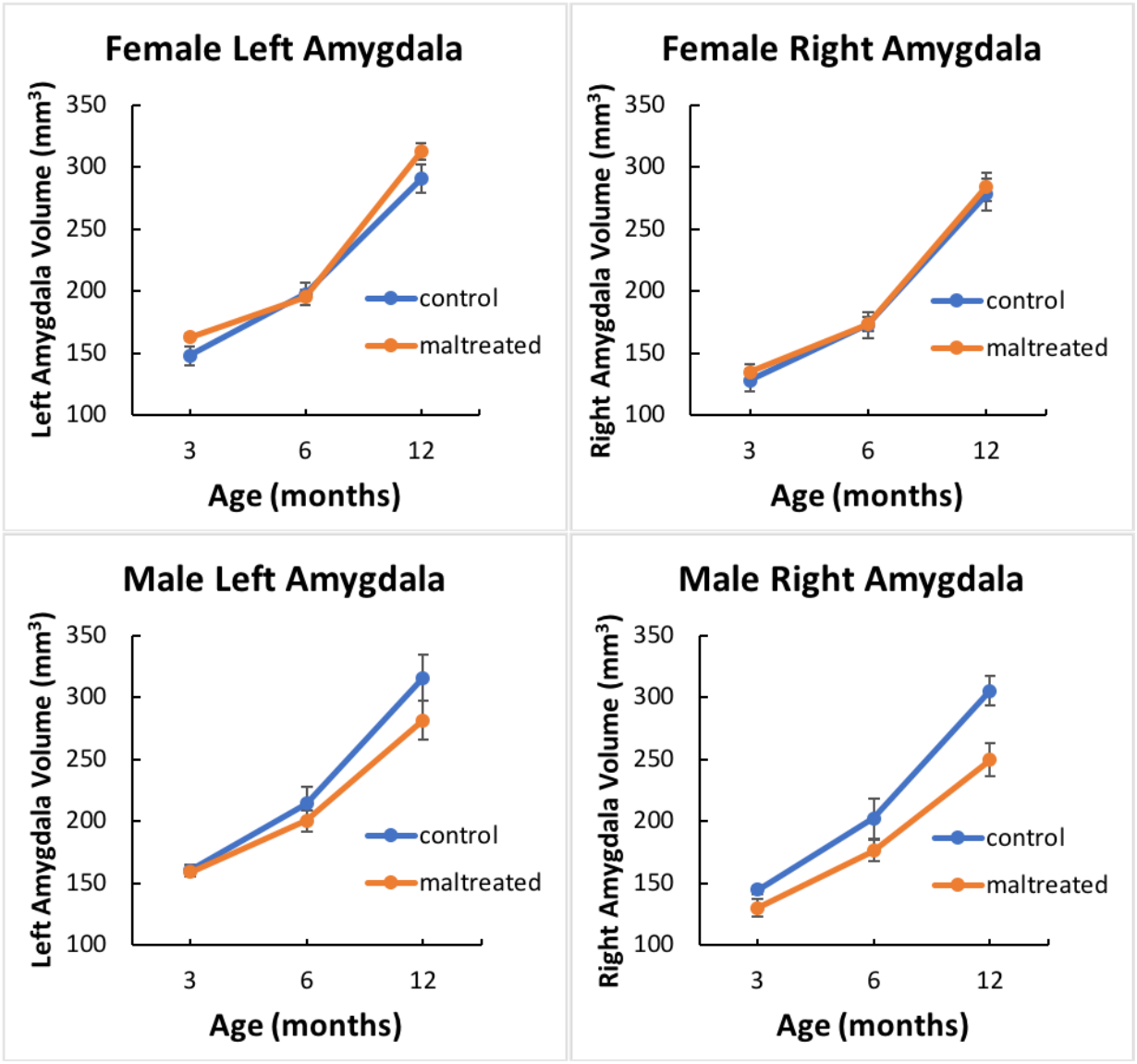

Figure 10. Developmental effects of infant maltreatment on amygdalar volume, separated by sex. Plots suggest that in the males, the maltreated animals have reduced volumes over time. But for the females, the groups are not different. Plots represent mean± SEM.

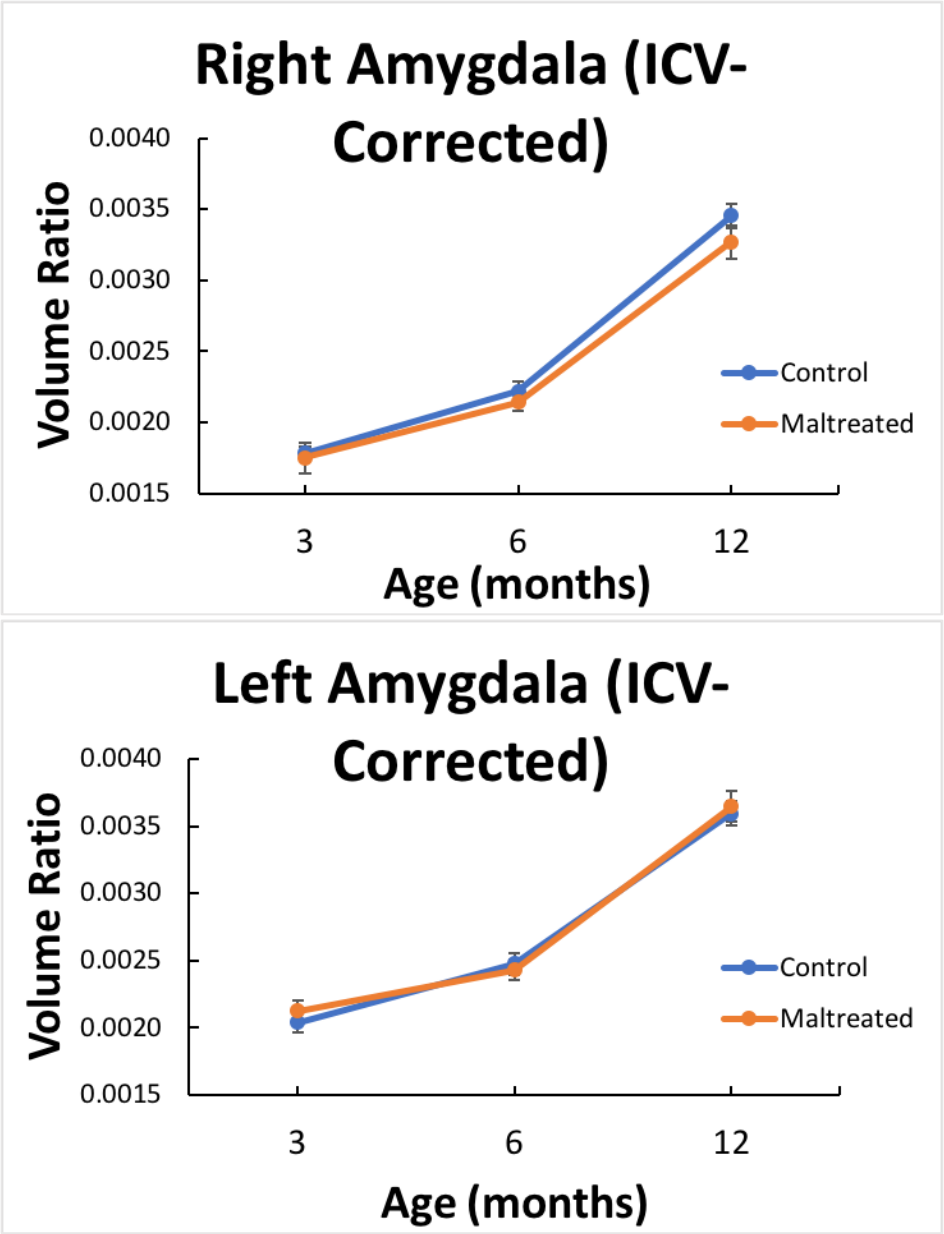

Figure 11. Developmental effects of infant maltreatment on ICV-corrected amygdalar volumes. significant main effect of age was detected, ($F_{2,13} = 560.861$, $p = 4.13x10^{-22}$, $\eta^2 = 0.977$) showing that volumes increase with age, independent of ICV growth. A main effect of hemisphere was detected ($F_{2,13} = 131.57$, $p = 3.58x10^{-8}$, $\eta^2 = 0.91$) as well as a hemisphere by group interaction was detected ($F_{2,13} = 6.76$, $p = 0.02$, $\eta^2 = 0.34$). Plots represent volume ratios (Amygdalar volume/ICV volume) ±standard error of the mean (SEM).

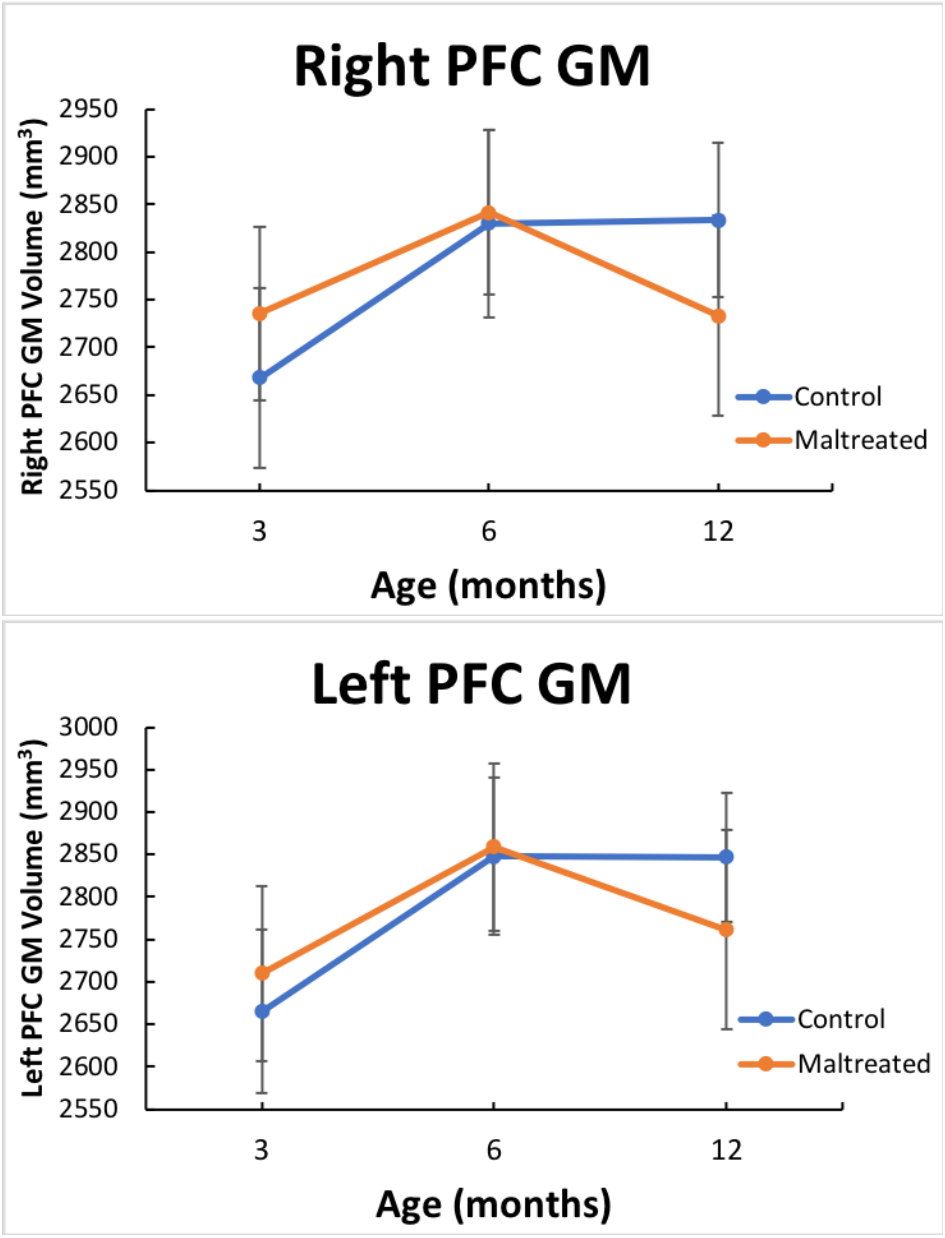

Figure 12. Developmental effects of infant maltreatment on PFC GM volumes. Main effects of age were detected ($F_{2,13} = 21.564$, $p = 3.01x10^{-6}$, $\eta^2 = 0.624$). There is also a significant age by group interaction in PFC GM ($F_{2,13} = 5.445$, $p = 0.011$, $\eta^2 = 0.295$), and the interaction follows a linear trend (right: $F_{2,13} = 9.465$, $p = 0.009$, $\eta^2 = 0.421$). There was a significant age by hemisphere interaction ($F_{2,13} = 4.792$, $p = 0.017$, $\eta^2 = 0.269$).Main effects of group were not detected. Plots represent mean± SEM.

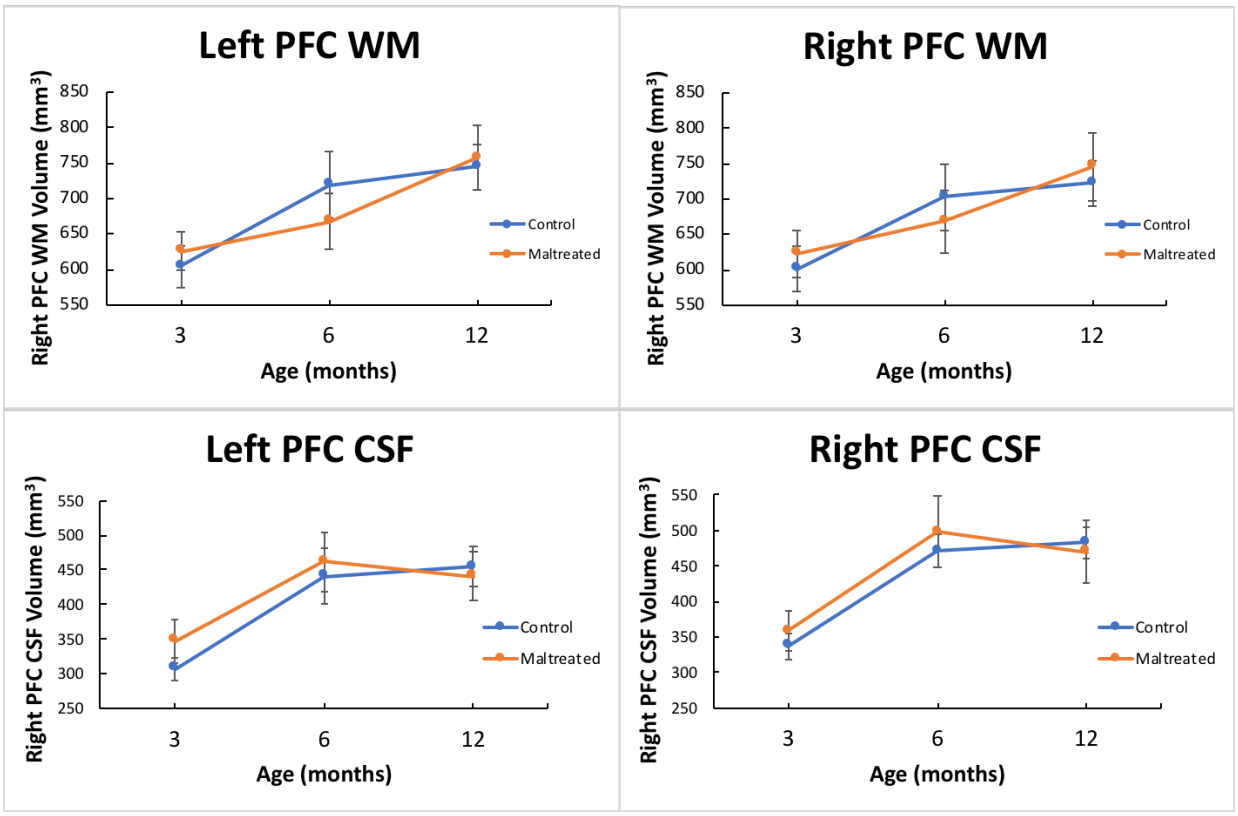

Figure 13. Developmental effects of infant maltreatment on PFC WM and CSF volumes. A significant main effect of age was detected in in WM ($F_{2,13} = 21.786$, $p = 2.77x10^{-6}$, $\eta^2 = .626$), and CSF ($F_{2,13} = 20.234$, $p = 5.02x10^{-6}$, $\eta^2 = 0.609$). A main effect of hemisphere was detected in PFC CSF ($F_{2,13} = 7.801$, $p = 0.015$, $\eta^2 = 0.375$). Plots represent mean± SEM.

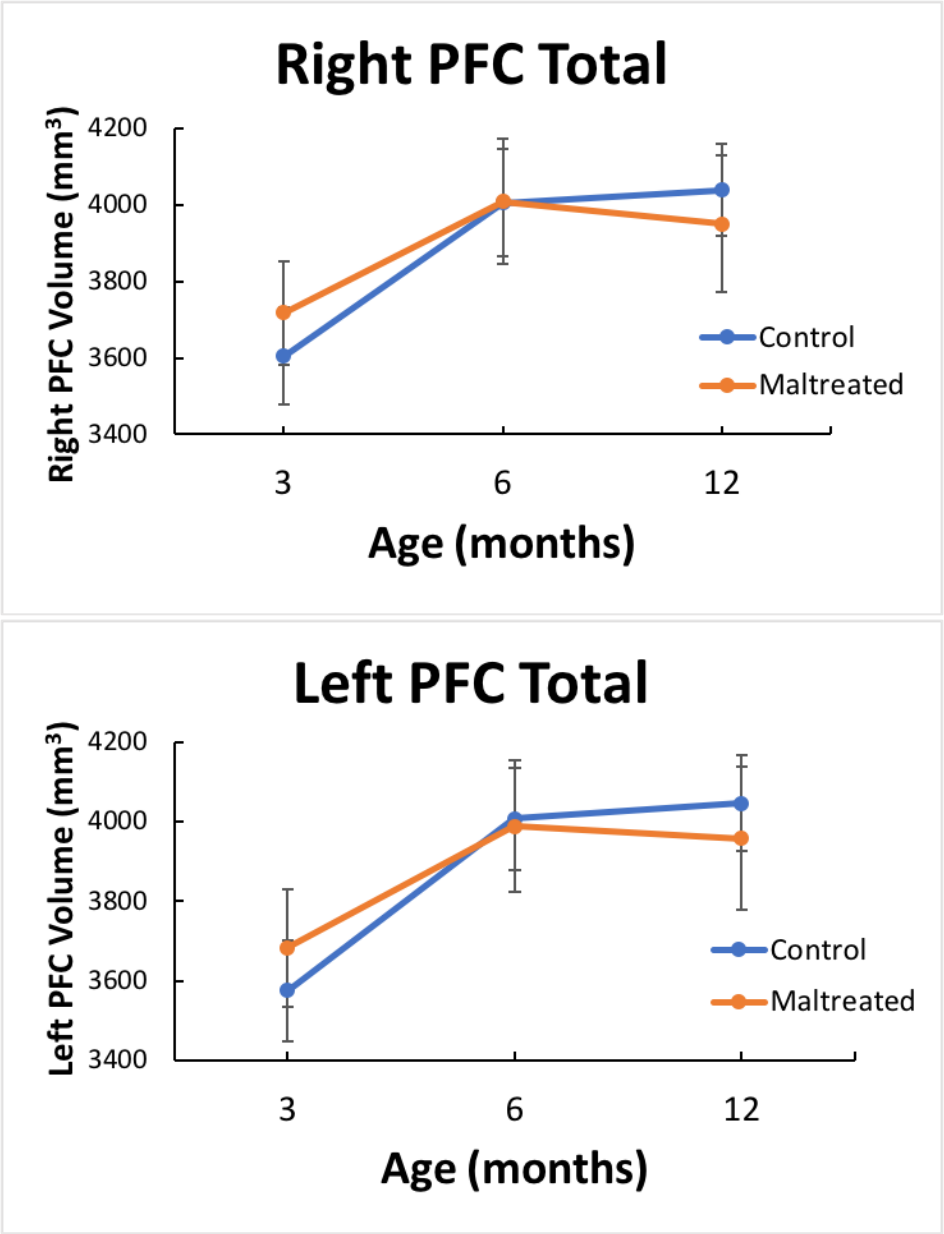

Figure 14. Developmental effects of infant maltreatment on PFC volume. Significant main effect of age were detected ($F_{2,13} = 43.828$, $p = 4.69x10^{-9}$, $\eta^2 = 0.771$) demonstrating volume growth as age increases. There were no group effects or age by group interactions effects detected. Plots represent mean± SEM.

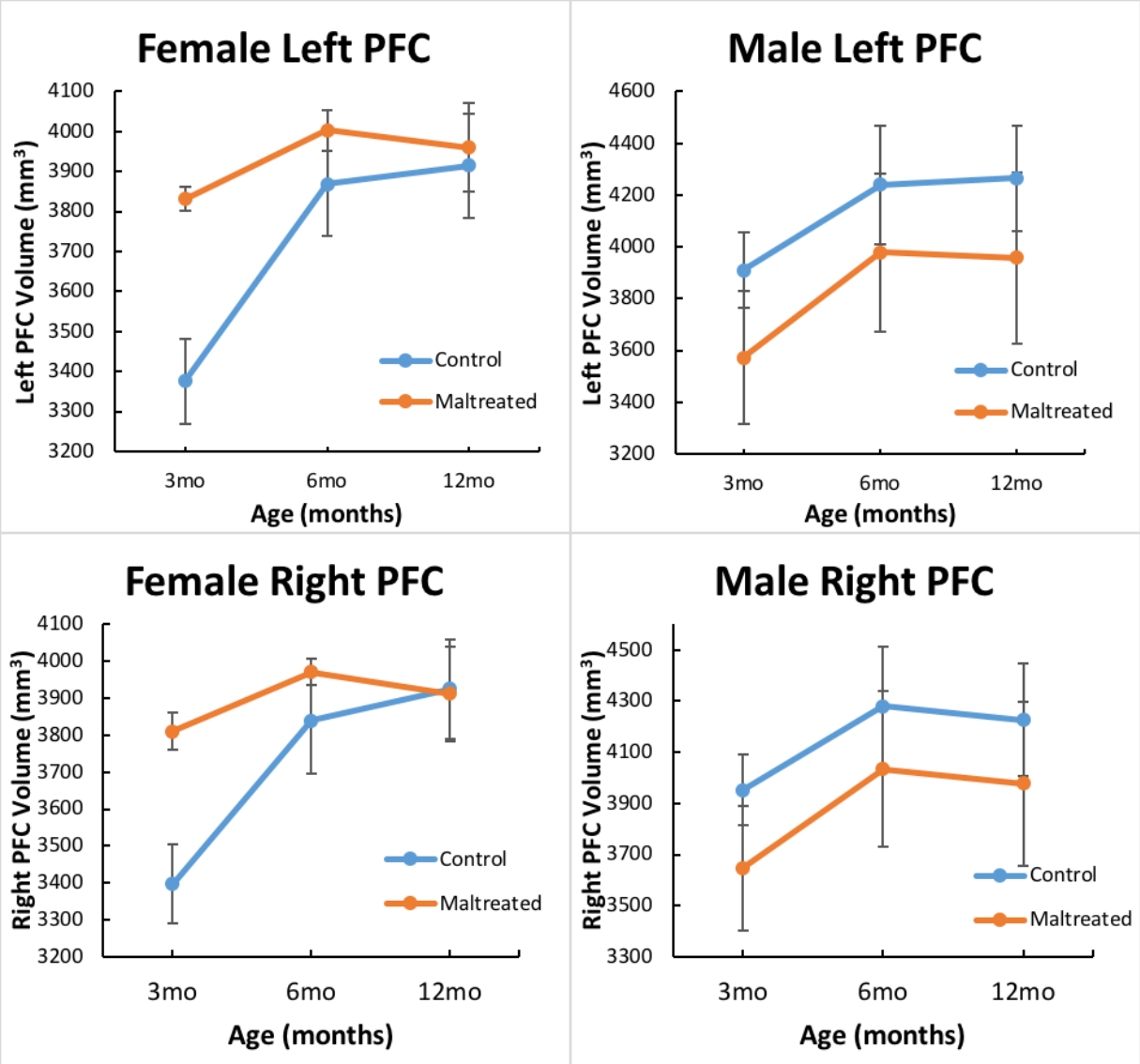

Figure 15. Developmental effects of infant maltreatment on PFC volume, separated by sex. This suggests that the age by group interaction found in the PFC is dependent on the sex. The age by group interaction seems to be present in the females, but no the males. Also, for females, the maltreated animals have larger PFC volumes, but for males, the maltreated animals have smaller volumes. Plots represent mean± SEM.

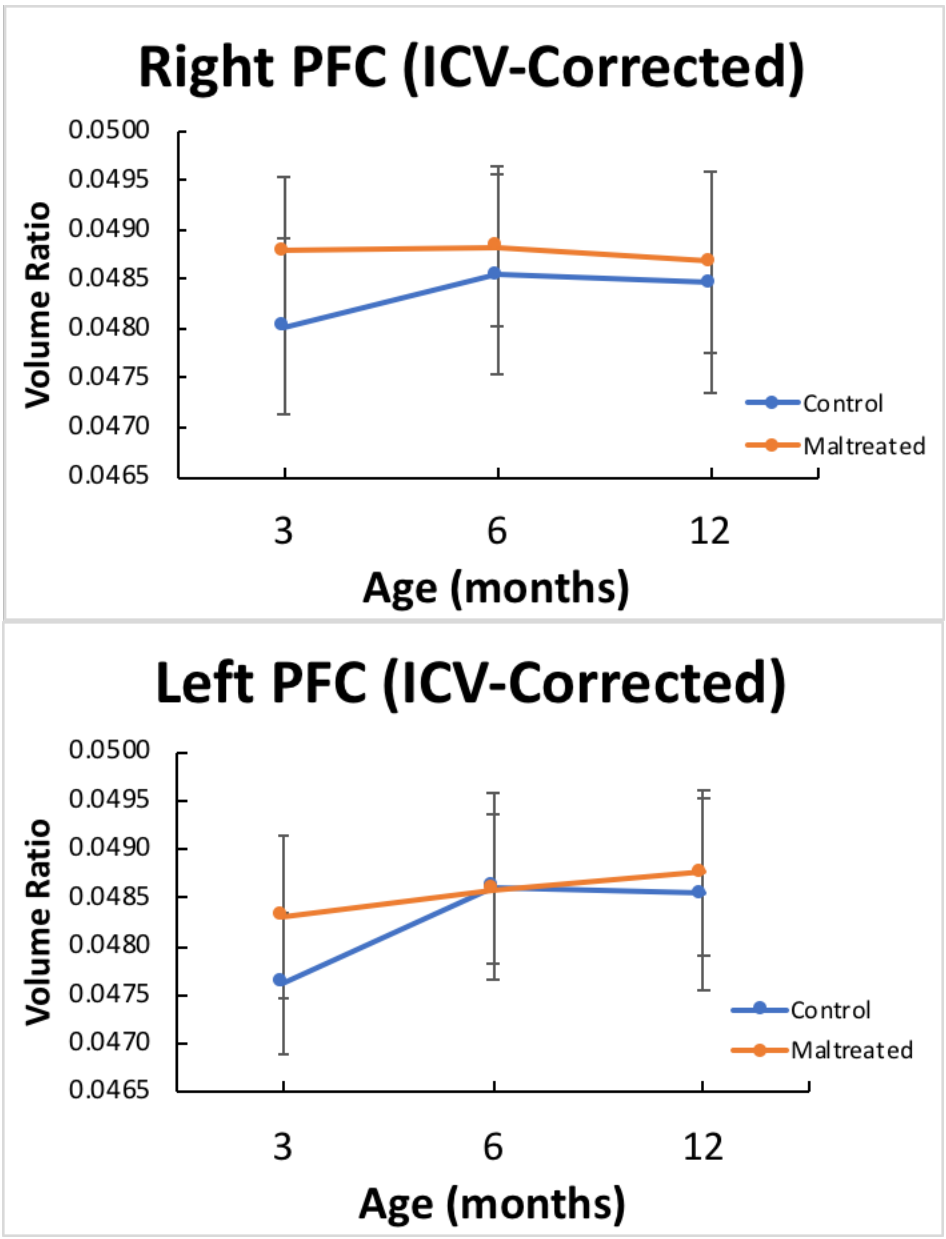

Figure 16. Developmental effects of infant maltreatment on ICV-corrected PFC volumes. No age, group, or age by group effects were detected. Plots represent volume ratios (PFC volume/ICV volume) ±standard error of the mean (SEM).

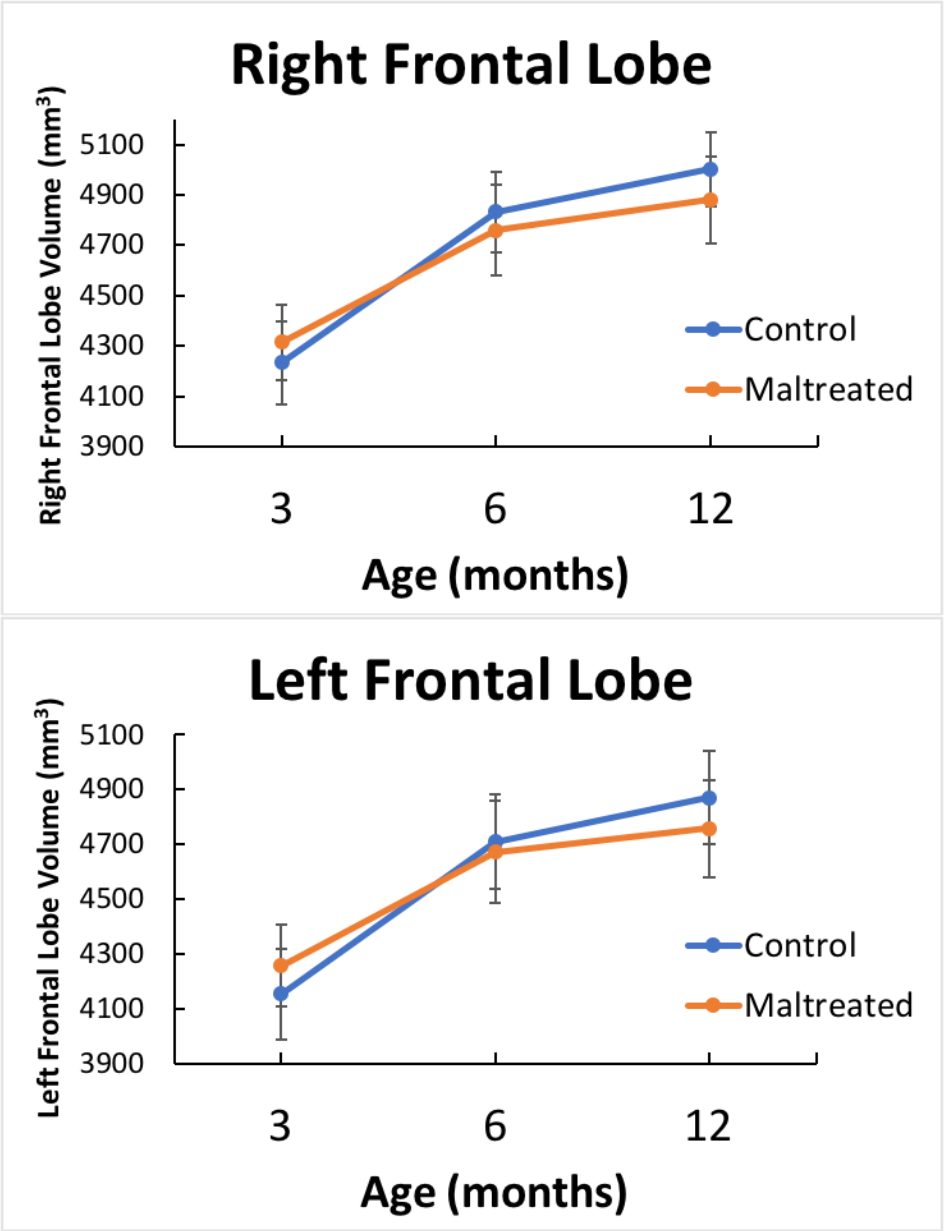

Figure 17. Developmental effects of infant maltreatment on frontal lobe volume. A significant main effect of age ($F_{2,13} = 106.171$, $p = 3.09x10^{-13}$, $\eta^2 = 0.891$) was detected. A significant main effect of hemisphere ($F_{2,13} = 23.857$, $p = 2.98x10^{-4}$, $\eta^2 = 0.647$) and an age by hemisphere interaction ($F_{2,13} = 4.309$, $p = 0.024$, $\eta^2 = 0.249$) was detected. Plots represent mean± SEM.

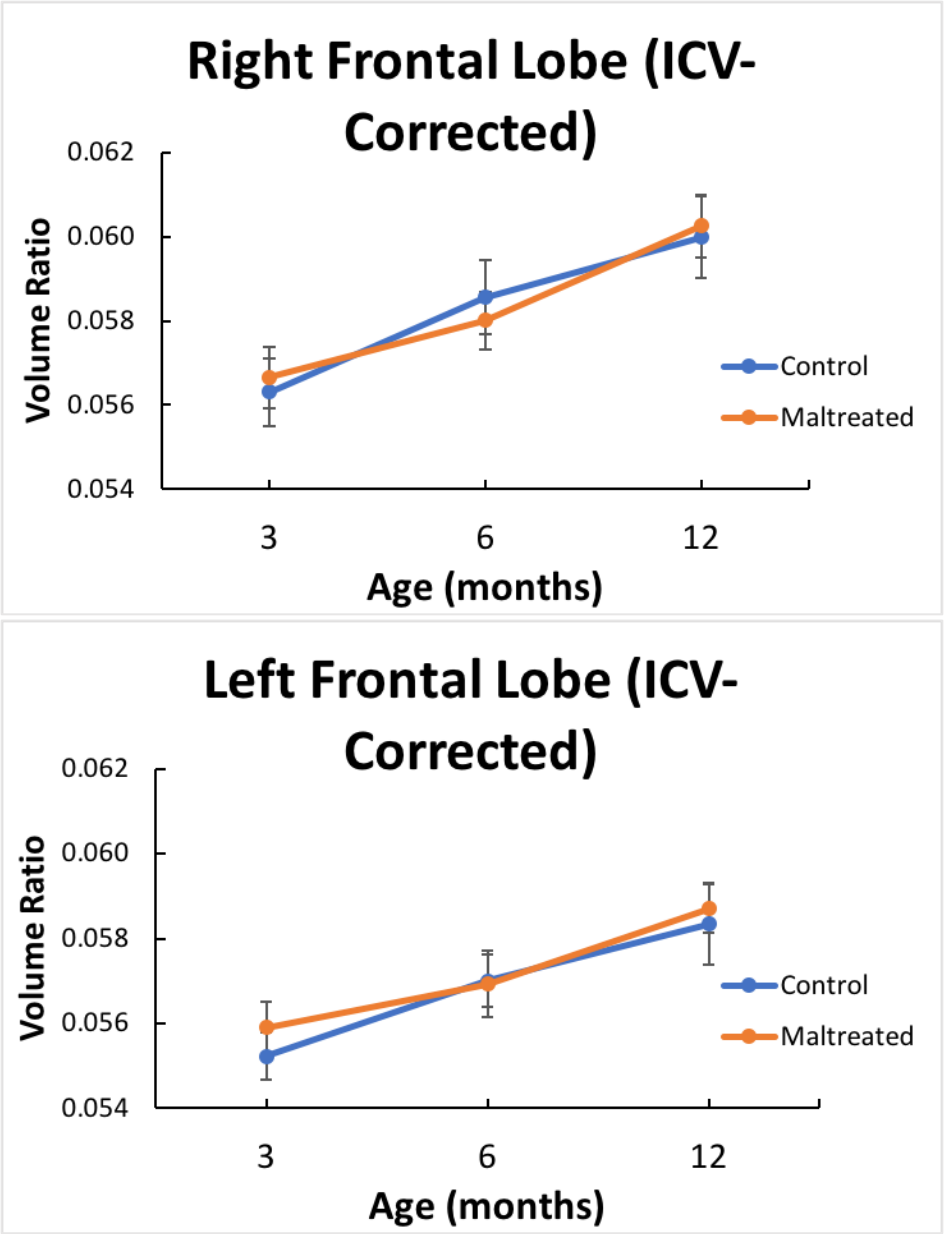

Figure 18. Developmental effects of infant maltreatment on ICV-Corrected frontal lobe volumes. Main effects of age were detected ($F_{2,13} = 58.69$, $p = 2.49x10^{-10}$, $\eta^2 = 0.82$) showing that volumes increase with age, independent of ICV growth. A main effect of hemisphere ($F_{2,13} = 24.19$, $p = 2.81x10^{-4}$, $\eta^2 = 0.65$)There were no group or age by group interactions effects detected. Plots represent volume ratios (frontal lobe volume/ICV volume) ±standard error of the mean (SEM).

Table 1. Descriptive Statistics for Hair Cortisol Levels, Abuse Rates, and Rejection Rates.

	Hair Cortisol (pg/mg hair) Birth	Hair Cortisol (pg/mg hair) 6 months	Abuse (mean rates/hr; mo1-3)	Rejection (mean rates/hr; mo1-3)
Control	$\bar{x}$=559.922 SE=12.759	$\bar{x}$=111.664 SE=4.639	$\bar{x}$=0.007 SE=0.003	$\bar{x}$=0.203 SE=0.060
Maltreated	$\bar{x}$=534.980 SE=19.675	$\bar{x}$=133.081 SE=9.272	$\bar{x}$=1.383 SE=0.254	$\bar{x}$=3.317 SE=0.450

$\bar{x}$ = mean

SE = standard error of the mean

Table 2. Pearson's Correlations Between increased ROI Volumes from 3 to 12 months and Hair Cortisol Levels, Abuse Rates, and Rejection Rates.

	Hair Cortisol (pg/mg hair) Birth	Hair Cortisol (pg/mg hair) 6 months	Abuse (mean rates/hr; mo1-3)	Rejection (mean rates/hr; mo1-3)
Δ ICV	r=-0.189 p=0.577	r=0.310 p=0.384	r=0.116 p=0.734	r=-0.456 p=0.158
Δ R Hipp	r=0.394 p=0.230	r=0.366 p=0.298	r=-0.030 p=0.931	r=-.638* p=0.035
Δ L Hipp	r=0.158 p=0.643	r=0.480 p=0.161	r=0.041 p=0.904	r=-0.591 p=0.056
Δ R Amy	r=0.546 p=0.082	r=-0.046 p=0.900	r=-0.135 p=0.691	r=-0.492 p=0.124
Δ L Amy	r=0.402 p=0.221	r=0.127 p=0.726	r=0.045 p=0.896	r=-0.293 p=0.382
Δ R PFC	r=-0.215 p=0.525	r=0.308 p=0.386	r=0.040 p=0.908	r=-0.398 p=0.226
Δ L PFC	r=-0.305 p=0.362	r=0.260 p=0.469	r=0.031 p=0.929	r=-0.397 p=0.226
Δ R Front	r=-0.193 p=0.570	r=0.129 p=0.722	r=0.138 p=0.686	r=-0.312 p=0.350
Δ L Front	r=-0.154 p=0.651	r=0.070 p=0.847	r=0.043 p=0.900	r=-0.411 p=0.209

* denotes significance at $p < 0.05$ level
L = Left
R= Right
Hipp=hippocampus
Amy=amygdala
Front=frontal lobe

Discussion

The goal of this study was to examine the effects of infant maltreatment on brain development by focusing on the structural development of the hippocampus, amygdala, and prefrontal cortex, due to their critical role in stress and emotional regulation and vulnerability to ELS. This was done by using a naturalistic infant maltreatment model in rhesus monkeys and comparing the volumetric changes of these brain regions longitudinally, at 3, 6 and 12 months of age (equivalent to 1, 2, and 4 years of age in human children) using structural MRI approaches. Because maltreatment is a stressful experience for the infants and results in increased release of the stress hormone cortisol (Drury et al., 2017), and both prenatal and postnatal cortisol have programming effects on brain development (Lupien et al., 2009), I also examined the associations between hair cortisol levels (accumulated prenatally and over the first six months of life) and brain structural changes with age. In addition, associations between abuse and rejection rates received during infancy and MRI volumetric changes were investigated. Overall, significant increases in total brain and regional volumes were detected by MRI from 3 to 12 months of age in both control and maltreated groups. However, maltreated macaques showed slower growth rates in total brain volume (measured as ICV) and hippocampal volumes emerging with increasing age. Although no associations were found between prenatal or postnatal cortisol exposure and MRI volumetric measures, the blunted hippocampal volume growth detected in the maltreated group was predicted by maternal rejection rates experienced early in life. In addition, lateralized group effect were found in the amygdala, which persisted when corrected for total brain size. These findings suggest that ELS in the form of infant maltreatment resulted in global developmental effects that affected brain volume growth, as well as hippocampal and amygdala-specific impact that emerged during infancy and the juvenile

period. The region-specific effects of the ELS experience (i.e. impact on hippocampal and amygdala, but not PFC volumetric growth) during this developmental period, as well as the potential that differences in directionality, magnitude and developmental trajectory of the changes might vary with age, has been previously discussed for humans (Tottenham & Sheridan, 2010).

Total brain size, measured as ICV in this study (GM + WM + CSF) showed a significant increase with age during infancy and the early juvenile period (from 3 to 12 months of age) in both control and maltreated groups. These findings were expected and are consistent with previous reports in humans and in macaques (Giedd & Rapoport, 2010; Knickmeyer et al., 2010; C. Liu et al., 2015; Malkova et al., 2006), with this being a critical period for brain development (Gale et al., 2004). Specifically, the increase in ICV detected in our sample is in agreement with two longitudinal structural MRI studies in macaques that show significant increases in ICV across the first year of life, although particularly rapid during the first 4 postnatal months (C. Liu et al., 2015; Malkova et al., 2006). In humans, ICV follows an inverted U shaped growth curve, with volumes increasing throughout childhood and peaking at age 10.5 years in females and 14.5 in males and then decreasing thereafter (Giedd & Rapoport, 2010). Thus, our findings match the expected ICV increases throughout infancy and the early juvenile period, and it is possible that a peak and decrease can be seen at later ages not included in this study (2-3 years). As shown in Figure 5, the age-related increase in ICV is driven by increases in total brain GM, WM and CSF volumes, which is consistent with both reports in humans and macaques. For example, the total brain WM increases with age are consistent 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., 2011; Liu et al., 2015), total GM volume increased with age, with significant increases in GM volume reported during the first year of life in humans (equivalent to the first 3 months in rhesus), followed by much smaller increase in GM volume the second year of life (Gilmore et al., 2011). A study done with older human children showed an inverted U-shape pattern of development of GM volume, with volumes peaking during late childhood (at approximately 8 years in girls and 11 years in boys) and declining thereafter (Giedd & Rapoport, 2010), phenomenon replicated in juvenile macaques (Liu et al., 2015), although at later ages than those studied here.

A significant linear age by group interaction was detected in ICV as well. This interaction suggests smaller ICV in maltreated animals in comparison to controls, but the difference emerging after 6 months and becoming more pronounced with age. This interaction seems driven by maltreatment effects on total GM, which is the only tissue class that showed the same interaction effect detected in ICV, suggesting that GM growth may be more vulnerable to the effects of ELS than other tissue classes at these ages. The smaller ICV and GM volumes in maltreated animals are consistent with decreased volumes in people who have experienced childhood maltreatment, and the effects may become more pronounced in later childhood or around adolescence (Teicher et al., 2006).

Although sex could not be added to the statistical models as a factor due to the small sample size, when sex was included as a covariate in the RM ANCOVA, it did not change any of the group effects except for the ICV. In fact the ICV plots for males and females suggest potential sex-differences. Males appeared to have overall bigger brain volumes compared to females. These findings are consistent with previous studies in macaques (Scott et al., 2016;

Malkova et al., 2006; Franklin et al., 2000), as well as humans (Giedd & Rapoport, 2010). The graphs also suggest potential sex differences in ELS impact, with maltreated males showing reduced ICV compared to control males across all ages, while maltreated females show bigger ICVs compared to controls initially (at 3 months), followed by a growth slow from 6-12 months. These findings may support previous reports of sex-related differences in ELS vulnerability and patterns of developmental impacts(Samplin et al., 2013; Loi et al., 2017).

A significant increase in hippocampal volume was detected with age, indicating that the hippocampus is growing as the young macaques get older. The hippocampal growth is slow until 2 years of age in humans (equivalent to 6 months old in rhesus macaques) and accelerates thereafter, growing until adulthood (Gogtay et al., 2006; Tottenham et al., 2010; Knickmeyer et al., 2008), which is consistent with our findings of stronger hippocampal growth occurring from 6 to 12 months, than from 3 to 6 months shown in Figure 6. In addition, the developmental changes in hippocampal volume do not seem to show sex differences, as shown in Figure 7, which is consistent with the lack of sex effects previously reported in infant rhesus monkey hippocampal development (Payne et al., 2010).

Significant effects of maltreatment were also detected in the hippocampus, showing a region-specific impact. The linear age by rearing group interaction effect detected suggest that the impact of the adverse maternal care on the hippocampus emerged with age, specifically at 6 months (Figure 6). These findings support the hypothesis that the ELS experience would impact hippocampal development, and it is consistent with previous reports in humans showing that hippocampal volumes are smaller in people who experienced adversity early in life (Lupien et al., 2009; Hanson et al., 2015; Tottenham & Sheridan, 2010), although findings in children are inconsistent and other studies report no ELS impact on hippocampal volume during development

(De Bellis et al., 2001; Tottenham & Sheridan, 2010). And if hippocampal growth is slow during infancy and accelerates after 6 months, one would expect the differences between groups to become more pronounced over time as well. Although the biological mechanism for stress-related cellular changes in the brain are still not fully understood, a potential mechanism for the reduced volumes detected in the hippocampus of the maltreated group explored in this thesis was the potential association with the increased levels of the stress hormone cortisol reported during infancy in the maltreated group (see Table 1 and Drury et al., 2017). According to Popoli and colleagues, increased stress-induced glucocorticoid release can lead to increased glutamate release. When glutamate levels are too high, they can result in excitotoxicity, with damage/shrinkage of PFC and hippocampal dendrites, while the opposite –dendritic growth- can take place in the amygdala (Popoli et al., 2011). However, no correlation was found between cortisol accumulated in hair grown from birth to 6 months of age, suggesting that other biological mechanisms may be involved. Interestingly, stress has been reported to affect hippocampal neural structure not just through increased cortisol, but direct increase in glutamate excitotoxicity (Sapolsky et al., 1985; McEwen, 1998). Unfortunately, we don't have any measure of glutamatergic function at the level of the hippocampus in this study to demonstrate this potential alternative, which will have to be tested in future studies. When hippocampal volumes were ICV-corrected to account for individual and group differences in total brain size, the interaction effect disappeared. This suggests that the maltreatment impact on hippocampal growth may track along the global effects of the ELS experience on overall brain growth.

Although no associations were detected between postnatal hair cortisol and hippocampal volumes, we did find a significant negative correlation between rejection rates experienced during infancy and hippocampal volume growth between 3 and 12 months, such that animals

who experienced more rejection showed blunted hippocampal growth. It is possible that reduced maternal tactile stimulation affected the structural development of the hippocampus, as previously reported in rats, where maternal licking and grooming during the first week of life promotes structural development of rat pups hippocampus (Bredy et al., 2003; D. Liu et al., 2000; see Howell & Sanchez, 2011 for review), apparently via increased neonatal brain 5HT release –which works as a neurotrophic factor during brain development- in the hippocampus (Smythe et al., 1994; Cenci & Kalen, 2000). Thus, tactile stimulation provided by the mother can regulate the development of brain systems involved in emotional and stress responses, independently of glucocorticoids (van Oers et al., 1998). Interestingly, our lab has previously reported that that maternal rejection is more prolonged than abuse in this infant maltreatment model and is a better predictor of poor developmental outcomes, including reduced brain serotonin (5HT) (Sanchez et al., 2007; Howell et al, 2016), which could have impacted hippocampal structural development.

In the amygdala, a main effect of age was detected, which was expected due to the normative growth of the brain during development. Payne and colleagues have shown that in macaques, amygdalar volume increases during the first 2 years of life, with a higher rate of growth during the first 2 weeks (Payne et al., 2010). Similar to macaques, humans show amygdalar volume growth during childhood, and particularly rapid growth soon after birth (Tottenham & Sheridan, 2010). Lateralized effects of the early rearing experience were also detected so that the amygdala volume is smaller in the right, but not in the left, hemisphere of maltreated animals than in control suggesting that maternal care did not affect the right and left amygdalae growth the same way. Other studies have shown lateralization effects in the amygdala

associated with ELS, such as Hanson and colleagues' findings of a smaller left amygdala in children who have experience ELS, but no effect on the right amygdala (Hanson et al., 2015).

Although multiple studies indicate that amygdala volumes increase as a result of maltreatment in humans (Mitra et al., 2005; Tottenham et al., 2010) and even in a previous cohort of adolescent macaques studied by our groups (Howell et al, 2014), this is not consistent in all studies, and sometimes smaller amygdala volumes are reported in children with ELS with laterality effects (Hanson et al., 2015). or no effects (Cohen et al., 2006; Howell et al., 2014). The inconsistency in the literature makes it difficult to interpret the amygdala findings in our study. A possible explanation for this inconsistency could be that the amygdala experiences rapid growth in response to ELS, but throughout development, the increased activity leads to dendritic and synaptic damage, resulting in reduced structural volumes. (Hanson et al., 2015; McEwen, 2005; Tottenham & Sheridan, 2010). Since the amygdala is active and still being fine-tuned until adulthood (Tottenham & Sheridan, 2010), it is possible that the differences will emerge at a later age (Howell et al., 2014).

Although our study is underpowered to make any conclusions about sex-effects, and the results adding sex as covariate in the ANCOVA models did not change the overall findings, the visual inspection of the graphs may support previous reports of sex-related differences in ELS vulnerability and patterns of developmental impacts. Figure 10 suggests that adverse care might have a stronger developmental impact on the amygdala of males than females because the amygdala is still developing in these animals until adulthood, and the development is protracted in the males, maybe the sex difference suggested by Figure 10 is due to their different developmental maturity. PFC volume was found to increase with age, similar to the other regions of interest. Typically PFC growth in macaques shows an inverted U shape driven by both PFC

WM and GM, with the peak occurring during adolescence (Rebecca C. Knickmeyer et al., 2010). In humans, PFC GM also has an inverted U growth trajectory peaking at adolescence, but PFC WM continues to grow past adolescence (Giedd & Rapoport, 2010). When investigating the WM, GM, and CSF in the PFC, a linear age by group interaction effect was detected in PFC GM. This interaction suggests reduced PFC GM volumes at 12 months in the maltreated group, as shown in Figure 12. Additionally, another study had found a decrease in total GM after 10 months of age in rhesus macaques (C. Liu et al., 2015), so perhaps the decrease in PFC GM is a common effect found in late infancy in rhesus macaques and the effect is exacerbated by maltreatment. No effect of maternal care quality was detected in PFC WM or CSF, but the PFC continues to develop past early childhood (Hanson et al., 2012), therefore, it is possible that these structural changes emerged after 12 months because the PFC was not finished developing at the last time point of this study.

A major limitation of this study is the small sample size of 15 animals, which may not be representative of the total population; therefore, the findings reported here should be considered preliminary until analyses in the full cohort of 42 animals are completed. Another limitation is the short period of development being analyzed (3-12 months). There is additional longitudinal data being analyzed at 2 weeks, 18 months, and 5 years of age with the same subjects. Including those additional time points will provide a better understanding of the developmental effects of early life infant maltreatment from infancy through adolescence.

In summary, the findings of this study suggest that maternal care affects the structural development of the brain. ELS animals in this study were maltreated during infancy, with the highest rates of abuse and rejection experienced during the first 3 months of life. Using a cross fostering design to reduce the confounding effects of heritability, we found reductions in ICV,

PFC GM, and hippocampal volumes of maltreated animals in comparison to controls, but the differences emerged with increasing age. For ICV and PFC GM, maltreated rhesus macaques showed smaller volumes at 12 months than controls, while the maltreatment-related effects emerged at 6 months in the hippocampus. Rejection rates predicted reduced hippocampal volumes, further supporting the association of smaller hippocampal volumes with ELS. Lateralized group effects were found in the amygdala, suggesting reduced volumes in the right amygdala of the maltreated animals. Additional studies with these animals as well as future longitudinal studies will be necessary to fully understand ELS's impact on brain structural development.

Literature Cited

Amaral, D. G., & Bassett, J. L. (1989). Cholinergic innervation of the monkey amygdala: an immunohistochemical analysis with antisera to choline acetyltransferase. *Journal of comparative Neurology, 281*(3), 337-361.

Arnsten, A. F. T. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nature reviews. Neuroscience, 10*(6), 410-422. doi:10.1038/nrn2648

Bredy, T. W., Grant, R. J., Champagne, D. L., & Meaney, M. J. (2003). Maternal care influences neuronal survival in the hippocampus of the rat. *European Journal of Neuroscience, 18*(10), 2903-2909.

Bremner, J. D., & Vermetten, E. (2001). Stress and development: behavioral and biological consequences. *Development and Psychopathology, 13*(3), 473-489.

Brent, L., Koban, T., & Ramirez, S. (2002). Abnormal, abusive, and stress-related behaviors in baboon mothers. *Biological Psychiatry, 52*(11), 1047-1056.

Cenci, M. A., & Kalen, P. (2000). Serotonin release from mesencephalic raphe neurons grafted to the 5, 7-dihydroxytryptamine-lesioned rat hippocampus: effects of behavioral activation and stress. *Experimental neurology, 164*(2), 351-361.

Cohen, P., Brown, J., & Smailes, E. (2001). Child abuse and neglect and the development of mental disorders in the general population. *Development and Psychopathology, 13*(4), 981-999.

Cohen, R. A., Grieve, S., Hoth, K. F., Paul, R. H., Sweet, L., Tate, D., . . . Williams, L. M. (2006). Early Life Stress and Morphometry of the Adult Anterior Cingulate Cortex and Caudate Nuclei. *Biological Psychiatry, 59*(10), 975-982. doi:https://doi.org/10.1016/j.biopsych.2005.12.016

Davenport, M. D., Tiefenbacher, S., Lutz, C. K., Novak, M. A., & Meyer, J. S. (2006). Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *General and Comparative Endocrinology, 147*(3), 255-261. doi:https://doi.org/10.1016/j.ygcen.2006.01.005

De Bellis, M. D., Hall, J., Boring, A. M., Frustaci, K., & Moritz, G. (2001). A pilot longitudinal study of hippocampal volumes in pediatric maltreatment-related posttraumatic stress disorder. *Biological Psychiatry, 50*(4), 305-309. doi:https://doi.org/10.1016/S0006-3223(01)01105-2

Doom, J. R., & Gunnar, M. R. (2013). Stress physiology and developmental psychopathology: past, present, and future. *Development and Psychopathology, 25*(4pt2), 1359-1373.

Drury, S. S., Howell, B. R., Jones, C., Esteves, K., Morin, E., Schlesinger, R., . . . Sanchez, M. M. (2017). Shaping long-term primate development: Telomere length trajectory as an indicator of early maternal maltreatment and predictor of future physiologic regulation. *Development and Psychopathology, 29*(5), 1539-1551. doi:10.1017/S0954579417001225

Ehlert, U. (2013). Enduring psychobiological effects of childhood adversity. *Psychoneuroendocrinology, 38*(9), 1850-1857. doi:https://doi.org/10.1016/j.psyneuen.2013.06.007

Franklin, M. S., Kraemer, G. W., Shelton, S. E., Baker, E., Kalin, N. H., & Uno, H. (2000). Gender differences in brain volume and size of corpus callosum and amygdala of rhesus monkey measured from MRI images. *Brain research, 852*(2), 263-267.

Funahashi, S., & Andreau, J. M. (2013). Prefrontal cortex and neural mechanisms of executive function. *Journal of Physiology-Paris, 107*(6), 471-482. doi:https://doi.org/10.1016/j.jphysparis.2013.05.001

Gale, C. R., O'Callaghan, F. J., Godfrey, K. M., Law, C. M., & Martyn, C. N. (2004). Critical periods of brain growth and cognitive function in children. *Brain, 127*(2), 321-329.

Geuze, E., Vermetten, E., & Bremner, J. D. (2005). MR-based in vivo hippocampal volumetrics: 2. Findings in neuropsychiatric disorders. *Molecular Psychiatry, 10*(2), 160-184. doi:10.1038/sj.mp.4001579

Giedd, J. N., & Rapoport, J. L. (2010). Structural MRI of Pediatric Brain Development: What Have We Learned and Where Are We Going? *Neuron, 67*(5), 728-734. doi:https://doi.org/10.1016/j.neuron.2010.08.040

Gilmore, J. H., Shi, F., Woolson, S. L., Knickmeyer, R. C., Short, S. J., Lin, W., . . . Shen, D. (2011). Longitudinal development of cortical and subcortical gray matter from birth to 2 years. *Cerebral cortex, 22*(11), 2478-2485.

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

Gogtay, N., Nugent, T. F., Herman, D. H., Ordonez, A., Greenstein, D., Hayashi, K. M., . . . Thompson, P. M. (2006). Dynamic mapping of normal human hippocampal development. *Hippocampus, 16*(8), 664-672. doi:10.1002/hipo.20193

Grotegerd, D., Stuhrmann, A., Kugel, H., Schmidt, S., Redlich, R., Zwanzger, P., . . . Dannlowski, U. (2014). Amygdala excitability to subliminally presented emotional faces distinguishes unipolar and bipolar depression: An fMRI and pattern classification study. *Human Brain Mapping, 35*(7), 2995-3007. doi:10.1002/hbm.22380

Gunnar, M. R., & Vazquez, D. (2006). Stress neurobiology and developmental psychopathology.

Hanson, J. L., Chung, M. K., Avants, B. B., Rudolph, K. D., Shirtcliff, E. A., Gee, J. C., . . . Pollak, S. D. (2012). Structural variations in prefrontal cortex mediate the relationship

between early childhood stress and spatial working memory. *The Journal of Neuroscience, 32*(23), 7917-7925. doi:10.1523/JNEUROSCI.0307-12.2012

Hanson, J. L., Nacewicz, B. M., Sutterer, M. J., Cayo, A. A., Schaefer, S. M., Rudolph, K. D., . . . Davidson, R. J. (2015). Behavior Problems After Early Life Stress: Contributions of the Hippocampus and Amygdala. *Biological Psychiatry, 77*(4), 314-323. doi:10.1016/j.biopsych.2014.04.020

Heim, C., & Nemeroff, C. B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biological Psychiatry, 49*(12), 1023-1039.

Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D. C., & Cullinan, W. E. (2003). Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo–pituitary–adrenocortical responsiveness. *Frontiers in neuroendocrinology, 24*(3), 151-180.

Herman, J. P., Ostrander, M. M., Mueller, N. K., & Figueiredo, H. (2005). Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 29*(8), 1201-1213.

Hinde, R. A., & Spencer-Booth, Y. (1967). The behaviour of socially living rhesus monkeys in their first two and a half years. *Animal Behaviour, 15*(1), 169-196.

Howell, B. R., Grand, A. P., McCormack, K. M., Shi, Y., LaPrarie, J., Maestripieri, D., . . . Sanchez, M. M. (2014). Early Adverse Experience Increases Emotional Reactivity in Juvenile Rhesus Macaques: Relation to Amygdala Volume. *Developmental Psychobiology, 56*(8), 1735-1746. doi:10.1002/dev.21237

Howell, B. R., McCormack, K. M., Grand, A. P., Sawyer, N. T., Zhang, X., Maestripieri, D., . . . Sanchez, M. M. (2013). 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, 3*, 21-21. doi:10.1186/2045-5380-3-21

Howell, B. R., McMurray, M. S., Guzman, D. B., Nair, G., Shi, Y., McCormack, K. M., . . . Sanchez, M. M. (2017). Maternal buffering beyond glucocorticoids: impact of early life stress on corticolimbic circuits that control infant responses to novelty. *Social neuroscience, 12*(1), 50-64. doi:10.1080/17470919.2016.1200481

Howell, B. R., Neigh, G., & Sanchez, M. M. (2016). Animal models of developmental psychopathology. In D. Cicchetti (Ed.), *Developmental Psychopathology* (3 ed., Vol. 2, pp. 166-201). New Jersey: John Wiley & Sons Press.

Howell, B. R., & Sanchez, M. M. (2011). Understanding behavioral effects of early life stress using the reactive scope and allostatic load models. *Development and Psychopathology, 23*(4), 1001-1016.

Kaplow, J. B., & Widom, C. S. (2007). Age of onset of child maltreatment predicts long-term mental health outcomes. *Journal of Abnormal Psychology, 116*(1), 176-187. doi:10.1037/0021-843X.116.1.176

Kaufman, J., Plotsky, P. M., Nemeroff, C. B., & Charney, D. S. (2000). Effects of early adverse experiences on brain structure and function: clinical implications. *Biological Psychiatry, 48*(8), 778-790.

Knickmeyer, R. C., Gouttard, S., Kang, C., Evans, D., Wilber, K., Smith, J. K., . . . Gilmore, J. H. (2008). A Structural MRI Study of Human Brain Development from Birth to 2 Years.

The Journal of neuroscience : the official journal of the Society for Neuroscience, 28(47), 12176-12182. doi:10.1523/JNEUROSCI.3479-08.2008

Knickmeyer, R. C., Styner, M., Short, S. J., Lubach, G. R., Kang, C., Hamer, R., . . . Gilmore, J. H. (2010). Maturational trajectories of cortical brain development through the pubertal transition: unique species and sex differences in the monkey revealed through structural magnetic resonance imaging. *Cereb Cortex, 20*(5), 1053-1063. doi:10.1093/cercor/bhp166

Knickmeyer, R. C., Styner, M., Short, S. J., Lubach, G. R., Kang, C., Hamer, R., . . . Gilmore, J. H. (2010). Maturational Trajectories of Cortical Brain Development through the Pubertal Transition: Unique Species and Sex Differences in the Monkey Revealed through Structural Magnetic Resonance Imaging. *Cerebral Cortex (New York, NY), 20*(5), 1053-1063. doi:10.1093/cercor/bhp166

Kumaran, D., Warren, D. E., & Tranel, D. (2015). Damage to the Ventromedial Prefrontal Cortex Impairs Learning from Observed Outcomes. *Cerebral Cortex (New York, NY), 25*(11), 4504-4518. doi:10.1093/cercor/bhv080

LeDoux, J. E. (1993). Emotional memory systems in the brain. *Behavioural Brain Research, 58*(1), 69-79. doi:https://doi.org/10.1016/0166-4328(93)90091-4

Liu, C., Tian, X., Liu, H., Mo, Y., Bai, F., Zhao, X., . . . Wang, J. (2015). Rhesus monkey brain development during late infancy and the effect of phencyclidine: A longitudinal MRI and DTI study. *NeuroImage, 107*, 65-75. doi:https://doi.org/10.1016/j.neuroimage.2014.11.056

Liu, D., Diorio, J., Day, J. C., Francis, D. D., & Meaney, M. J. (2000). Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nature neuroscience, 3*(8), 799.

Loi, M., Mossink, J. C. L., Meerhoff, G. F., Den Blaauwen, J. L., Lucassen, P. J., & Joëls, M. (2017). Effects of early-life stress on cognitive function and hippocampal structure in female rodents. *Neuroscience, 342*, 101-119. doi:https://doi.org/10.1016/j.neuroscience.2015.08.024

López, J. F., Akil, H., & Watson, S. J. (1999). Neural circuits mediating stress. *Biological Psychiatry, 46*(11), 1461-1471.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience, 10*, 434. doi:10.1038/nrn2639

Machado, C., J., & Bachevalier, J. (2002). Non-human primate models of childhood psychopathology: the promise and the limitations. *Journal of Child Psychology and Psychiatry, 44*(1), 64-87. doi:10.1111/1469-7610.00103

Maestripieri, D. (1998). Parenting styles of abusive mothers in group-living rhesus macaques. *Anim Behav, 55*(1), 1-11.

Maestripieri, D. (1998). Parenting styles of abusive mothers in group-living rhesus macaques. *Animal Behaviour, 55*(1), 1-11. doi:https://doi.org/10.1006/anbe.1997.0578

Maestripieri, D., & Carroll, K. A. (1998). Risk factors for infant abuse and neglect in group-living rhesus monkeys. *Psychol Sci, 9*(2), 143-145.

Maestripieri, D., Higley, J. D., Lindell, S. G., Newman, T. K., McCormack, K. M., & Sanchez, M. M. (2006). Early maternal rejection affects the development of monoaminergic

systems and adult abusive parenting in rhesus macaques (Macaca mulatta). *Behavioral Neuroscience, 120*(5), 1017-1024. doi:10.1037/0735-7044.120.5.1017

Maguire-Jack, K., Lanier, P., Johnson-Motoyama, M., Welch, H., & Dineen, M. (2015). Geographic variation in racial disparities in child maltreatment: The influence of county poverty and population density. *Child Abuse & Neglect, 47*, 1-13. doi:https://doi.org/10.1016/j.chiabu.2015.05.020

Malkova, L., Heuer, E., & Saunders, R. C. (2006). Longitudinal magnetic resonance imaging study of rhesus monkey brain development. *European Journal of Neuroscience, 24*(11), 3204-3212. doi:10.1111/j.1460-9568.2006.05175.x

Manly, J. T., Kim, J. E., Rogosch, F. A., & Cicchetti, D. (2001). Dimensions of child maltreatment and children's adjustment: Contributions of developmental timing and subtype. *Development and Psychopathology, 13*(4), 759-782.

McCormack, K., Newman, T., Higley, J., Maestripieri, D., & Sanchez, M. (2009). Serotonin transporter gene variation, infant abuse, and responsiveness to stress in rhesus macaque mothers and infants. *Horm Behav, 55*(4), 538-547.

McCormack, K., Sanchez, M. M., Bardi, M., & Maestripieri, D. (2006). Maternal care patterns and behavioral development of rhesus macaque abused infants in the first 6 months of life. *Developmental Psychobiology, 48*(7), 537-550. doi:10.1002/dev.20157

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *New England journal of medicine, 338*(3), 171-179.

McEwen, B. S. (2005). Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. *Metabolism-Clinical and Experimental, 54*(5), 20-23.

Meyer, J., Novak, M., Hamel, A., & Rosenberg, K. (2014). Extraction and analysis of cortisol from human and monkey hair. *Journal of visualized experiments: JoVE*(83).

Mitra, R., Jadhav, S., McEwen, B. S., Vyas, A., & Chattarji, S. (2005). Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America, 102*(26), 9371-9376. doi:10.1073/pnas.0504011102

Nadel, L., Campbell, J., & Ryan, L. (2007). Autobiographical Memory Retrieval and Hippocampal Activation as a Function of Repetition and the Passage of Time. *Neural Plasticity, 2007*, 90472. doi:10.1155/2007/90472

Neigh, G., Gillespie, C., & Nemeroff, C. (2009). *The Neurobiological Toll of Child Abuse and Neglect* (Vol. 10).

Patel, P. D., Lopez, J. F., Lyons, D. M., Burke, S., Wallace, M., & Schatzberg, A. F. (2000). Glucocorticoid and mineralocorticoid receptor mRNA expression in squirrel monkey brain. *Journal of Psychiatric Research, 34*(6), 383-392.

Payne, C., Machado, C. J., Bliwise, N. G., & Bachevalier, J. (2010). Maturation of the hippocampal formation and amygdala in Macaca mulatta: a volumetric magnetic resonance imaging study. *Hippocampus, 20*(8), 922-935.

Popoli, M., Yan, Z., McEwen, B., & Sanacora, G. (2011). The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nature reviews. Neuroscience, 13*(1), 22-37. doi:10.1038/nrn3138

Price, J. L. (1987). The limbic region. II: The amygdaloid complex. *Integrated systems of the CNS, Part I. Hypothalamus, hippocampus, amygdala, retina, Handbook of Chemical Neuroanatomy, 5*, 280-388.

Radley, J. J. (2012). Toward a limbic cortical inhibitory network: implications for hypothalamic-pituitary-adrenal responses following chronic stress. *Frontiers in Behavioral Neuroscience, 6*, 7. doi:10.3389/fnbeh.2012.00007

Rapisarda, J. J., Bergman, K. S., Steiner, R. A., & Foster, D. L. (1983). Response to estradiol inhibition of tonic luteinizing hormone secretion decreases during the final stage of puberty in the rhesus monkey. *Endocrinology, 112*(4), 1172-1179.

Rosene, D., & Hoesen, G. (1987). The Hippocampal Formation of the Primate Brain. In E. Jones & A. Peters (Eds.), *Cerebral Cortex* (Vol. 6, pp. 345-456): Springer US.

Samplin, E., Ikuta, T., Malhotra, A. K., Szeszko, P. R., & DeRosse, P. (2013). Sex Differences in Resilience to Childhood Maltreatment: Effects of trauma history on hippocampal volume, general cognition and subclinical psychosis in healthy adults. *Journal of Psychiatric Research, 47*(9), 1174-1179. doi:10.1016/j.jpsychires.2013.05.008

Sanchez, M. M. (2006). The impact of early adverse care on HPA axis development: Nonhuman primate models. *Hormones and Behavior, 50*(4), 623-631. doi:https://doi.org/10.1016/j.yhbeh.2006.06.012

Sanchez, M. M., Alagbe, O., Felger, J. C., Zhang, J., Graff, A. E., Grand, A. P., . . . Miller, A. H. (2007). Activated p38 MAPK is associated with decreased CSF 5-HIAA and increased maternal rejection during infancy in rhesus monkeys. *Molecular Psychiatry, 12*(10), 895.

Sánchez, M. M., Young, L. J., Plotsky, P. M., & Insel, T. R. (2000). Distribution of Corticosteroid Receptors in the Rhesus Brain: Relative Absence of Glucocorticoid Receptors in the Hippocampal Formation. *The Journal of Neuroscience, 20*(12), 4657.

Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1985). Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. *The Journal of Neuroscience, 5*(5), 1222.

Sclafani, V., Simpson, E. A., Suomi, S. J., & Ferrari, P. F. (2015). Development of space perception in relation to the maturation of the motor system in infant rhesus macaques (Macaca mulatta). *Neuropsychologia, 70*, 429-441. doi:10.1016/j.neuropsychologia.2014.12.002

Scott, J. A., Grayson, D., Fletcher, E., Lee, A., Bauman, M. D., Schumann, C. M., . . . Amaral, D. G. (2016). Longitudinal analysis of the developing rhesus monkey brain using magnetic resonance imaging: birth to adulthood. *Brain Structure and Function, 221*(5), 2847-2871.

Shi, Y., Budin, F., Yapuncich, E., Rumple, A., Young, J. T., Payne, C., . . . Styner, M. A. (2016). UNC-Emory Infant Atlases for Macaque Brain Image Analysis: Postnatal Brain Development through 12 Months. *Frontiers in Neuroscience, 10*, 617. doi:10.3389/fnins.2016.00617

Shin, L. M., & Liberzon, I. (2010). The Neurocircuitry of Fear, Stress, and Anxiety Disorders. *Neuropsychopharmacology, 35*(1), 169-191. doi:10.1038/npp.2009.83

Short, S. J., Lubach, G. R., Karasin, A. I., Olsen, C. W., Styner, M., Knickmeyer, R. C., . . . Coe, C. L. (2010). Maternal influenza infection during pregnancy impacts postnatal brain development in the rhesus monkey. *Biological Psychiatry, 67*(10), 965-973.

Smythe, J. W., Rowe, W. B., & Meaney, M. J. (1994). Neonatal handling alters serotonin (5-HT) turnover and 5-HT2 receptor binding in selected brain regions: relationship to the

handling effect on glucocorticoid receptor expression. *Developmental Brain Research, 80*(1-2), 183-189.

Styner, M., Knickmeyer, R., Joshi, S., Coe, C., Short, S. J., & Gilmore, J. (2007). *Automatic brain segmentation in rhesus monkeys*.

Suomi, S. J. (2005). Mother-Infant Attachment, Peer Relationships, and the Development of Social Networks in Rhesus Monkeys. *Human Development, 48*(1-2), 67-79.

Teicher, M., H., Tomoda, A., & Andersen Susan, L. (2006). Neurobiological Consequences of Early Stress and Childhood Maltreatment: Are Results from Human and Animal Studies Comparable? *Annals of the New York Academy of Sciences, 1071*(1), 313-323. doi:10.1196/annals.1364.024

Teicher, M. H., Andersen, S. L., Polcari, A., Anderson, C. M., Navalta, C. P., & Kim, D. M. (2003). The neurobiological consequences of early stress and childhood maltreatment. *Neuroscience & Biobehavioral Reviews, 27*(1), 33-44.

Tottenham, N., Hare, T. A., Quinn, B. T., McCarry, T. W., Nurse, M., Gilhooly, T., . . . Casey, B. J. (2010). Prolonged institutional rearing is associated with atypically larger amygdala volume and difficulties in emotion regulation. *Developmental science, 13*(1), 46. doi:10.1111/j.1467-7687.2009.00852.x

Tottenham, N., & Sheridan, M. A. (2010). A review of adversity, the amygdala and the hippocampus: a consideration of developmental timing. *Frontiers in Human Neuroscience, 3*, 68.

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience, 10*(6), 397.

van Oers, H. J. J., de Kloet, E. R., Whelan, T., & Levine, S. (1998). Maternal deprivation effect on the infant's neural stress markers is reversed by tactile stimulation and feeding but not by suppressing corticosterone. *Journal of Neuroscience, 18*(23), 10171-10179.

Vyas, A., Jadhav, S., & Chattarji, S. (2006). Prolonged behavioral stress enhances synaptic connectivity in the basolateral amygdala. *Neuroscience, 143*(2), 387-393. doi:https://doi.org/10.1016/j.neuroscience.2006.08.003

Wang, J., Vachet, C., Rumple, A., Gouttard, S., Ouziel, C., Perrot, E., . . . Styner, M. A. (2014). Multi-atlas segmentation of subcortical brain structures via the AutoSeg software pipeline. *Frontiers in neuroinformatics, 8*, 7.