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Brik Kochoian March 27, 2019

# Effects of Adverse Maternal Care on Development of the Prefrontal Cortex, Amygdala, Hippocampus and Nucleus Accumbens in Rhesus Monkeys: Relations with Cocaine Self-Administration

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Abstract

Effects of Adverse Maternal Care on Development of the Prefrontal Cortex, Amygdala, Hippocampus and Nucleus Accumbens in Rhesus Monkeys: Relations with Cocaine Self-Administration

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Childhood is a critical period for neurodevelopment, which can be strongly influenced by social, sensory and stressful experiences. The goal of this study was to examine the impact of early life stress (ELS) on brain structural development by focusing on the development of the amygdala and hippocampus (due to their critical role in stress and emotional regulation and their connections with reward regions), the prefrontal cortex (PFC) (due to its critical role in cognitive control of behavior, goal-directed behaviors, impulse/perseverative control, reward associations, emotional regulation, response inhibition, and top-down control of reward centers), and the nucleus accumbens (NAcc) due to its role in reward processes and reinforcing effects of psychostimulants such as cocaine (COC). The main goal was to identify neurodevelopmental underpinnings linking ELS and psychostimulant escalation using a non-human primate (NHP) of infant maltreatment and a COC self-administration (SA) paradigm during adolescence. We examined the impact of ELS, defined by a naturalistic infant maltreatment model, on longitudinal structural development of those brain regions using structural MRI scans acquired at 3, 6, and 12 months of age. Confounding effects of heritability were controlled for by using a unique cross-fostering paradigm, in which all subjects were randomly assigned to either a maltreating or control mother at birth. Furthermore, we also examined whether subjects who had been maltreated during infancy were more vulnerable than controls on a COC SA escalation paradigm during adolescence. This study found region-specific impacts of ELS on brain development in the amygdala and NAcc, but not the PFC or hippocampus. Furthermore, we did not observe ELS-related differences on COC SA escalation measures, either. These findings suggest that ELS has a negative impact on neurodevelopment, with region-specific effects on amygdala and NAcc development, but not the PFC or hippocampus. Furthermore, our findings also suggest that all subjects, regardless of rearing, self-administer COC at comparable levels during the phases of the COC SA paradigm analyzed in this study.

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## INTRODUCTION

### ELS as a Risk for Developmental Psychopathology, Including Substance Use Disorders (SUDs): Impact on Prefrontal-Limbic Regions that Regulate Emotional/Stress Responses & Reward Processes

Childhood is a critical period for neurodevelopment, which can be strongly influenced by social, sensory and stressful experiences. Previous studies have shown that early life stress (ELS) alters the brain's function and structural development (e.g. neuronal dendritic arborization, synapses, myelination), affecting the volumes of cortical regions, including grey and white matter (GM; WM) and limbic regions (Howell et al., 2014; van Harmelen et al., 2010; Sanchez et al., 2011). ELS has been shown to decrease the volume of the prefrontal cortex (PFC) (Frodl et al., 2010), in part due to its protracted development and because of the high expression of stress hormone receptors (Radley, 2012; McEwen, 1998; Arnsten, 2009; Popoli et al., 2011; Sanchez et al, 2000). Furthermore, previous studies have shown that damage to the PFC impairs decision-making and executive function skills, emotional and stress regulation, and regulatory processes related to substance abuse (Funahashi and Andreau, 2013). Different brain structures, neural circuits, and tissue types have distinct developmental trajectories, which undergo dynamic changes throughout life (Giedd and Rapoport, 2010) and could make them more susceptible to stress at different times (Lupien et al., 2009; Tottenham and Sheridan, 2010). Since many psychiatric disorders have been linked to neurodevelopmental alterations, examining developmental trajectories as phenotypes in studies of psychopathology is of great interest (Giedd et al., 2008).

In rhesus monkeys, like in other primates, normal development of the PFC follows an inverted "U" growth pattern, with gray matter (GM) volumes increasing until adolescence and

decreasing thereafter, and white matter (WM) still increasing through the juvenile and adolescent periods, into adulthood (Knickmeyer et al., 2010). Some studies report that the macaque PFC GM volume decreases even earlier, after 10 months of age (late infancy) (Liu et al., 2015). Thus, total brain volume is mostly driven by WM volume increases for the first four years of life in macaques, from birth through adolescence (Malkova et al., 2006). These developmental patterns are very similar to those in humans, where WM volume also increases throughout childhood and adolescence, while GM volume trajectories follow an inverted U-shape developmental trajectory (Lenroot et al., 2007; Giedd and Rapoport, 2010). The prolonged and protracted development of the primate PFC explains why previous studies have shown a high vulnerability of the PFC to stress exposure, resulting, for example, in reduced PFC GM volumes and impaired cognitive functions thereafter (Arnsten, 2009; Hanson et al., 2012). Furthermore, different developmental trajectories between males and females have been observed, which may help identify gender-specific vulnerabilities to psychopathology observed during adolescence (Paus, Keshavan and Giedd, 2008; Bramen et al., 2011). Higher order association cortices, such as the PFC develop fully later in life, whereas primary, lower order cortices (e.g. motor cortex) reach cortical maturation sooner (Giedd et al., 1999; Giedd, 2004; Gogtay et al., 2004; Reiss et al., 1996). This developmental pattern suggests that more anterior regions of the PFC, which have protracted or prolonged development, may be more impacted by ELS than more caudal regions (Andersen, 2003; Crews et al., 2007). Timing of stress exposure may be a critical moderator of the effect of ELS on behavioral development (Manly et al., 2001; Campbell et al., 2014). Therefore, investigating the impact of stress longitudinally is necessary to understand how and when alterations in brain development manifest. Because many details of the discrete developmental trajectories of different brain structures and tissues are still unclear, how those typical

maturational changes are impacted by chronic stress and adversity early in life still remains poorly understood and should be further investigated.

The dorsolateral PFC (DLPFC) is a PFC subregion with critical role in executive function, including attention and working memory, and impulse control in primates, both nonhuman species (Goldman-Rakic, 1987; Petrides, 1994; Fuster, 1997) and humans, where the maturation of these functions have been strongly linked to the maturation of DLPFC (Giedd and Rapoport, 2010; Paus, Keshavan and Giedd, 2008). As an example, in a study with children ranging from 6-13-years of age, DLPFC cortical thickness was associated with enhanced cognitive control over impulses (Steinbeis, Bernhardt and Singer, 2012, Toshio et al., 2017). Cognitive control is essential for solving difficult/complex tasks, regulating negative emotional reactivity and impulses and overcoming habitual responses, and the DLPFC plays integral roles in these functions (MacDonald et al., 2000). In fact, Steinbeis and colleagues found that as the DLPFC develops and increases in volume with age, children are better able to control their selfish drive and adjust behavior to anticipated negative consequences (Steinbeis, Bernhardt and Singer, 2012). In addition to the DLPFC, other PFC subregions, the orbitofrontal cortex (OFC), the medial PFC (mPFC), and the anterior cingulate cortex (ACC) also have protracted developments and are very important for: perseverative behaviors, goal-directed behaviors, impulse control, reward associations, emotional regulation, and response inhibition (Andersen, 2003; Crews et al., 2007).

In a previously conducted study that measured rats' plasma GC levels response to a 20-min restraint stress, rats with lesions to the mPFC had significantly increased plasma levels of CORT, suggesting that the mPFC regulates stress-induced GC elevations and it is a target site for negative-feedback of GCs on HPA activity in response to stress (Diorio, Viau and Meaney,

1993). Furthermore, abundant evidence in studies conducted with rodents have revealed dendritic retraction on pyramidal neurons and reduced spine densities following chronic stress in the mPFC (for review, see Barfield and Gourley, 2018; Radley et al., 2008; Liu and Aghajanian, 2008; Anderson et al., 2016). This stress-induced structural remodeling of the mPFC is associated with deficits in attentional set-shifting (Liston et al., 2006) and failures in goal-directed decision making (Dias-Ferreira et al., 2009). Because of the mPFC's critical role in emotional regulation and goal-directed decision making, it was also a focus of this study.

ELS has also been linked to altered development of limbic regions, particularly the amygdala -a critical region for detecting salient and threatening stimuli and regulating stress and emotional responses (LeDoux, 1993)-, and the hippocampus -an important region for learning, memory and stress neuroendocrine regulation (Tottenham and Sheridan, 2010; Teicher et al., 2003)-. Chronic stress has been associated with increased amygdala volumes (Mitra et al., 2005; Tottenham et al., 2010), and ELS can result in a hyperactive amygdala and increased stress/emotional reactivity (for a review, see Wakeford et al., 2018). This volume increase may be mediated by stress-induced increase in glutamate release following higher levels of glucocorticoids (GC). In fact, previous studies have observed that amygdalar dendrites increased in length and branching in response to high levels of glutamate (Popoli et al., 2011). Amygdalar dysfunction is also found in numerous people struggling with post-traumatic stress disorder (PTSD), depression and drug addiction (LeDoux, 1993; Shin and Liberzon, 2010; Grotegerd et al., 2014). In contrast to the amygdala, chronic stress exposure has been shown to reduce hippocampal volumes through different mechanisms ranging from reductions of dendritic fields and synapses (Sapolsky, Krey and McEwen, 1985; McEwen, 1998; Lupien et al., 2009) to neuronal death (Hanson et al., 2015), which is believed to be mediated by prolonged GC

exposure (Sapolsky, Krey and McEwen, 1985). One study with vervet monkeys demonstrated atrophy in hippocampal CA1 and CA3 pyramidal neurons following prolonged social stress (Uno et al., 1989). The effects of chronic stress have more long-term detrimental effects when stress is experienced during development (ELS), partially because both the amygdala and hippocampus are still developing during infancy, childhood and even through adulthood, both in humans (Tottenham and Sheridan, 2010) and non-human primates (NHPs) (Payne et al., 2010; Knickmeyer et al., 2010; Scott et al., 2016).

It has also been suggested that ELS critically alters the development of neural circuitry involved with reward and inhibitory control (Cicchetti and Toth, 2005; Hein, Cohen and Campbell, 2005). A likely substrate that is affected by this is the PFC connections with nucleus accumbens (NAcc); therefore, utilizing a NHP ELS model would be beneficial to greater understand the neurobiological alterations that render one more vulnerable to psychostimulant drug initiation and addiction during adolescence. Exposure to stress can also have a detrimental impact on reward circuitry, including reduced connectivity and signaling between the PFC and the NAcc, which has been demonstrated to result in addiction-related behaviors (MacAskill et al., 2014). This may be related to the decreases in functional coupling between subregions of the PFC (involved in behavioral control) and the NAcc (reward processing region), which has been linked with self-reported risky behavior across adolescence (Qu et al., 2015). In fact, it has been proposed that loss of PFC and NAcc functional connectivity is a critical underlying characteristic of cocaine use disorder (CUD) (Murnane et al., 2014). Furthermore, stress-induced increases in hypothalamic-pituitary-adrenal (HPA) axis activity have been linked to decreased density in mesolimbic dopamine receptors (DR) in the NAcc (for review, see Goff and Tottenham, 2014). In another study, larger NAcc volumes were observed in subjects who experienced higher levels

of stress (Kühn, Schubert and Gallinat, 2011), which the authors concluded corresponded to greater trait anxiety. Dysregulation of the emotional systems of the brain, which mediate arousal and stress and interface with reward systems is a key component of addiction (for review, see Koob, 2009). Since a relationship between the stress, HPA axis and the NAcc has been observed, and because of the NAcc's role in reward-related brain function and addiction, it was also a primary focus of this analysis.

Childhood maltreatment is a devastating ELS experience that has been linked to an increased risk of developing psychopathology (for reviews, see Sanchez, 2006; Wakeford et al., 2018; Glaser, 2000). People who experience ELS are at higher risk to develop psychiatric illnesses such as anxiety, PTSD, depression, attention deficit and hyperactivity disorder (ADHD), and substance use disorder (SUD) (Heim et al., 2001; Bremmer and Vermetten, 2001; Glaser, 2000; Gunnar and Vazquez, 2006). Furthermore, it has been previously demonstrated that earlier exposure to ELS results in more severe symptoms (Kaplow and Widom, 2007).

According to the 2015 Nation Survey on Drug Abuse and Health (NSRAH) and Substance Abuse and Mental Health Service Administration (SAMHSA), 19.6 million adults had SUDs in the US alone; so, this is a significant health issue. (http://www.samhsa.gov/data/). Key symptoms of SUDs include compulsive drug taking and an intense drive to take the drug despite negative consequences. Notably, SUDs are marked by a shift from recreational use to compulsive use (American Psychiatric Association, 1994). Compulsivity is defined as the manifestation of "perseverative, repetitive actions that are excessive and inappropriate" (Berlin and Hollander, 2014). It is this "compulsive" feature of drug intake that is considered a hallmark in addiction. Among drugs of abuse, psychostimulants, like cocaine (COC), are some of the most highly abused drugs in the country (http://www.samhsa.gov/data/). According to the SAMHSA

in 2017, 966,000 people aged 12 or older had a CUD (http://www.samhsa.gov/data/). Despite the obvious need for effective treatment, there is no approved pharmacotherapy to treat CUDs (Ciccarone, 2011; Grabowski et al., 2004; Howell and Cunningham, 2015). This is partially due to the fact that not all COC users develop CUDs, suggesting potential environmental, genetic, and neurobiological vulnerabilities that may predispose some users to CUD, including ELS. A major limitation in the development of an effective treatment is the fact that examining the link between ELS and CUD involves prospective, longitudinal studies prior to initiation of drug use, which are very difficult to conduct with adolescent and pre-adolescent children. Therefore, research utilizing preclinical models of SUDs is needed to increase our understanding of the link between ELS and SUDs and to generate better therapies and interventions.

## Macaque Model of ELS—Induced Risk for Adolescence Drug Addiction

Experimental manipulations using NHP models of ELS, specifically rhesus macaques (*Macaca mulatta*), allow a more in-depth analysis of potential risk factors for the development of psychopathologies in comparison to studies with humans (Gibbs et al., 2007). Rhesus macaques are an ideal model due to the species' complex social interactions, strong mother-infant bonds, and biological, phylogenetic, and brain similarities (e.g. organization, neurochemistry and development) with humans, particularly as ELS models of risk for SUDs in humans (Sanchez, 2006; Wakeford et al, 2018). Rhesus macaques live in a matrilineal hierarchical social structure, (Altmann, 1962), and strong family relations and alliances are integral to maintain social status of the family in the group (Suomi, 2005). Furthermore, as in human infants, maternal care is critical for the development of infant rhesus monkeys, as these infants depend on their mothers during the first postnatal months. In fact, even when infant exploration and independence increases and the interaction with the mother declines during weaning (3-6 months of age),

mother-infant interaction remains high even into the juvenile years (1-4 years of age) (Suomi, 2005; Sanchez, 2006). Therefore, competent maternal care is integral to the proper development of the infant, and disruptions of maternal care in NHPs lead to alterations in stress and emotional reactivity, as well as in social relationships and reward processes that resemble psychopathology in children (Drury, Sanchez and Gonzalez, 2016; Sanchez et al., 2001; Wakeford et al., 2018). A very detrimental form of adverse caregiving, infant maltreatment, not only occurs in human populations but also in NHPs, both in captivity and in the wild (Brent, Koban and Ramirez, 2002; Johnson et al., 1996; Maestripieri, 1998; Sanchez, 2006; Troisi and D'Amato, 1983). Similar prevalence rates of maltreatment occur in rhesus macaques and human populations, between 2-5% (Maestripieri, 1999). In these studies, maltreatment is defined as maternal neglect and physical abuse, the later operationalized as violent behaviors of the mother towards the infant, such as crushing, dragging, or throwing of the infant (Maestripieri, 1998; Troisi and D'Amator, 1983). These aversive early experiences have been linked to socioemotional alterations, e.g., increased emotional reactivity (Maestripieri and Carroll, 1998; Maestripieri, Jovanovic and Gouzoules, 2000; McCormack et al., 2009; McCormack et al., 2006). Studies show that infant maltreatment in macaques occurs early in development (during the first 3-6 months postpartum) with long-term negative effects on development and these behaviors serve as effective models for child maltreatment in humans (Sanchez, 2006).

It has been previously demonstrated that infant maltreatment produces adverse effects on the macaque HPA axis (Sanchez, 2006; Howell et al., 2013), including elevated baseline levels of the stress hormone cortisol, and heightened stress-induced- cortisol responses. The HPA axis is activated in response to threatening/stressful stimuli, resulting in the release of GCs, e.g. cortisol in primates, from the adrenal cortex (for review, see Herman et al., 2003; Sanchez,

2006). GCs are catabolic steroid hormones that mobilize energy substrates and affect multiple bodily functions, including anti-inflammatory effects, to promote survival responses (Sanchez, 2006). Our lab has reported that, although the presence of competent (responsive/sensitive, protective) mothers buffers the infant's cortisol stress responses, a maltreating mother does not buffer the infant's response to stress (McCormack et al., 2003).

Using NHPs to model aspects of stress neuroendocrine function is advantageous because of the large amount of homology between humans and NHP species, in comparison to rodents, which is critical in studying the relationship between ELS and the risk for drug addiction (Patel et al., 2000; Pryce et al., 2005; Sanchez et al., 1999; Sanchez et al., 2000; Sanchez, 2006). The PFC modulates stress and emotional reactivity (exerts top-down control on the amygdala; Kim et al., 2011), and because it has protracted or prolonged maturation, it may be the most impacted by ELS (Anderson, 2003; Crews et al., 2007); resulting in higher emotional reactivity and risky behavior, which is typical in adolescence, and is likely in part due to this protracted development (Somerville et al., 2010). It has been suggested that increased sensitivity to the PFC during adolescence may make it more sensitive to ELS, altering the PFC-amygdala functional connectivity, and contributing to drug abuse (O'Connor and Cameron, 2006). Since, NHPs and humans have more homologous PFCs than either share with rodents (Fuster, 1997; Haber et al., 2006; Ongur and Price, 2000; Preuss and Goldman-Rakic 1991a, 1991b; Preuss, 1995), this makes them an ideal preclinical model for studying structural PFC and PFC-amygdala functional connectivity alterations in the context of drug addiction.

**Adolescence Brain Remodeling & Risk for Psychostimulant Drug Initiation and Addiction:**

Adolescence represents a crucial stage in development in which drug use is widely initiated and when functional neurobiological alterations occur. Adolescents exhibit increased

risk-taking and novelty-seeking, and these behaviors can increase the risk for substance abuse (Adams, Montemayer and Gullotta, 1989; Savin-Williams, 1987). The increased risk has been proposed to stem, in part, from the drastic remodeling the PFC undergoes during this developmental period (for review, see Barfield and Gourley, 2018). The PFC exerts top-down regulation of limbic regions such as the NAcc and amygdala, which are critical to process reward and emotional/stress responses, respectively and develop at a faster rate than the PFC (Giedd et al., 1999; Giedd, 2004; Gogtay et al., 2004; Reiss et al., 1996). Furthermore, it has been demonstrated that dysregulation of cognitive control is a key component that drives compulsive drug seeking (Koob, Loyd and Mason, 2009). In fact, the differential maturational rate between PFC and subcortical reward/emotional centers such as the NAcc and amygdala led to the proposal of a "developmental imbalance model" of these brain regions during adolescence that explain heighted risk-taking and novelty seeking, as well as emotional reactivity at this stage (Casey et al., 2010; Casey et al., 2008).

ELS has been identified as a risk factor to adolescent drug use, including in individuals with childhood maltreatment (Sinha and Li, 2008, 2009; Wakeford et al., 2018). However, the neurodevelopmental alterations following ELS and how these alterations surmount in a higher risk for SUDs in adolescence is poorly understood. Experiments with humans are not feasible, because perspective, longitudinal studies of children at risk before they initiate drug consumption would be required to understand the neurobiological mechanisms underlying the increased risk of SUDs following ELS. Although no animal model of SUDs fully emulates the human condition, animal models do permit investigation of specific elements of the process of drug reinforcement, seeking, escalation, extinction and reinstatement. Because brain development and maturation in NHPs follows comparable trajectory patterns with humans—despite developing at

approximately 4 times the speed of human children—NHPs are an excellent model to study the effects of early experiences on neurodevelopment (Hayashi, 1992; Huttenlocher and Dabholkar, 1997; Kilb, 2012; Sanchez, 2016). Drugs abused by humans increase dopamine (DA) in the reward circuit, and this is believed to underlie the rewarding effects of drugs (see review, Goldstein and Volkow, 2011). Drugs that are self-administered by animals correspond well with those that have high abuse potential in humans in that they also increase DA, and intravenous drug self-administration (SA) is considered an animal model that is predictive of abuse potential (Collins et al., 1984). Drug SA involves operant schedules of reinforcement, in which NHPs are trained to respond to certain response requirements that result in a reinforcing stimulus (Brady et al., 1987; Collins et al., 1983). Drug SA in animal models has been validated extensively as a model for various aspects of human drug use and abuse, including binge/intoxication aspects during chronic drug intake (Ator and Griffiths, 2003; Banks et al., 2017; Haney and Spealman, 2008; Howell and Murnane, 2008; Mello and Negus, 1996; Wakeford et al, 2018). Furthermore, many variables can be controlled by using drug SA (Yokel, 1987), which can reveal how these variables are potential risk factors for drug abuse (for review, see Wakeford et al., 2019). Using this procedure, the dose, and cost of responding can be manipulated to determine the value of the reward.

Equally compelling are studies that show drug taking in the presence of aversive consequences, e.g. punishment, in animals given extended access (EA) to the drug. EA to COC SA in rodents has been proposed to model the escalating patterns of drug intake seen in COC dependence (Ahmed and Koob, 1998, 1999; Deroche-Gamonet et al., 2004; Koob et al., 2014). Studies in which rats were given EA to COC found that they did not suppress drug seeking in the presence of an aversive conditioned stimulus or punishment, which has face validity for the

DSM-IV criteria of "continued substance use despite knowledge of having a persistent physical or psychological problem" (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; APA, 1994). Following this logic, circuits related to perseverative behavior such as the OFC, are likely to be involved in this aspect of drug use (Stuss and Benson, 1986). This has been validated in neuroimaging studies associating the disruption of striatal, thalamic and orbitofrontal brain regions as important regions of the brain for preservative and compulsive behavior (Volkow et al., 1996a). It has also been previously demonstrated that chronic stress exposure induces hypertrophy of apical dendrites in the OFC (Liston et al., 2006; Dias-Fierreira et al., 2009), and this can surmount into abnormal function of the OFC which is linked with compulsive behavior and disinhibition (Volkow and Fowler, 2000). Gourley et al. (2013) also demonstrated that chronic corticosterone (CORT) reduced dendritic spines on neurons, suggesting that stress negatively alters the structure of the OFC. The OFC does not only process information about the rewarding properties of stimuli (Aou et al., 1983; Tremblay and Schultz, 2000), but it also modifies animal behavior when reinforcing characteristics of these stimuli change (Thorpe et al., 1983) and when learning stimulus reinforcement associations (Rolls, 1996; Schoenbaum et al., 1998). The OFC provides dense projections to the NAcc, the target of the reinforcing effects of COC (Koob and Bloom, 1988; Pontieri et al., 1996) and a core region where COC has its action, (Haber et al., 1995). Because of the extensive connectivity between these two brain regions and their respective involvement in processes critically related to addiction, these areas are primary focuses of the present study.

Dysregulation of the HPA axis, e.g. due to excessive stress exposure, has not only been associated with reduced activity in PFC (Volkow, Wang and Telang et al., 2008), but also with decreases in striatal DR $D_2$ (D2R) (Lemieux and al'Absi, 2016). Reductions of D2R availability

within the striatum has been observed in CUDs (for review, see Koob and Volkow, 2016), as well as in preclinical studies in rodents and NHPs with repeated COC exposure (Volkow et al., 2009). Furthermore, low D2R availability has been associated with decreases in baseline glucose metabolism, a marker of brain function, in the DLPFC, ACC and OFC (Volkow, Wang and Telang et al., 2007; Volkow, Fowler and Wang et al., 1993; Volkow, Chang and Wang et al., 2015) and compulsive COC SA in rodents (for review, see Everitt et al., 2008). Additionally, the mPFC is a crucial regulator of reward-directed behaviors and a major component of the mesocorticolimbic DA pathway. Specifically, it receives dopaminergic projections from the ventral tegmental area (VTA) and sends key glutamatergic projections to the NAcc (Albertin et al., 2000; McGinty and Grace, 2009; Hamel et al., 2017; Morrison et al., 2017). Loss or reduction of signaling in this circuit has been associated with SUDs in humans (Volkow et al., 2010).

Previous findings suggest that impulsivity predicts the escalation of COC SA in rats, such that rats with higher impulsivity had higher rates of COC SA (Anker et al., 2009; Dalley et al., 2007). Dalley and colleagues (2007) also suggested that deficits in inhibitory control were related to vulnerability to engage in binge-like drug intake. Previous research has demonstrated the involvement of the ACC in response inhibition (Anker et al., 2009). Furthermore, deficits in response inhibition are thought to be a leading factor in the risk of compulsive and impulsive behaviors, contributing factors to SUDs (APA, 1994). Because of the ACC's role in response inhibition, its alterations are linked with impulsivity associated with SUDs (Anker et al., 2009; Stuss and Benson, 1986). In addition, adolescent exposure to chronic stress also results in a dendritic retraction on pyramidal neurons and reduced spine densities in the ACC (Barfield and Gourley, 2018; Anderson et al., 2016). Following this logic, it is reasonable to suggest that

impairments in this region, e.g. altered structural development caused by ELS (maltreatment), would predict higher responding, i.e. more COC infusions in maltreated animals in comparison to controls, during the EA phase of COC SA.

Previous data have shown a lack of gradual escalation in the number of COC infusions earned over a 60-day EA condition in NHPs (Kirkland, Davis and Howell, 2009; Henry and Howell, 2009). Kirkland, Davis and Howell (2009) reported that there is a significant increase in COC SA in NHPs when switching from limited access (LA) to EA, but that these higher levels are maintained across the 60-day period, without signs of escalation. This result is surprising because similar experiments conducted with rodents have demonstrated gradual patterns of increased COC intake across days of testing with subjects given up to 6 hours of EA (Ahmed and Koob, 1999; Ferrario et al., 2005; Knackstedt and Kalivas, 2007). Kirkland, Davis and Howell's (2009) previous findings were corroborated with a study in which, when given months of EA, NHPs did not show escalation patterns with increasing trials (Henry and Howell, 2009). This could indicate a divergence between the two animal models under the EA condition, suggesting that primates may have self-regulatory abilities (i.e. inhibitory control of behavior) not reported in rodents. One reason for this may be the differences between rodents and primates in the overall complexity of the PFC. Although it is generally accepted that the PFC of primates is significantly more developed and complex than that of rodents' (Preuss, 1995), the similarities are still hotly debated (for review, see Uylings, Groenewegen and Kolb, 2003). Furthermore, this highlights the translational relevance of the NHP model to examine questions in humans and brings up issues that should be considered when determining duration of EA and the animal model used.

## GOALS AND HYPOTHESES OF THIS STUDY:

The goal of present study is to examine the neurodevelopmental underpinnings linking ELS and psychostimulant addiction using a NHP model of infant maltreatment and a COC SA paradigm during adolescence. In particular it will examine ELS impact on development of corticolimbic regions that regulate stress and emotional responses, as well as reward processes (i.e. PFC, amygdala, hippocampus and NAcc) in relation to psychostimulant escalation (i.e. "binging").

These are the hypotheses that will be tested:

(1) Brain regions controlling reward processes and their interface with stress and emotion regulation pathways, particularly the PFC and its subregions, the hippocampus, and the NAcc will show developmental alterations in structural growth (blunted volumetric growth) in subjects that experienced ELS.

(2) Conversely, the amygdala will have larger volumes in subjects that experienced ELS compared to controls.

(3) Additionally, NHPs that experience maltreatment (ELS) will require a shorter time to take 20 reinforcers than controls (i.e. faster intake of reinforcers), mimicking "binging" in humans.

## METHODS

### Subjects and Housing

Thirty-four rhesus monkeys (*Macaca mulatta*), 18 controls (9 males, 9 females) and 16 maltreated (8 males, 8 females), were studied from 3 to 12 months of age as part of a larger longitudinal study of neurodevelopmental impact of ELS (maltreatment) from birth through the juvenile, prepubertal period (McCormack et al., 2015; Howell et al., 2017; Drury et al., 2017);

see **Figure 1** for experimental design. A subset of these animals (n=14; 7 controls (4 males, 3 females) and 7 maltreated (4 males, 3 females) underwent cocaine self-administration (COC SA) procedures during adolescence (described below). Animals were born and raised with their mothers and families in complex social groups housed in outdoor compounds with access to climate-controlled indoor housing at the Yerkes National Primate Research Center (YNPRC) Field Station in Lawrenceville, GA. Social groups in each compound consisted of 2-3 adult males and 15-50 adult females and offspring. Water was available *ad libitum*, and animals were fed twice daily with monkey chow (Purina Mills Int., Lab Diets, St. Louis, MO), supplemented with vegetables and fresh fruit.

At approximately 4-5 years of age, subjects were transferred to the YNPRC Main Research Center for the COC SA studies. Upon arrival, subjects were pair-housed, fed Purina monkey chow (Purina Mills Int., Lab Diets, St. Louis, MO), supplemented with fruit and vegetables daily, and water was available *ad libitum* in their home cages. Environmental enrichment was provided on a regular basis. Ambient conditions within the colony were maintained at a temperature of 22 ± 2°C and at 45-50% humidity. The room lighting was set to a 12 h light/dark cycle, with the light period from 7:00 A.M. to 7:00 P.M. All subjects were naïve to COC as well as the SA apparatus and procedure. Following a period of acclimation to the new housing environment, the animals underwent several other tasks as part of a larger study that are not included here. Subsequently, animals were fitted with primate collars, chair-trained to sit comfortably in chairs designed for NHPs (Primate Products, Miami, FL, USA), and implanted with intravenous (I.V.) catheters for the COC SA procedures described below, at approximately 5-6 years of age. All protocols and animal care and handling strictly follow the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition, revised

2011) and the recommendations of the American Association for Accreditation of Laboratory Animal Care and were approved by the Emory University Animal Care and Use Committee (IACUC).

**Cross-fostering Design & Naturalistic Maltreatment Model**

A cross-fostering experimental design was used to randomly assign infants to either a control (CONT) or maltreating (MALT) mother at birth following previously published protocols (Howell et al., 2017; Drury et al., 2017), to control for confounding effects of heritable and prenatal factors on postnatal maternal care impact on animal's development. 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. In addition, social rank was counterbalanced across groups to control for its potential confounding effects on our measures. After cross-fostering, focal observations of maternal care were performed during the first 3 postnatal months to measure the rates of abuse and rejection that mothers showed toward their fostered infants. Focal observations, which were 30 mins long, were performed on separate days (5 days/week during month 1, 2 days/week during month 2 and 1 day/week during month 3) for a total of 16 hours/mother-infant pair; given that physical abuse occurs during the first 3 postnatal months with the highest rates in the first month, this protocol is optimal for early infant maltreatment documentation in this species (Drury, *et al.*, 2017; Howell, *et al.*, 2017; Maestripieri D, 1998; McCormack, *et al.*, 2006). Experienced coders (interobserver reliability >90% agreement) collected these behavioral observations. Maltreatment in this model was defined as the co-morbid experience of maternal physical abuse and early infant rejection during the first three months of life, following previously published criteria: (1) the infant receiving physical abuse from the mother, operationalized as violent behaviors exhibited by the mother

towards the infant, which cause pain and distress, such as dragging, crushing, throwing, stepping or sitting on, or rough grooming of the infants; and (2) early maternal rejection of the infant, defined by the mother pushing away or blocking contact with the infant (Maestripieri et al., 2005; McCormack et al., 2006; McCormack et al., 2009; Sanchez, 2006; Howell et al, 2017; Drury et al., 2017). Both abuse and rejection cause high levels of distress in infants, (e.g. scream vocalizations) and elevations in stress hormones (Drury et al., 2017; Howell et al., 2013; Maestripieri D, 1998; McCormack et al., 2006; Sanchez, 2006). CONT mothers never exhibit these physical abuse or rejection behaviors (Drury et al., 2017; Howell et al., 2017); competent maternal care was defined by species-typical behaviors such as nursing, cradling, grooming, ventral contact and protection (retrieve from potential danger, restrain) of the infant (McCormack et al., 2015).

**Structural MRI Image Acquisition**

Each subject was transported with its mother from the YNPRC Field Station to the Yerkes Main Center either the day before or the morning of their scheduled scan. Structural Magnetic Resonance Imaging (sMRI) scans of the subjects' brains (T1- and T2-weighted MRIs) were acquired in the same session longitudinally, at 2 weeks, 3, 6, 12, and 18 months of age. The present study will focus specifically on sMRI scans collected at 3, 6, and 12 months, due to missing data at 2 weeks and 18 months due to technical difficulties with the scans. A 3T Siemens TRIO scanner (Siemens Med. Sol., Malvern, PA, USA) and an 8-channel phase array knee coil located at the YNPRC Imaging Center were used to acquire the T1-weighted scan with a 3-dimensional (3D) magnetization-prepared rapid gradient-echo (3D-MPRAGE) sequence with parallel imaging and the following parameters: TR/TE=3000/3.31ms, voxel-size=0.6mm$^3$, isotropic, 6 averages. A T2-weighted scan was collected in the same direction as the T1

(TR/TE=7,900/125ms, voxel size=0.5x0.5x1.0mm$^3$, 10 averages) to improve anatomical identification of white matter (WM), gray matter (GM) and cerebrospinal fluid (CSF) borders, and delineation of regions of interest (ROIs) (Knickmeyer et al., 2010; Rapisarda et al., 1983). To limit motion artifacts, subjects were scanned under isoflurane anesthesia (0.8-1%, inhalation, to effect) following induction with telazol (approx. 2-3mg/Kg BW, i.m.) and endotracheal intubation, and placement on a custom-made head holder with ear bars and a mouth piece to secure and prevent movement of the head. To identify the right brain hemisphere, a vitamin E capsule was taped to the right temple. 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. After each subject completed the scan and completely recovered from anesthesia, it was immediately returned to its mother. The pair was returned to their social group on the following day.

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

T1- and T2-weighted structural MRI data were processed and analyzed using the AutoSeg (version 3.3.2) processing and analysis open-source pipeline, which was developed in house by our collaborator, Dr. Martin Styner, at the Neuro Image Research and Analysis Laboratories of the University of North Carolina (Wang et al., 2014). AutoSeg was used to (1) perform automatic brain tissue structural segmentation into tissue classes: GM, WM and CSF tissues, (2) generate parcellations of cortical lobes (for this study: the prefrontal cortex (PFC) and selected PFC subregions: ACC Brodmann Area (BA) 24, OFC BA11 and BA13, mPFC BA25 and BA32 and the DLPFC BA 46 ) and subcortical structures (amygdala, hippocampus and the NAcc ) to

compute their respective volumes, following methods previously described (Howell et al., 2014; Knickmeyer et al., 2010;Wang et al., 2014; Shi et al., 2016).

In the first step, brain images were corrected for signal intensity inhomogeneity using N4-ITK. Next, the subject's images were registered to age-specific, population-based, infant and juvenile rhesus brain T1- and T2 MRI atlases using BRAINSFit for rigid body and affine registration (Wang et al., 2014; Styner et al., 2007; Short et al., 2010, Shi et al., 2017). The 3, 6, and 12 months UNC-Emory rhesus brain atlases were used to register the subjects' images to the age-corresponding atlas, due to drastic developmental changes in GM/WM signal contrast across these ages (Shi et al., 2017). Longitudinal scans from 48 animals were used to generate these atlases (**Figure 2**). The atlases were used to obtain atlas probabilistic tissue maps (WM, GM, CSF, no-brain) and parcellations of cortical and subcortical regions. For this we applied Atlas-Based Classification (ABC), an automatic tissue segmentation step, for its classification based on probabilistic tissue classes including brain tissue (GM, WM, or CSF) or non-brain tissue (skull, vessels, muscle) and for removal of non-brain tissue (skull-stripping) for analysis. This step works 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 edits. Next, AutoSeg uses ANTs registration to perform cortical lobar and subcortical ROI parcellations in the skull-stripped brain image by warping the ROIs in the atlas to the subject's image. The tissue class segmentations generated (GM, WM, CSF) are also applied to the cortical parcellations to generate GM, WM, and CSF segmentations for cortical regions (see example in **Figure 3**). AutoSeg then automatically computed volumes for all ROIs in this study. The present study analyzed volumetric data generated from 3, 6 and 12 months of age, and one subject had to be dropped from the analysis due to a persisting technical error and

time constraints (n=33; 18 controls (9 males, 9 females) and 15 maltreated (7 males, 8 females)). Total Intracranial volume (ICV) was also generated as the sum of total GM+total WM+total CSF to control for potential group and sex differences in total brain size.

For this study, ITK-Snap software was used for the manual edits of the skull-stripped mask by four raters blind to the experimental group and trained for interrater reliability to ensure accurate neuroanatomical delineation of brain versus non-brain tissue. Cases were counterbalanced by experimental group and sex to avoid rater segmentation bias on our measures.

**Neuroanatomical Definitions of Regions of Interest (ROI) in the Atlas**

ROI delineation was guided by macaque brain atlases (Saleem and Logothetis, 2012; Paxinos, Huang and Toga, 1999 Schmahmann and Pandya, 2006) and region-specific neuroanatomical criteria. The amygdala boundaries were defined rostrally by the anterior limit of the periamygdaloid cortex, posteriorly by the hippocampus, ventrally by white matter and CSF, ventrolaterally by WM (Amaral and Bassett, 1989; Price, 1987). The hippocampus boundaries were described in Rosene and Hoesen (1987) and defined superiorly by the lateral ventricle/temporal horn, except for its most lateral extent (subiculum) in which the superior boundary was WM, inferiorly by WM, anteriorly by the amygdala and the lateral ventricle/temporal horn, posteriorly by lateral ventricle or WM, and laterally by WM (Knickmeyer et al., 2010). The ACC, OFC, mPFC, DLPFC and the NAcc were anatomically defined based on the published Paxinos ROIs landmarks (Paxinos, Huang, and Toga, 1999) and the Paxinos rhesus stereotaxic atlas (Paxinos, Huang, and Toga, 1999).

## Cocaine self-administration (COC SA)

### Drugs

Cocaine hydrochloride (COC, generously provided by the National Institute on Drug Abuse –NIDA-, Bethesda, MD, USA) was dissolved in 0.9% sterile saline and administered intravenously. The doses of the drug were calculated and expressed as the salt form. Brevital (sodium methohexital; 3mg/kg I.V.) was used to test catheter patency on an as-needed basis, never less than two weeks apart. After injecting brevital, the subject is constantly monitored, and a catheter is deemed patent if the animal shows visible signs of ataxia within 30 seconds of administration.

### COC SA procedures

*Training*

During all SA sessions, animals were trained to use an operant response panel that included one lever and three stimulus lights (red, white, and green). At the start of each session a red stimulus light on the response panel set the occasion for an operant response (see **Figure 4** for illustration). Each animal began acquisition training during which they received 0.1 mg/kg/infusion I.V. of COC on a fixed-ratio (FR) 1 schedule of reinforcement followed by a 10 s timeout (TO), during which no drug was available. During this training, all sessions lasted 1 hr and animals were able to obtain a maximum of 5 infusions per session. Completion of three consecutive sessions whereby subjects received 4 or 5 infusions qualified an increase of the FR (1, 2, 4, 8, 16, 20) until FR20 was reached. When the FR20 was met, the TO was extended to 30 s, and subjects had to again receive 4 to 5 infusions for three consecutive sessions before the dose of COC was dropped to 0.03 mg/kg/infusion. Subjects were then allowed 20 maximum infusions per each 1 hr session until 19-20 infusions were received for 5 consecutive sessions,

and response rates did not vary by more than 25% across those 5 consecutive sessions. All inputs and outputs were recorded and controlled by a computer outside of the SA chambers.

Following acquisition, subjects were tested under a full dose response curve to determine the dose of COC that engendered maximal responding (i.e., the highest response rate). This dose of COC was termed the subject's EDMax dose of cocaine and was used in all experiments.

*Limited Access (LA)*

Time taken to obtain 20 reinforcers, or the maximum number of reinforcers obtainable under limited access (LA) conditions, and the average rate to take 20 reinforcers were the dependent measures of the present study. Each LA session lasted one hour, and subjects were tested under their EDMax dose of COC. LA consisted of 25 sessions, during which animals demonstrated stable responding.

*Extended Access (EA)*

Once LA was completed, subjects progressed to extended access (EA). In comparison to LA sessions, EA sessions lasted up to four hours or until the subject obtained the maximum number of reinforcers (60). The maximum session intake was capped at 6 mg/kg to prevent any untoward effects that could compromise the health of the subjects. All other parameters were identical to LA. EA consisted of 25 sessions, during which animals demonstrated stable responding. Time to obtain 20 reinforcers and the average response rate to take 20 reinforcers were the dependent measures of the present study. Time to obtain 20 reinforcers during EA (instead of the full 60 reinforcers) was used to allow for direct comparison between LA (in which only 20 infusions could be reinforced) and EA. LA and EA were compared to determine differences in a subject's responding when switching phases.

## Data Analyses

*Structural MRI data*

Developmental, group rearing and sex-related differences in ICV (proxy for total brain size) were analyzed using a Repeated Measures Analysis of Variance (RM ANOVA) with maternal caregiving group (CONT –competent maternal care-, MALT) and sex (male, female) as fixed factors and age (3 months, 6 months, 12 months) as the repeated measure. Left and right PFC (total, as well as PFC GM and PFC WM), hippocampus, and amygdala were also analyzed using RM ANOVA with caregiving group and sex as the fixed factors and age (3, 6, 12 months) and laterality (right vs. left hemisphere) as repeated measures. In contrast, left and right ACC BA24, OFC BA11 and BA13, mPFC BA25 and BA32, DLPFC BA46, and the NAcc volumes were only analyzed at 12 months because the 3 and 6 months data were not available, using RM ANOVA with group and sex as the fixed factors and laterality as the repeated measure.

In cases where a significant interaction effect was detected, the nature of this interaction was examined by reporting whether a linear or quadratic fit was detected. Due to the potential confounding effects of sex or group differences in brain size as well as to control for developmental changes in ICV on ROIs volumes, the statistical analyses were done both on the uncorrected measures (i.e. raw volumetric values) and ICV-corrected (i.e. raw ROI volume divided by ICV).

Kolmogorov-Smirnoff tests were run to examine normal distribution of sMRI data and, if violated, data were log10 transformed. If the assumption of homogeneity of variances was violated according to Levene's Test of Equal Variances (Independent samples t-test) or Mauchly's Test of Sphericity (RMANOVA) data were reported with equal variances not assumed using the Greenhouse-Geisser (G-G) correction. Statistical analyses were performed

using SPSS (version 24.0). Significant p values were set at $p < 0.05$, and all data are presented as mean ± SEM unless otherwise stated.

*Cocaine Self-Administration (COC SA)*

Average response rate to take 20 reinforcers was analyzed using a RM ANOVA, in which caregiving group and sex were the fixed variables, and week (1,2,3,4,5) and phase (LA, EA) were the repeated measures in the omnibus model. If no significant effect of week was found, data were collapsed across the 5 weeks to obtain an average for each subject and week was dropped from the final statistical model as a factor (i.e. phase was the only repeated measure). Average time to obtain 20 reinforcers was analyzed using a RM ANOVA, in which group and sex were the fixed variables, and week (1,2,3,4,5) and phase (LA, EA) were the repeated measures. As described above, if no week effects were detected in the omnibus model, data were collapsed across the 5 weeks to obtain an average for each subject and week was dropped from the final statistical model (i.e. phase was the only repeated measure).

Kolmogorov-Smirnoff tests were also run to examine normal distribution of COC SA data and, if violated, data were log10 transformed. If the assumption of homogeneity of variances was violated according to Levene's Test of Equal Variances (Independent samples t-test) or Mauchly's Test of Sphericity (RMANOVA) data were reported with equal variances not assumed using the G-G correction. Statistical analyses were performed using SPSS (version 24.0). Significant p values were set at $p < 0.05$, and all data are presented as mean ± SEM unless otherwise stated.

## RESULTS

- **Structural MRI Measures**

Results from Kolmogorov-Smirnoff normality tests showed that all brain sMRI data were normally distributed, therefore, was not log10-transformed.

Intracranial Volume (ICV)

A significant main effect of age ($F_{1.47,44.089}$ =65.156, p = 3.43E-12, $\eta^2$ = 0.685 -G-G corrected-) was found for total ICV (defined as total CSF+GM+WM), with volume increasing with age (**Figure 5**). A main effect of sex ($F_{1,30}$ = 14.070, p = 0.001, $\eta^2$ = 0.319) was also detected, with males showing significantly larger ICV than females overall. No other main or interaction effects were found.

Total WM, GM, and CSF Volumes

A significant main effect of age was detected in total WM, GM, and CSF (GM: $F_{1.532,45.947}$ = 8.179, p = 0.002, $\eta^2$ = 0.214 -G-G corrected-;WM: $F_{2,60.}$ = 174.255, p = 1.0196E-25, $\eta^2$ = 0.853; CSF: $F_{1.637,49.102}$ = 65.743, p = 2.201E-13, $\eta^2$ = 0.687 -G-G corrected-), where volumes increase with age, as shown in **Figure 6**. A significant main effect of sex was detected in total GM, WM, and CSF (GM: $F_{1,30}$ = 11.821, p = 0.002, $\eta^2$ = 0.283; WM: $F_{1,30}$ = 13.268, p = 0.001, $\eta^2$ = 0.307; CSF: $F_{1,30}$ = 5.044, p = 0.032, $\eta^2$ = 0.144), with males having larger volumes that females. No other main or interaction effects were found.

Hippocampal Volume

In the hippocampus, a significant main effect of age was found ($F_{1.647,49.421}$ =1449.164, p = 7.351E-43, $\eta^2$ =0.980 -G-G corrected-), showing that volumes increase with age (**Figure 7**). A main effect of sex was detected ($F_{1,30}$ = 10.374, p = 0.003, $\eta^2$ = 0.257), with larger hippocampal

volumes in males than females. An age by sex interaction was detected ($F_{1.647,30} = 5.121$, $p = 0.014$, $\eta^2 = 0.146$ -G-G corrected-), which fitted a linear trend ($F_{1,30} = 6.865$, $p = 0.014$, $\eta^2 = 0.002$), suggesting that hippocampi in females grew at a slower rate. A main effect of laterality ($F_{1,30} = 6.912$, $p = 0.013$, $\eta^2 = 0.187$ -G-G corrected-) was also detected, showing faster growth of the right than left hippocampus. An age by laterality interaction effect was also detected ($F_{1.907,57.215} = 6.784$, $p = 0.003$, $\eta^2 = 0.184$ -G-G corrected-), fitting a quadratic trend ($F_{1,30} = 14.028$, $p = 0.001$, $\eta^2 = 0.319$), showing faster growth in the right hemisphere from 6-12 months of age. No other main or interaction effects were found.

When hippocampal volumes were ICV-corrected, the main effect of age persisted ($F_{2,60} = 2109.556$, $p = 2.535E\text{-}56$, $\eta^2 = 0.986$), showing region-specific hippocampal volumes increases with age, either independent of ICV growth or at higher rates than ICV (**Figure 8**). Neither the main sex effect nor the age by sex interactions survived ICV-correction, suggesting that the smaller hippocampal size in females is driven by general smaller brain sizes compared to males. The main effect of laterality persisted ($F_{1,30} = 8.012$, $p = 0.009$, $\eta^2 = 0.211$), further suggesting different rates of growth by hemisphere in the hippocampus. Age by laterality interaction effect also persisted ($F_{2,60} = 7.634$, $p = 0.001$, $\eta^2 = 0.23$), and it followed a quadratic trend ($F_{1,60} = 15.513$, $p = 0.000452$, $\eta^2 = 0.341$), showing that there are differential rates of brain growth of each hemisphere of the hippocampus at different ages. No other main or interaction effects were found.

Amygdala Volume

A main effect of age was detected ($F_{1.447,43.412} = 817.589$, $p = 1.0839E\text{-}32$, $\eta^2 = 0.965$-G-G corrected-), with volumes increasing with age (**Figure 9**). A group by laterality interaction effect ($F_{1,30} = 5.961$, $p = 0.021$, $\eta^2 = 0.166$) was also observed, which followed a linear trend

($F_{1,30} = 5.961$, $p = 0.021$, $\eta^2 = 0.166$), with bigger left amygdala volumes in MALT than CONT animals. Main effects of sex ($F_{1,30} = 9.874$, $p = 0.004$, $\eta^2 = 0.248$), with larger amygdalae in males than females, and laterality ($F_{1,30} = 329.448$, $p = 9.994E\text{-}18$, $\eta^2 = 0.917$ -GG corrected-) were also detected. Additional age by laterality ($F_{1.696,50.881} = 14.511$, $p = 0.000028$, $\eta^2 = 0.326$ – G-G corrected-; following a linear trend ($F_{1,30} = 9.366$, $p = 0.005$, $\eta^2 = 0.238$) and age by laterality by sex interaction effects ($F_{1.696,30} = 3.187$, $p = 0.057$, $\eta^2 = 0.096$ -G-G corrected-; following a linear trend ($F_{1,30} = 4.278$, $p = 0.047$, $\eta^2 = 0.125$) were found. No other main or interaction effects were found.

The age main effect persisted after ICV-correction ($F_{1.884,56.512} = 1656.495$, $p = 2.8563E\text{-}50$, $\eta^2 = 0.982$ -G-G corrected-), showing specific amygdala growth with age, independent of ICV increases (**Figure 10**). Group by laterality interaction effects were also preserved ($F_{1,30} = 6.646$, $p = 0.015$, $\eta^2 = 0.181$ -G-G corrected-), and followed a linear trend ($F_{1,30} = 6.646$, $p = 0.015$, $\eta^2 = 0.181$), suggesting that MALT effects on left amygdala are region-specific. As for the hippocampus, the main effect of sex did not survive ICV-correction, suggesting that the smaller amygdalar size in females than males is driven by overall sex differences in brain size. Laterality ($F_{1,30} = 325.533$, $p = 1.178E\text{-}17$, $\eta^2 = 0.916$ -G-G corrected-) and age by laterality interaction effects ($F_{1.634,49.021} = 10.722$, $p = 0.000340$, $\eta^2 = 0.263$ -G-G corrected- following a quadratic linear trend ($F_{1,30} = 32.759$, $p = 0.000003$, $\eta^2 = 0.522$) also remained after ICV correction. The age by laterality by sex interaction did not survive ICV correction, either. No other main or interaction effects were detected after ICV correction.

PFC Volume

*Total PFC Volume (GM+WM)*

A main effect of age was detected ($F_{1.366,40.966}$ = 29.882, p = 2.513E-7, $\eta^2$ = 0.499 -G-G corrected-), with PFC volumes increasing with age (**Figure 11**). An age by laterality interaction effect ($F_{1.731,51.940}$ = 14.135, p = 0.000030, $\eta^2$ = 0.320 -G-G corrected-, showing a linear trend ($F_{1,30}$ = 25.212, p = 0.000022, $\eta^2$ = 0.457) indicated that the left PFC grows faster than the right (12 months: right=3678.604±104.7961, left=3708.963±100.1443; 6 months: right=3638.305±104.7193, =3649.05±99.10676; 3 months: right=3408.017±90.15278, left=3381.642±85.60252). A main effect of sex ($F_{1,30}$ = 7.517, p = 0.010, $\eta^2$ = 0.200) was also detected, with larger PFC volumes in males than females. No other main or interaction effects were found.

The main age effect persisted after correcting for brain size (ICV) ($F_{1.549,46.468}$ = 15.325, p = 0.000035, $\eta^2$ = 0.338 -G-G corrected-) (**Figure 12**). The age by laterality interaction effect also persisted after ICV correction ($F_{1.734,}$ = 14.260, p = 0.000027, $\eta^2$ = 0.322 -G-G corrected-; fitting a linear trend ($F_{1,30}$ = 23.874, p = 0.000032, $\eta^2$ = 0.443). The main effect of sex did not survive ICV-correction. No other main or interaction effects were found.

*Total PFC GM*

A main effect of age was detected ($F_{1.407,42.203}$ = 10.115, p = 0.001, $\eta^2$ = 0.252 -G-G corrected-), so that volumes increased with age (**Figure 13**). An age by laterality interaction effect ($F_{1.914,57.428}$ = 13.547, p = 0.000020, $\eta^2$ = 0.311 -G-G corrected-) was detected, and this interaction effect fit a linear trend ($F_{1,30}$ = 21.424, p = 0.000066, $\eta^2$ = 0.417) indicating that the left PFC GM grows faster than the right (12 months: right=2935.404±78.83911, left=2946.291±76.63069; 6 months: right=2958.425±81.50464, left=2961.645±78.40304; 3

months: right=2806.735±76.64363, left=2775.136±72.67681). A main effect of sex was also detected ($F_{1,30} = 5.935$, $p = 0.021$, $\eta^2 = 0.165$), with larger PFC GM volumes in males than females. No other main or interaction effects were found.

*Total PFC WM*

Main effects of age ($F_{1.881,56.433} = 79.016$, $p = 1.2402E\text{-}16$, $\eta^2 = 0.725$ -G-G corrected-) and sex were detected ($F_{1,30} = 8.825$, $p = 0.006$, $\eta^2 = 0.227$) with larger PFC WM volumes in males than females (**Figure 14**). Group by age by sex ($F_{1.881,56.433} = 5.099$, $p = 0.010$, $\eta^2 = 0.145$ -G-G corrected-, fitting a linear trend ($F_{1,30} = 7.240$, $p = 0.012$, $\eta^2 = 0.194$) and group by sex by age by laterality interaction effects were found ($F_{1.376,41.278} = 5.079$, $p = 0.020$, $\eta^2 = 0.145$, showing a quadratic linear trend ($F_{1,30} = 6.868$, $p = 0.014$, $\eta^2 = 0.186$), which seem to be driven by CONT males (12 months: CONT males=827.684±44.53908, CONT females=706.6819±23.36024, MALT males=813.4304±47.77987, MALT females=663.9459±32.45824; 6 months: CONT males=755.1321111±44.05648236, CONT females=660.4477778±41.62844067, MALT males=718.8651875±43.33640383, MALT females=600.12575±22.01871158; 3 months: CONT males=671.5686111±40.93862686, CONT females=554.2556667±35.33640684, MALT males=601.0669375±30.77682372, MALT females=588.6866875±25.60606468), and indicating that the left PFC WM grows faster than the right (12 months: right= 743.1998299±32.39755819, left=762.6719618±30.03176078; 6 months: right=679.880941±30.60200493, left=687.4044722±29.09929598; 3 months: right=601.2822257±21.69223364, left=606.5067257±27.9766931). An age by sex interaction effect ($F_{1.881,56.433} = 4.452$, $p = 0.018$, $\eta^2 = 0.129$ -G-G corrected-) was also detected, following a linear trend ($F_{1,30} = 8.064$, $p = 0.008$, $\eta^2 = 0.212$). A main effect of laterality ($F_{1,30} = 10.140$, $p = 0.003$, $\eta^2 = 0.253$-G-G corrected-) as well as laterality by sex interaction effect ($F_{1,30} = 4.890$, $p =$

0.035, $\eta^2 = 0.140$ -G-G corrected-, fitting a linear trend ($F_{1,30} = 4.890$, $p = 0.035$, $\eta^2 = 0.140$) were detected. No other main or interaction effects were found.

*OFC Volume*

- *OFC Brodmann Area 11 Volume*

A main laterality effect ($F_{1,29} = 22.073$, $p = 0.000059$, $\eta^2 = 0.432$) was detected, with larger left than right BA 11 volumes (**Figure 15**). No other main or interaction effects were found. After correcting for ICV volume, the main laterality effect persisted ($F_{1,29} = 21.394$, $p = 0.000072$, $\eta^2 = 0.425$) (**Figure 16**). No other main or interaction effects were found.

- *OFC Brodmann Area 13 Volume*

A main effect of sex ($F_{1,29} = 6.107$, $p = 0.020$, $\eta^2 = 0.174$) was detected, with larger OFC BA13 in males than females (**Figure 17**). No other main or interaction effects were found. After ICV-correction, the main effect of sex disappeared, suggesting that the sex effects were accounted for sex differences in brain size (**Figure 18**). No other main or interaction effects were found.

*ACC Brodmann Area 24 Volume*

No main or interaction effects were found for either raw or ICV-corrected volumes (**Figures 19 & 20**).

*mPFC Volume*

- *mPFC Brodmann Area 25*

A main effect of laterality ($F_{1,29} = 49.471$, $p = 9.7896E\text{-}8$, $\eta^2 = 0.630$) was detected, with larger volumes in left than right BA 25 (**Figure 21**). No other main or interaction effects were found. The main effect of laterality persisted after ICV-correction ($F_{1,29} = 53.060$, $p = 5.0605E\text{-}8$, $\eta^2 = 0.647$) (**Figure 22**). No other main or interaction effects were found.

- *mPFC Brodmann Area 32*

A main effect of sex ($F_{1,29} = 5.526$, $p = .026$, $\eta^2 = 0.160$) was detected, showing males with larger volumes than females (**Figure 23**). A main effect of laterality ($F_{1,29} = 7.671$, $p = 0.010$, $\eta^2 = 0.209$) was also detected, showing larger volumes in the right hemisphere than the left. No other main or interaction effects were found. ICV-correction made the main sex effect disappear, suggesting that it was driven by overall sex differences in brain size (**Figure 24**). The main effect of laterality persisted ($F_{1,29} = 8.062$, $p = 0.008$, $\eta^2 = 0.218$). No other main or interaction effects were found.

*DLPFC Brodmann Area 46 Volume*

A main effect of laterality ($F_{1,29} = 4.418$, $p = 0.044$, $\eta^2 = 0.132$) was detected (**Figure 25**). No other main or interaction effects were found. The main laterality effect persisted following ICV-correction ($F_{1,29} = 4.777$, $p = 0.037$, $\eta^2 = 0.141$) (**Figure 26**). No other main or interaction effects were found.

<u>NAcc Volume</u>

A main effect of sex ($F_{1,29} = 5.643$, $p = 0.024$, $\eta^2 = 0.163$) was detected, with males showing larger NAcc volumes than females (**Figure 27**). No other main or interaction effects were found. A main group effect emerged following ICV-correction ($F_{1,29} = 6.632$, $p=0.015$, $\eta^2 = 0.186$) , showing larger NAcc to ICV volume ratio than CONT subjects (**Figure 28**). The main effect of sex did not survive ICV-correction, suggesting that it was driven by sex differences in brain size. No other main or interaction effects were found.

- **Cocaine Self-Administration**

Results from Kolmogorov-Smirnoff normality tests showed that all COC SA data were normally distributed, therefore, was not log10-transformed.

Average Response Rate

*Changes from LA to EA*

A RM ANOVA was also used to analyze potential changes in response rates from LA to EA. No main or interaction effects were found (**Figure 29**) even after collapsing data across the five weeks in both phases (limited access or extended access). No main or interaction effects were found (**Figure 30**).

*LA Average Response Rate*

Average response rates over the five weeks of LA were analyzed using a RM ANOVA, and no main or interaction effects were found (**Figure 29**).

*EA Average Response Rate*

Average response rates over the five weeks of EA were analyzed using a RM ANOVA, and no main or interaction effects were found (**Figure 29**).

Average Time to Obtain 20 Reinforcers

*Changes from LA to EA*

A repeated measures analysis of variance (RM ANOVA) analyzed potential changes in the time to obtain 20 reinforcers from LA to EA. The RM ANOVA detected no main or interaction effects (**Figure 29**), even after collapsing across the five weeks in each phase (**Figure 30**).

*LA Average Time to Obtain 20 Reinforcers*

Average time to obtain 20 reinforcers across the five weeks of LA were analyzed using an RMANOVA, in which no main or interaction effects were found (**Figure 29**).

*EA Average Time to obtain 20 Reinforcers*

Average time to obtain 20 reinforcers across the five weeks of EA were analyzed using an RMANOVA, in which no main or interaction effects were found (**Figure 29**).

## DISCUSSION

The goal of this study was to examine the impact of ELS on brain structural development by focusing on the development of the amygdala and hippocampus (due to their critical role in stress and emotional regulation and their connections with reward regions), the PFC (due to its critical role in cognitive control of behavior, goal-directed behaviors, impulse/perseverative control, reward associations, emotional regulation, response inhibition, and top-down control of reward centers), and the NAcc due to its role in reward processes and reinforcing effects of COC. The main aim was to identify neurodevelopmental underpinnings linking ELS and psychostimulant escalation using a NHP of infant maltreatment and a COC SA paradigm during adolescence. It has been suggested that neurodevelopmental trajectories rather than isolated individual timepoints in development are more closely related to behavioral outcomes (Shaw et al., 2006). Therefore, we examined the impact of ELS, defined by a naturalistic infant maltreatment model, on longitudinal structural development of those brain regions using MRI scans acquired at 3, 6, and 12 months of age, to generate volumetric growth data of these brain regions. Confounding effects of heritability were controlled for by using a cross-fostering

paradigm, in which all subjects were randomly assigned to either a maltreating or control mother at birth. Furthermore, this study also sought to examine whether animals who had been maltreated during infancy were more vulnerable than controls on a COC SA escalation paradigm during adolescence. The present study revealed bigger amygdala volumes in maltreated than control animals, which was consistent with previous findings (Mitra et al., 2005; Tottenham et al., 2010), and supported our hypotheses. Results also revealed increased NAcc volumes at 12 months of age in maltreated animals in comparison to controls, which was unexpected, but consistent with previous findings that have suggested larger NAcc volumes are indicative of greater trait anxiety (Kühn, Schubert and Gallinat, 2011). Failure to detect a significant impact of maternal care on PFC development could have been due to its slow maturation rate (Giedd and Rapoport, 2010), and maltreatment-related effects may arise later in development. Failure to detect an impact of ELS in the hippocampus was surprising, given previous findings that suggest reductions in hippocampal volume following early stress exposure (Lupien et al., 2009). The hypothesis that NHPs that experienced ELS would escalate during the COC SA EA phase (i.e. would require a shorter time to take 20 reinforcers than controls and have a higher response rate across trials (LA and EA)), was not supported, as no significant differences between groups were detected. It is possible that the gap between infancy (when subjects experienced ELS) and when they began testing under COC SA (adolescence) explain this lack of effects, although the link between ELS and risk for adolescence emergence of SUDs is well established (Heim et al., 2001; Bremmer and Vermetten, 2001; Glaser, 2000; Gunnar and Vazquez, 2006). Furthermore, it is possible that since adolescence is a critical period for the brain to reorganize itself, a second "hit" (i.e. stress, insult) experienced during adolescence is required to trigger the risk for developing an addictive phenotype (Romeo and McEwen, 2006; Spear, 2000). Total brain size, measured in

this study as ICV (GM+WM+CSF), showed a significant increase in volume from infancy to the early juvenile period (3 to 12 months of age). This finding was expected, based on previous studies in humans and macaques (Giedd and Rapoport, 2010; Knickmeyer et al., 2010; Liu et al., 2015; Malkova et al., 2006), with this being a critical period for brain development (Gale et al., 2004). This increase in brain size was driven by significant increases in GM, WM, and CSF volumes with age. As expected, males had significantly larger ICV volumes than females, consistent with previous reports in macaques (Scott et al., 2016; Malkova et al., 2006), and humans (Giedd and Rapoport, 2010). In summary, our findings indicate that ELS has a negative impact on neurodevelopment, with region-specific effects on amygdala and NAcc development, but not the PFC or hippocampus. Although we were unable to detect an effect of maternal care on the development of the PFC or hippocampus, previous research in humans has demonstrated variations in directionality, magnitude and developmental trajectories, suggesting that these alterations may arise at later timepoints, potentially into adulthood (Tottenham and Sheridan, 2010). Furthermore, we did not observe an impact of ELS on intake in COC SA, but were able to accurately replicate previous studies that utilized NHP EA conditions.

Significant increases in amygdala volume were detected with age in all groups, both in males and females, which was consistent with reports in macaques and humans (Payne et al, 2009; Chareyron et al., 2012; Tottenham and Sheridan, 2009; Tottenham, 2012). A linear group by laterality interaction effect was detected in the amygdala and preserved after ICV-correction (i.e. corrections for differences in brain size). This revealed that maltreated subjects had larger left amygdalar volumes than controls. This finding supports the hypothesis that the ELS experience impacts amygdalar development, and it is consistent with previous findings in humans and animal models, including macaques, that early life adversity/stress results in bigger

amygdala volumes (Mitra et al., 2005; Tottenham et al., 2010; Noble et al., 2012; Howell et al, 2014). Additionally, a linear age by laterality by sex interaction effect was detected, further showing that males have bigger left amygdalar volumes than females. This interaction effect did not survive ICV-correction, suggesting that the sex differences in amygdala volume were driven by overall sex differences in brain size.

Analysis of the NAcc at 12 months of age revealed larger NAcc volumes in males than females, although the effect did not survive ICV-correction, suggesting that these differences were driven by overall sex differences in brain size. More interesting, however, was a main ELS effect that survived ICV-correction, suggesting larger NAcc volumes in maltreated than control juveniles. The majority of studies assessing the impact of stress on the NAcc are primarily focused on gene expression and DA signaling, reporting downregulation of DA receptors and disrupted signaling between the PFC and NAcc (Cicchetti and Toth, 2005; Hein, Cohen and Campbell, 2005; MacAskill et al., 2014; Goff and Tottenham, 2014. Of the few studies that have analyzed the impact on NAcc volume a study using high-resolution MRI scans in humans reported that trait anxiety (considered a stress-related disorder) was positively correlated with bilateral NAcc volume (Kühn, Schubert and Gallinat, 2011). Therefore, the larger NAcc volumes observed in maltreated subjects compared to controls could be associated with not only COC SA measures, but with their elevated state anxiety, (measured by startle amplitude; Morin et al., in preparation).

The volume of the PFC (total, GM and WM) increased with age. Typically, the growth of the PFC in macaques shows an inverted U shape pattern which is driven by both PFC GM and WM, with the peak in GM occurring during adolescence and decreasing afterwards due to pruning, while the PFC WM volume continues to increase throughout the juvenile and

adolescence periods and through young adulthood (Knickmeyer et al., 2010). This is consistent with the developmental pattern reported for the human PFC (e.g. Giedd and Rapoport, 2010). Males had larger PFC volumes (total, GM, WM) than females, but this effect did not survive ICV-correction, suggesting that the sexual dimorphism in PFC volume was driven by overall sex differences in brain size. The lack of maltreatment effects on PFC volumes (total, GM, WM) was inconsistent with previous studies reporting chronic stress exposure, ELS and early adversity in general, linked to decreases in PFC (total, GM or WM) volumes in humans and animal models (Ansell et al., 2012; Sanchez et al., 1998; Spinelli et al., 2009; Hedges and Woon, 2011; Naninck et al., 2015; Lupien et al., 2009). Although inconsistent with those reports, our lack of PFC effects are not surprising given the early developmental stage of the animals in our studies (latest age: early juvenile period -12 months- equivalent to 4 years of age in children), and the protracted development of the PFC, which develops past childhood and adolescence into young adulthood (Hanson et al., 2012); therefore, the PFC is not fully developed at 12 months of age, the endpoint for this study, and a main effect may arise later in development (e.g. during adolescence).

When investigating subregions of the PFC, a main effect of sex in the OFC BA13 was detected, showing males with larger OFC BA13 volumes than females. This effect did not survive ICV-correction, suggesting that smaller OFC BA13 sizes is driven by general smaller brain sizes compared to males. Despite the evidence that exposure to chronic stress results in a dendritic retraction on pyramidal neurons and reduced spine densities in the ACC and the mPFC (Barfield and Gourley, 2018; Anderson et al., 2016), no group differences were observed. Few studies have analyzed whether morphological effects persist after the stress exposure ends (for review, see Barfield and Gourley, 2018). Furthermore, multiple reports have suggested that

dendrites and dendritic spines can recover with 1-3 weeks of rest (Radley et al., 2005; Bloss et al., 2010, 2011; Moench and Wellman, 2017). The analysis of the PFC subregions was only at 12 months of age, and since the highest levels of maltreatment occur from 3-6 months (Sanchez, 2006), this could be an explanation of why no group differences were detected. However, this does not seem to be the case, at least based on the lack of effects on total PFC, GM and WM at either 3 or 6 months. No main effects of group were detected in any of the subregions of the PFC at 12 months of age, suggesting that early OFC, ACC, mPFC, and DLPFC development is unaffected by maternal care quality. "Sleeper or programming effects" of ELS could still arise later in development, during or after adolescence given the protracted development of the PFC past childhood and previous evidence in humans (Hanson et al., 2012). A main effect of sex was also detected in the mPFC BA32, showing males have larger volumes than females, but this effect did not survive ICV-correction.

In the hippocampus, analysis revealed that the volume of this region significantly increases with age, and this finding persisted after ICV-correction. This finding was expected because it reflects the normative growth of the brain during development. A main effect of sex was detected, showing males with larger hippocampi volumes compared to females. This effect did not survive ICV-correction, suggesting that the sex differences in hippocampal volume were driven by overall sex differences in brain size. An age by sex interaction was also detected, showing that hippocampi in females grow at a slower rate than males. This effect did not remain after ICV-correction, which is consistent with the lack of sex effects previously reported in rhesus monkey hippocampal development (Payne et al., 2010). Despite previous research that has suggested that chronic stress exposure results in reduced hippocampal volumes (Lupien et al., 2009), the present study failed to detect significant effects of maternal care quality on the

volume of the hippocampi. Although maltreated subjects appeared to have smaller hippocampal volumes, these differences were not significant.

Analysis of the average response rate to obtain 20 reinforcers revealed no main effect of phase, suggesting that there is not a significant difference in the rate of responding between LA and EA in either experimental group. This finding is consistent with the previous research in NHPs that has showed stable COC intake throughout EA procedures suggesting that they don't "escalate" or "burst" intake (Kirkland, Davis and Howell, 2009), in contrast to rodents, which do increase response rates in EA in comparison to LA (Ahmed and Koob, 1998, 1999; Lynch et al., 2000). No main effect of week was detected, meaning that the rate in responding did not vary significantly from week to week. In the same study with Kirkland, Davis and Howell (2009), they found that higher levels of COC SA are maintained across the 60-day testing period, which is consistent with our findings. Similarly, analysis of the average time to obtain 20 reinforcers revealed no main effect of phase, suggesting that there is not a significant difference between LA and EA; that is there is no true "escalation" detected in either group. No main effect of week was detected, meaning that the time to obtain 20 reinforcers did not vary significantly from week to week. Our data suggest that maltreatment has no effect on COC SA throughout all phases examined in these experiments. Our data, in conjunction with results from Kirkland, Davis and Howell (2009), paint a very different picture of drug escalation in comparison to what has been reported in rodent models (Ahmed and Koob, 1999; Ferrario et al., 2005; Knackstedt and Kalivas, 2007). Specifically, response rates increase dramatically when rodents switch from a short access phase to an extended access phase, whereby they gradually escalate responding for COC over time (Ahmed and Koob, 1999; Ferrario et al., 2005; Knackstedt and Kalivas, 2007). In sharp contrast, NHPs demonstrate remarkably stable response rates throughout LA and EA SA

phases, despite taking almost all of the reinforcers (i.e. they seem to pace themselves). It is important to note, however, several differences in the NHP model of escalation used herein, and rodent models of escalation. Generally, rodents use lower fixed ratio (FR) schedules, e.g. FR1, than what we used in our study -i.e. FR20- (e.g. Roberts et al., 2002). In contrast to our study in which each subject received a different dose, intended to engender maximal responding (i.e. their EDMax dose), studies with rodents typically use the same dose for all subjects (e.g. Roberts et al., 2002).

A major limitation of this study was the sample size, which, although big for macaque studies, is small in comparison to human studies and limited our power to examine complex interactions between factors. The sample was even smaller for the COC SA studies, resulting in higher individual variable and reduced statistical power. This is a common concern in NHP research. Another limitation was the inability to analyze the structural changes in brain development of the PFC subregions and the NAcc at earlier ages (3 and 6 months of age). Analysis of additional longitudinal structural data collected in these subjects at other ages (not just at 3 and 6 months for PFC subregions, but additional scans at 2 weeks and 18 months of age) will likely provide a clearer understanding of the impact of infant maltreatment on brain development. Also, it should be mentioned that the findings reported here should be considered preliminary until analyses in the full cohort of 42 animals are completed. This is especially the case for the COC SA data analyzed, which was limited to data from only 14 out of 25 subjects in the study.

In summary, findings in this study suggest that maternal care quality does impact brain structural development. Thus, maltreated animals had larger left amygdalar volumes than controls, which supports our hypothesis of significantly larger amygdalar volumes in individuals

that experienced ELS and is consistent with previous findings in humans (Mitra et al., 2005; Tottenham et al., 2010). Interestingly, analysis of the NAcc at 12 months revealed that maltreated subjects also had larger NAcc volumes compared to controls, which has been associated with trait anxiety (Kühn, Schubert and Gallinat, 2011) and a predictive risk factor for the development of other psychiatric disorders. Surprisingly, no main effect of caregiving was found in either PFC or hippocampus but may emerge at later ages and after the remaining subjects are added to the analysis. This may be particularly true for the PFC, which is still developing past 12 months of age into young adulthood (Giedd and Rapoport, 2010). Since the analysis of the PFC subregions was limited to just the 12 months of age sMRI scans, data at 3 and 6 months need to be added to the analysis to examine earlier effects of caregiving. Maltreatment did not have a significant impact on the COC SA measures analyzed to examine vulnerability to escalation (response rate and time to obtain 20 reinforcers during the EA phase), suggesting that all subjects, regardless of rearing, self-administer COC at comparable levels. No main effects of phase were detected for average response rates or the time to obtain 20 reinforcers, suggesting that there is not a change in responding during COC SA when NHPs shift from LA to EA. This finding is consistent with the previous research in NHPs that has showed stable COC intake throughout escalation (Kirkland, Davis and Howell, 2009). Although not originally hypothesized, that NHPs demonstrated a lack of escalation is interesting and merits further examination. Specifically, whether this lack of escalation persists when a higher dose of COC is used or when a schedule of reinforcement that is more sensitive to the reinforcing efficacy of a stimulus (such as a progressive ratio schedule) may help researchers further understand what conditions are necessary to produce “binge-like” behavior in humans. Further

studies are needed with these subjects longitudinally to better understand the impact of ELS on brain structural development and measures of COC SA during adolescence.

## FIGURES AND TABLES

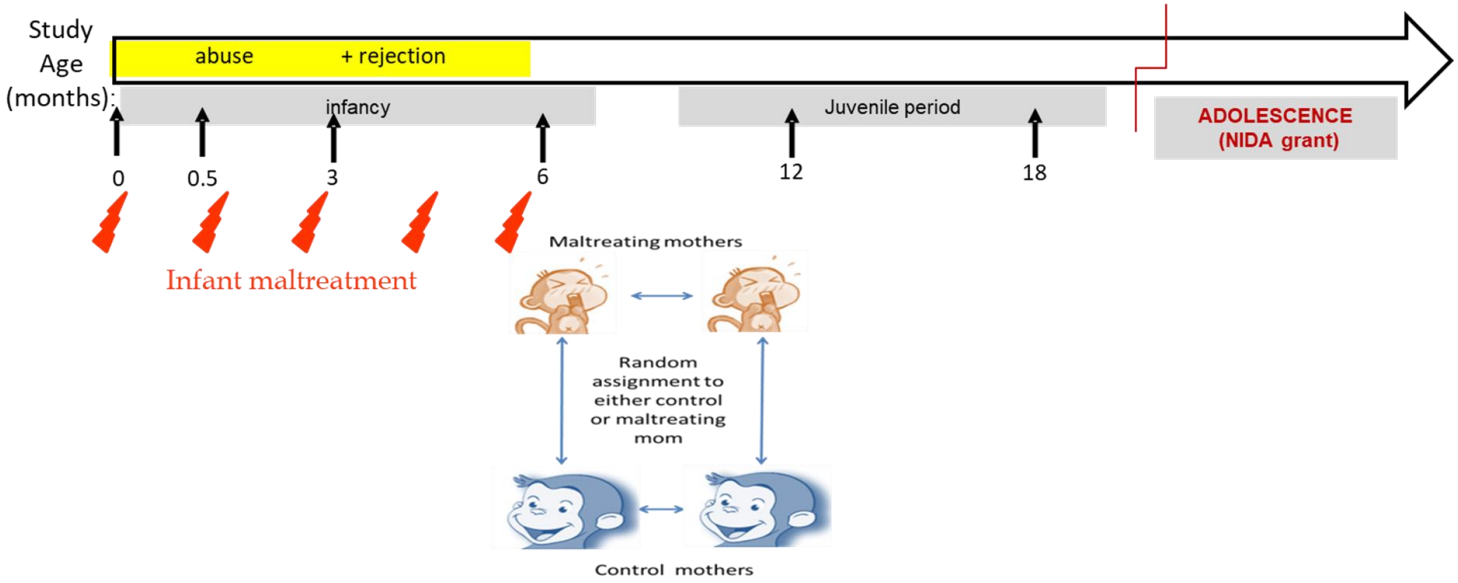


**Figure 1.** Schematic of the experimental design.

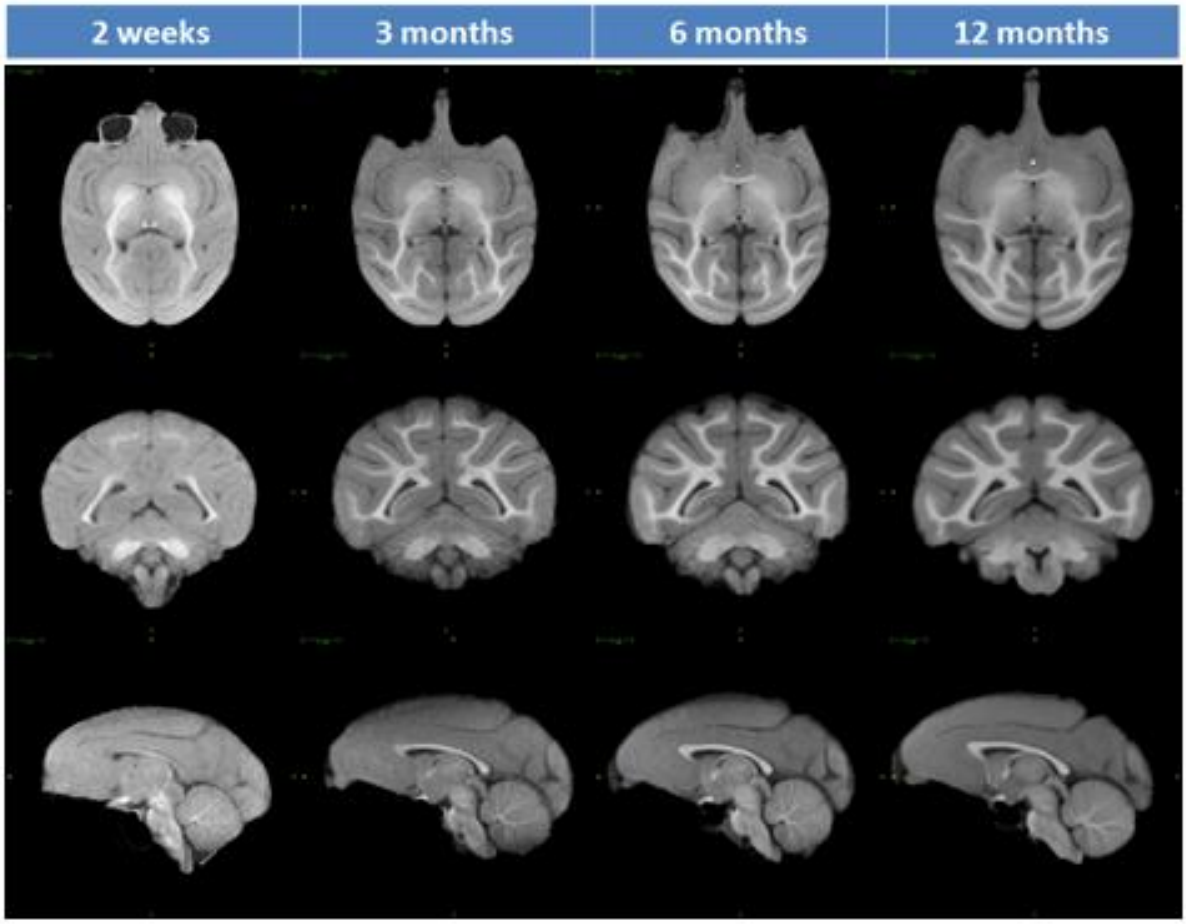


**Figure 2**. UNC-Emory infant rhesus structural MRI brain atlases. Reproduced from (Shi et al, 2017), with permission from the authors.

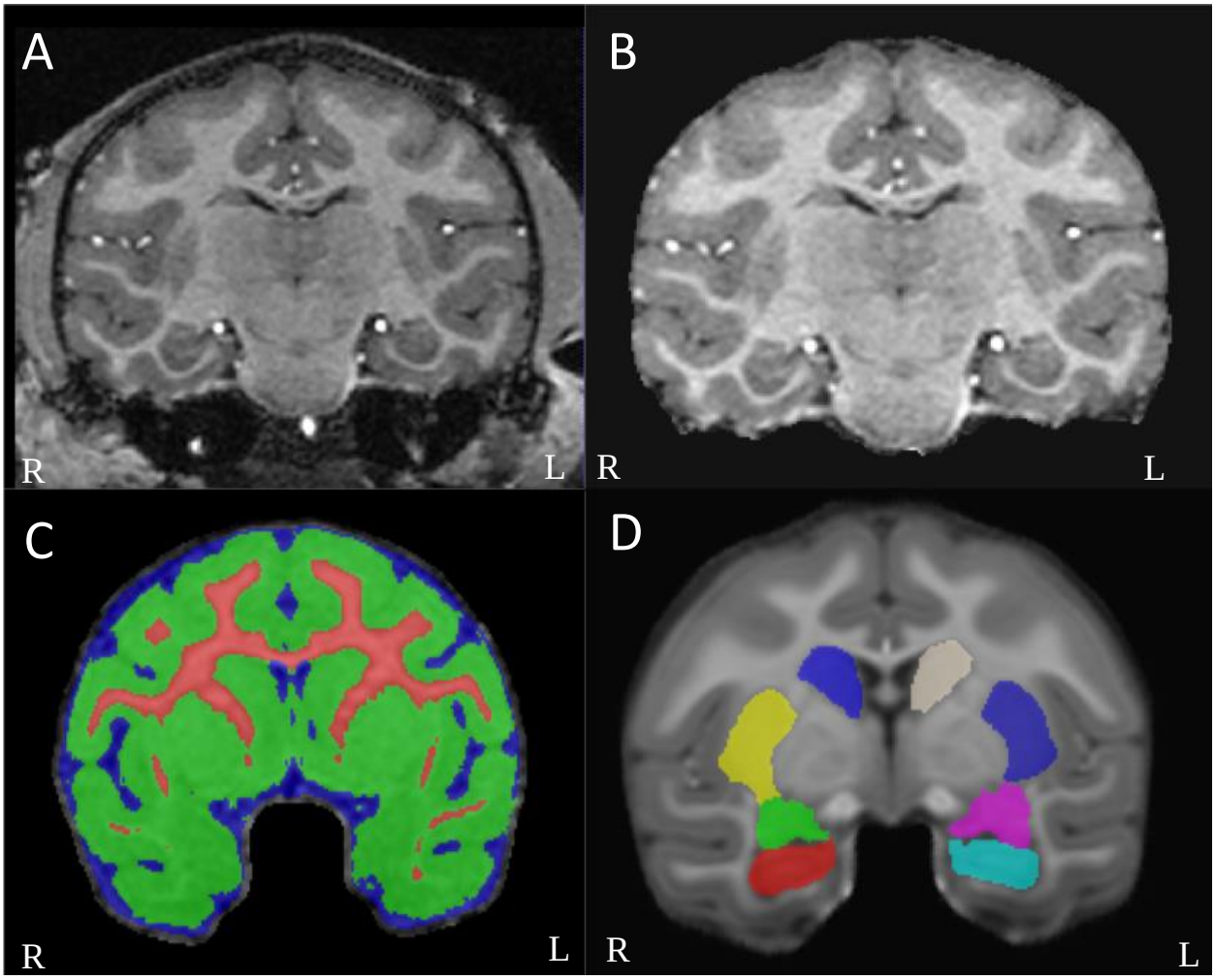


**Figure 3.** Representative images of skull-stripping, automatic tissue segmentation and subcortical ROI parcellation generated from AutoSeg. A) Coronal view of the brain in a T1-MRI non-skull stripped image. B) The T1 image as A, but skull-stripped. C) Coronal view of the brain, segmented into white 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).

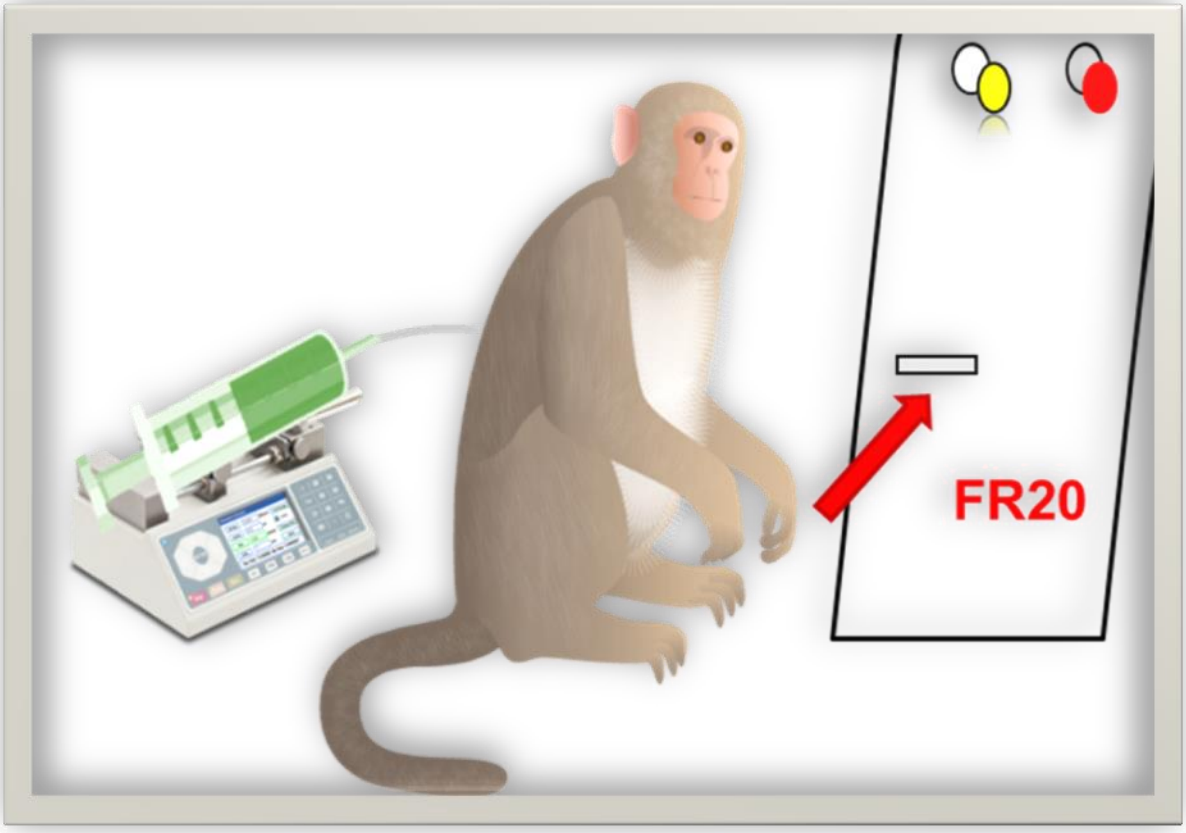


**Figure 4.** Schematic of the COC SA paradigm. (Schematic provided by Berro et al., 2017).

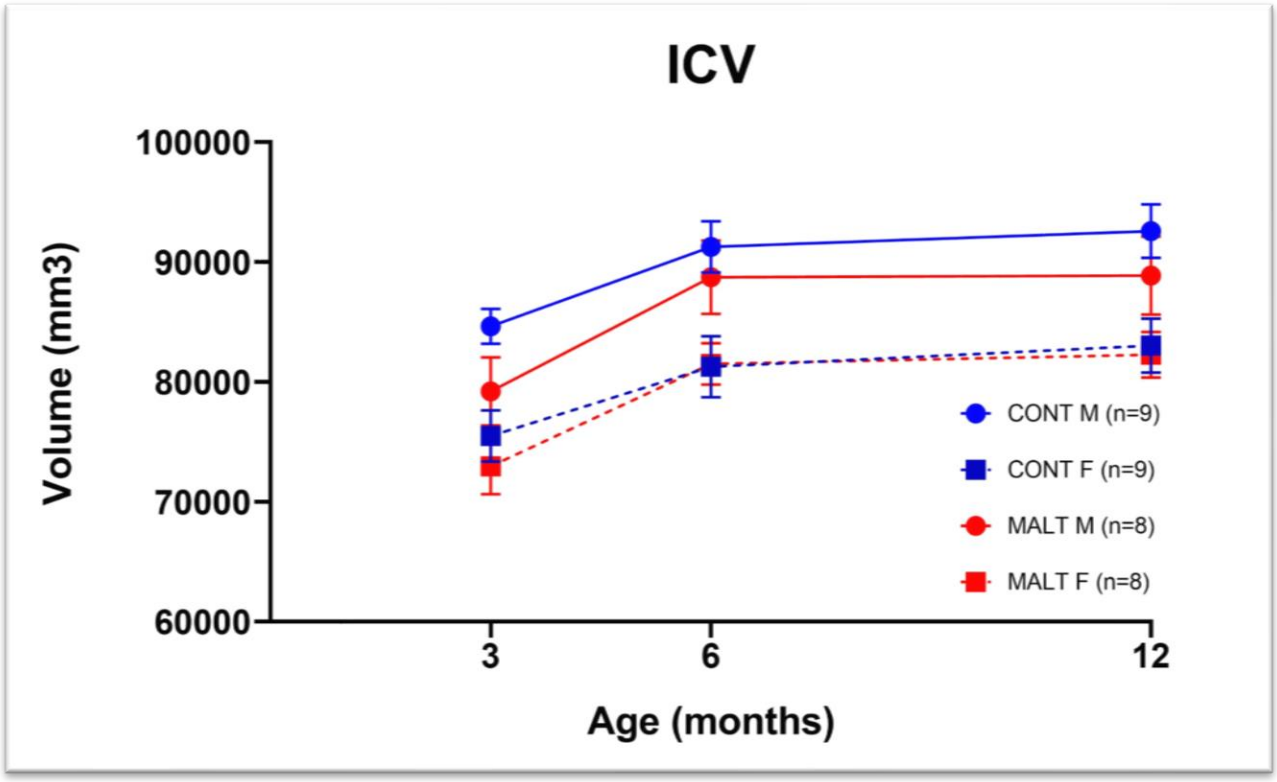


**Figure 5.** Developmental effects of infant maltreatment on total ICV, separated by group and sex. A significant effect of age ($F_{1.47,44.089}$ =65.156, p = 3.43E-12, $\eta^2$ = 0.685 -G-G corrected-) was found with ICV increasing with age. A main effect of sex ($F_{1,30}$ = 14.070, p = 0.001, $\eta^2$ = 0.319) was also detected, with larger ICV in males than females. Plots represent mean± SEM.

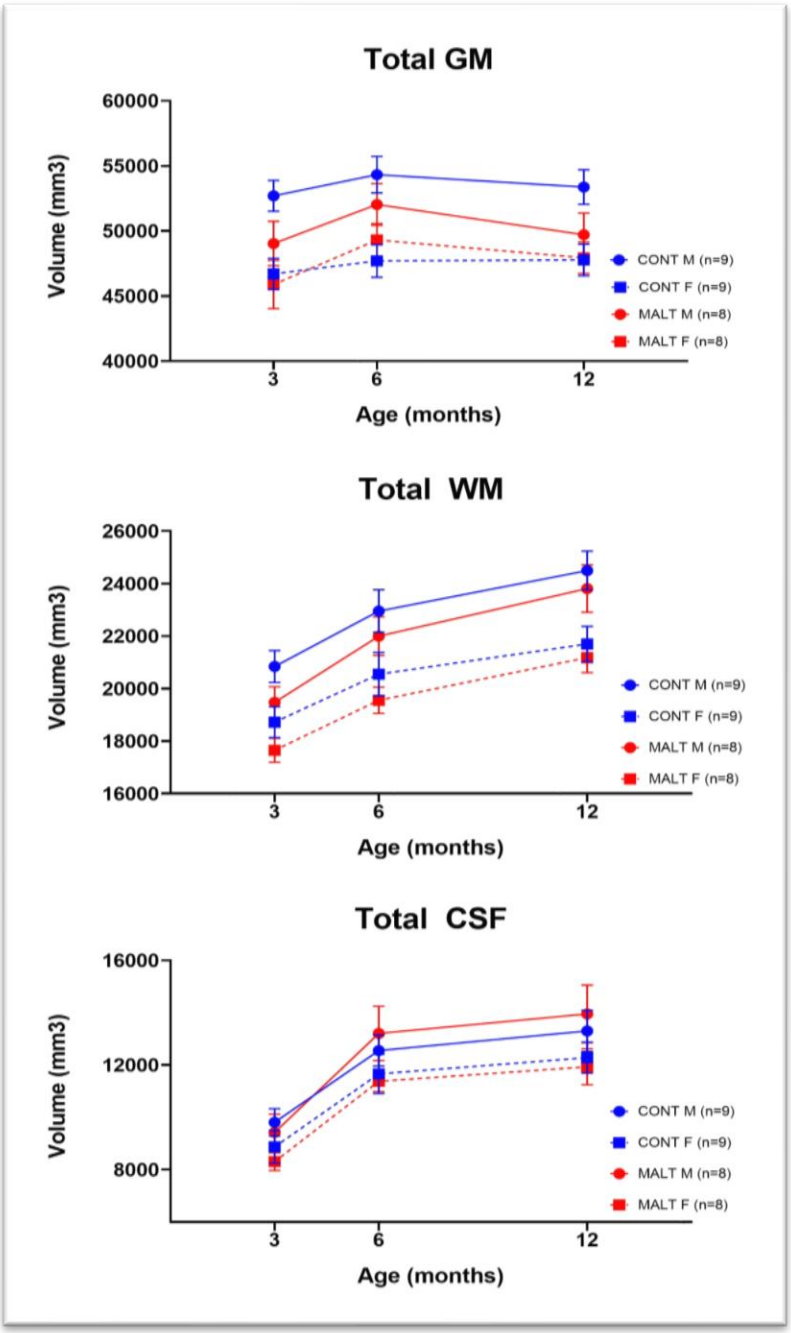


**Figure 6.** Developmental effects of infant maltreatment on total WM, GM, and CSF, separated by group and sex. A significant main effect of age was detected (GM: $F_{1.532,45.947} = 8.179$, $p = 0.002$, $\eta^2 = 0.214$ -G-G corrected-;WM: $F_{2,60.} = 174.255$, $p = 1.0196E\text{-}25$, $\eta^2 = 0.853$; CSF: $F_{1.637,49.102} = 65.743$, $p = 2.201E\text{-}13$, $\eta^2 = 0.687$ -G-G corrected-), with GM, WM, and CSF increasing with age. A significant main effect of sex was detected (GM: $F_{1,30} = 11.821$, $p = 0.002$, $\eta^2 = 0.283$; WM: $F_{1,30} = 13.268$, $p = 0.001$, $\eta^2 = 0.307$; CSF: $F_{1,30} = 5.044$, $p = 0.032$, $\eta^2 = 0.144$), with larger GM, WM, and CSF volumes in males compared to females. Plots represent mean± SEM.

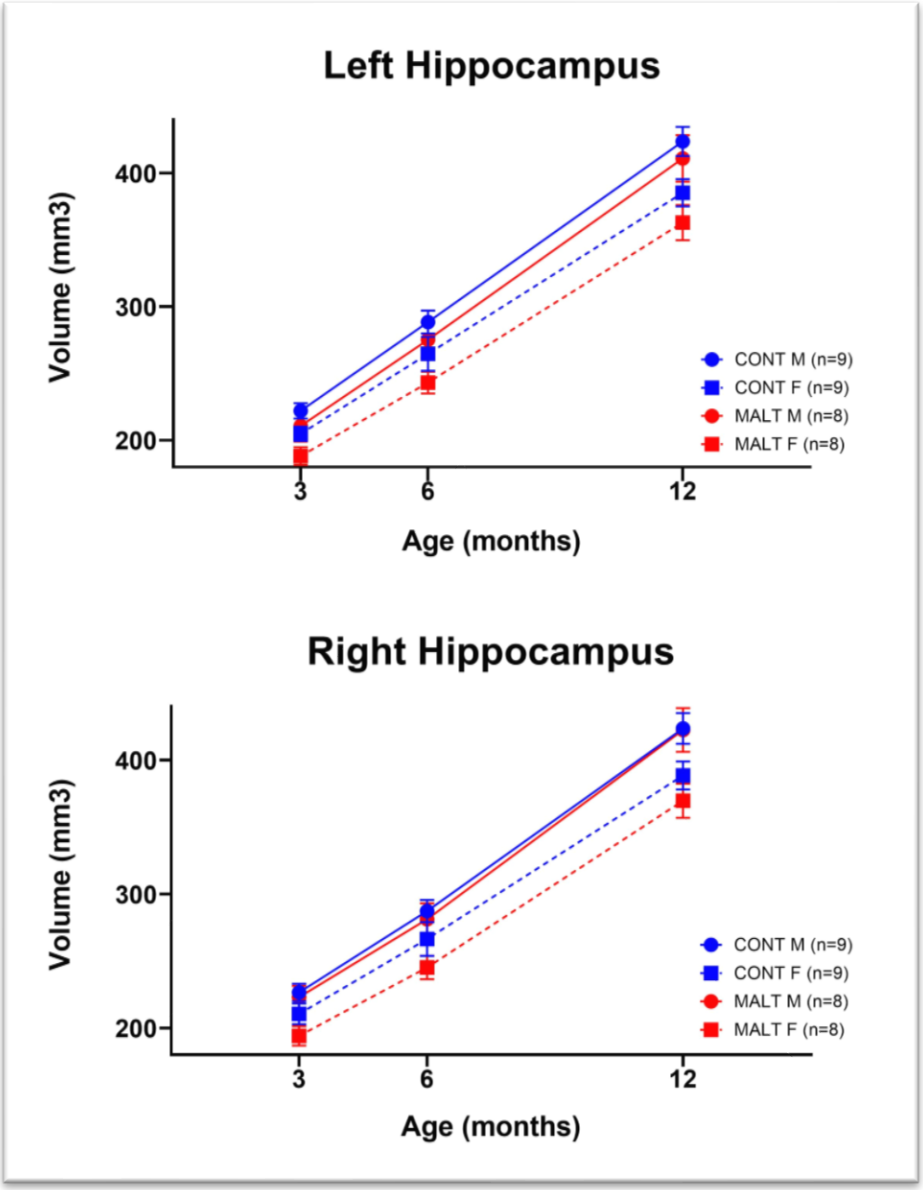


**Figure 7.** Developmental effects of infant maltreatment on total hippocampal volume, separated by group and sex. A significant main effect of age was found ($F_{1.647,49.421}$ =1449.164, p = 7.351E-43, $\eta^2$ =0.980 -G-G corrected-), showing hippocampal volumes increase with age. An age by sex interaction was detected ($F_{1.647,30}$ = 5.121, p =0.014, $\eta^2$ = 0.146 -G-G corrected-). The interaction fit a linear trend ($F_{1,30}$ = 6.865, p = 0.014, $\eta^2$ = 0.002), suggesting that hippocampi in females grew at a slower rate. A main effect of sex was detected ($F_{1,30}$ = 10.374, p = 0.003, $\eta^2$ = 0.257), showing larger hippocampal volumes in males. A main effect of laterality ($F_{1,30}$ = 6.912, p = 0.013, $\eta^2$ = 0.187 -G-G corrected-) was detected, showing faster growth of the right hippocampus. An age and laterality interaction effect was detected ($F_{1.907,57.215}$ = 6.784, p = 0.003, $\eta^2$ = 0.184 -G-G corrected- ), and the interaction fit a quadratic trend ($F_{1,30}$ = 14.028, p = 0.001, $\eta^2$ = 0.319). Plots represent mean± SEM.

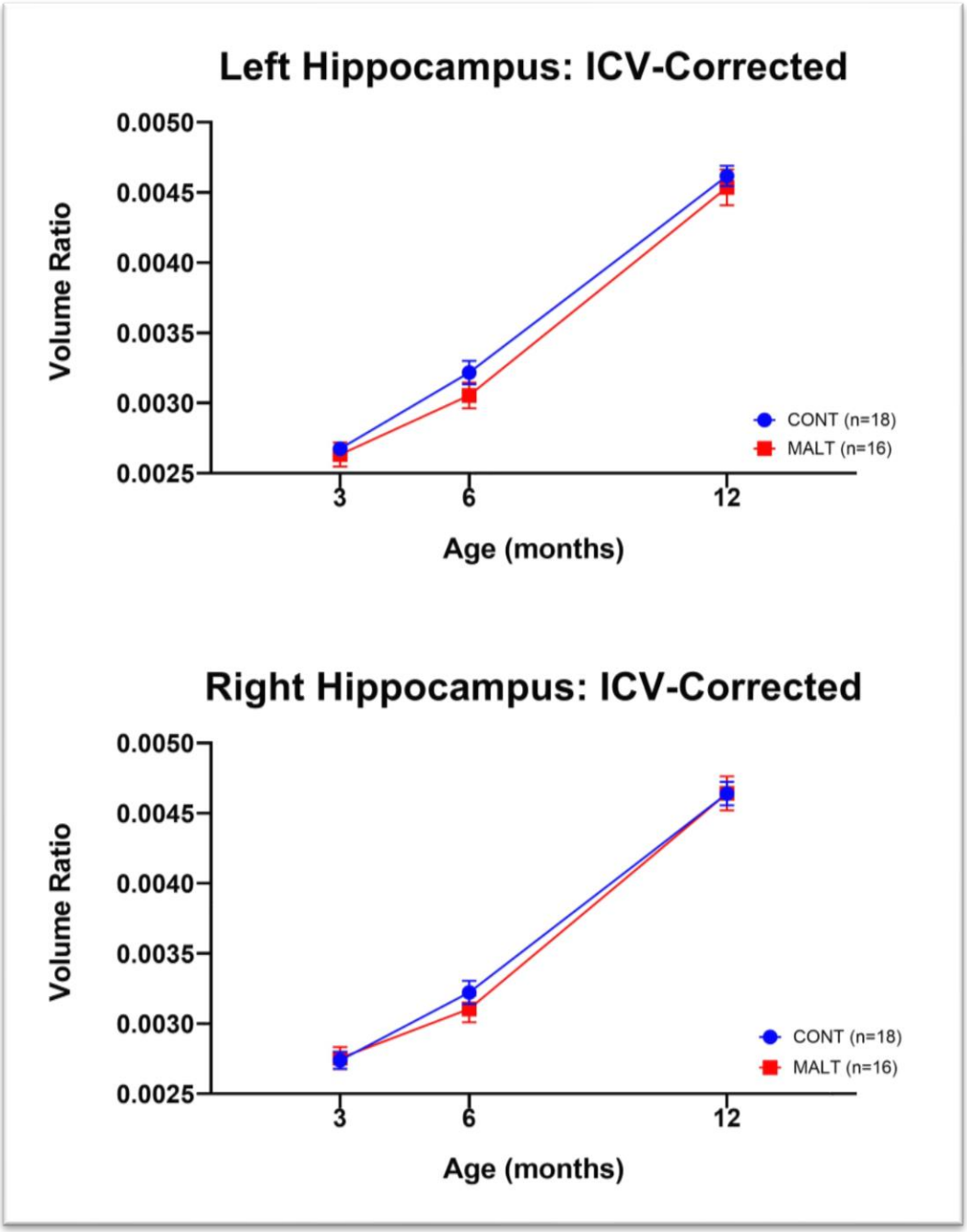


**Figure 8.** Developmental effects of infant maltreatment on ICV-Corrected hippocampal volumes. The main effect of age persisted ($F_{2,60}$ = 2109.556, p = 2.535E-56, $\eta^2$ = 0.986), showing region-specific hippocampal volumes increases with age. The main effect of laterality persisted ($F_{1,30}$ = 8.012, p = 0.009, $\eta^2$ = 0.211), further suggesting differential rates of growth by hemisphere in the hippocampus. An age and laterality interaction also persisted ($F_{2,60}$ = 7.634, p = 0.001, $\eta^2$ = 0.23), and it had a quadratic fit ($F_{1,60}$ = 15.513, p = 0.000452, $\eta^2$ = 0.341), showing differential rates of brain growth of each hemisphere of the hippocampus at different ages. Plots represent mean± SEM.

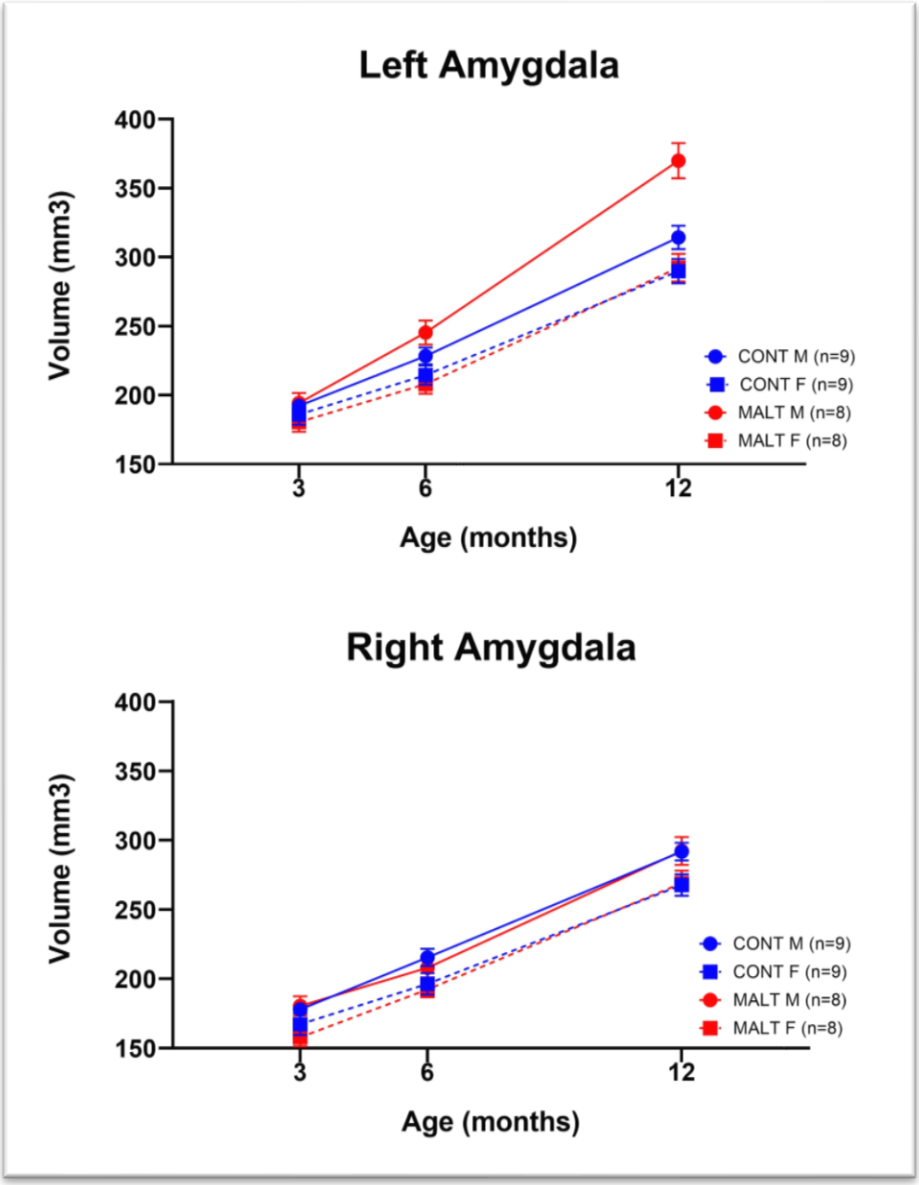


**Figure 9.** Developmental effects of infant maltreatment on total amygdalar volume, separated by group and sex. A main effect of age was detected ($F_{1.447,43.412}$ = 817.589, p = 1.0839E-32, $\eta^2$ = 0.965-G-G corrected-), with volumes increasing with age. A group by laterality interaction effect ($F_{1,30}$ = 5.961, p = 0.021, $\eta^2$ = 0.166) was also observed, which followed a linear trend ($F_{1,30}$ = 5.961, p = 0.021, $\eta^2$ = 0.166), with bigger left amygdala volumes in MALT than CONT animals. Main effects of sex ($F_{1,30}$ = 9.874, p = 0.004, $\eta^2$ = 0.248), with larger amygdalae in males than females, and laterality ($F_{1,30}$ = 329.448, p = 9.994E-18, $\eta^2$ = 0.917 -GG corrected-) were also detected. Additional age by laterality ($F_{1.696,50.881}$ = 14.511, p = 0.000028, $\eta^2$ = 0.326 –G-G corrected-; following a linear trend ($F_{1,30}$ = 9.366, p = 0.005, $\eta^2$ = 0.238) and age by laterality by sex interaction effects ($F_{1.696,30}$ = 3.187, p = 0.057, $\eta^2$ = 0.096 -G-G corrected-; following a linear trend ($F_{1,30}$ = 4.278, p = 0.047, $\eta^2$ = 0.125)) were found. Plots represent mean± SEM.

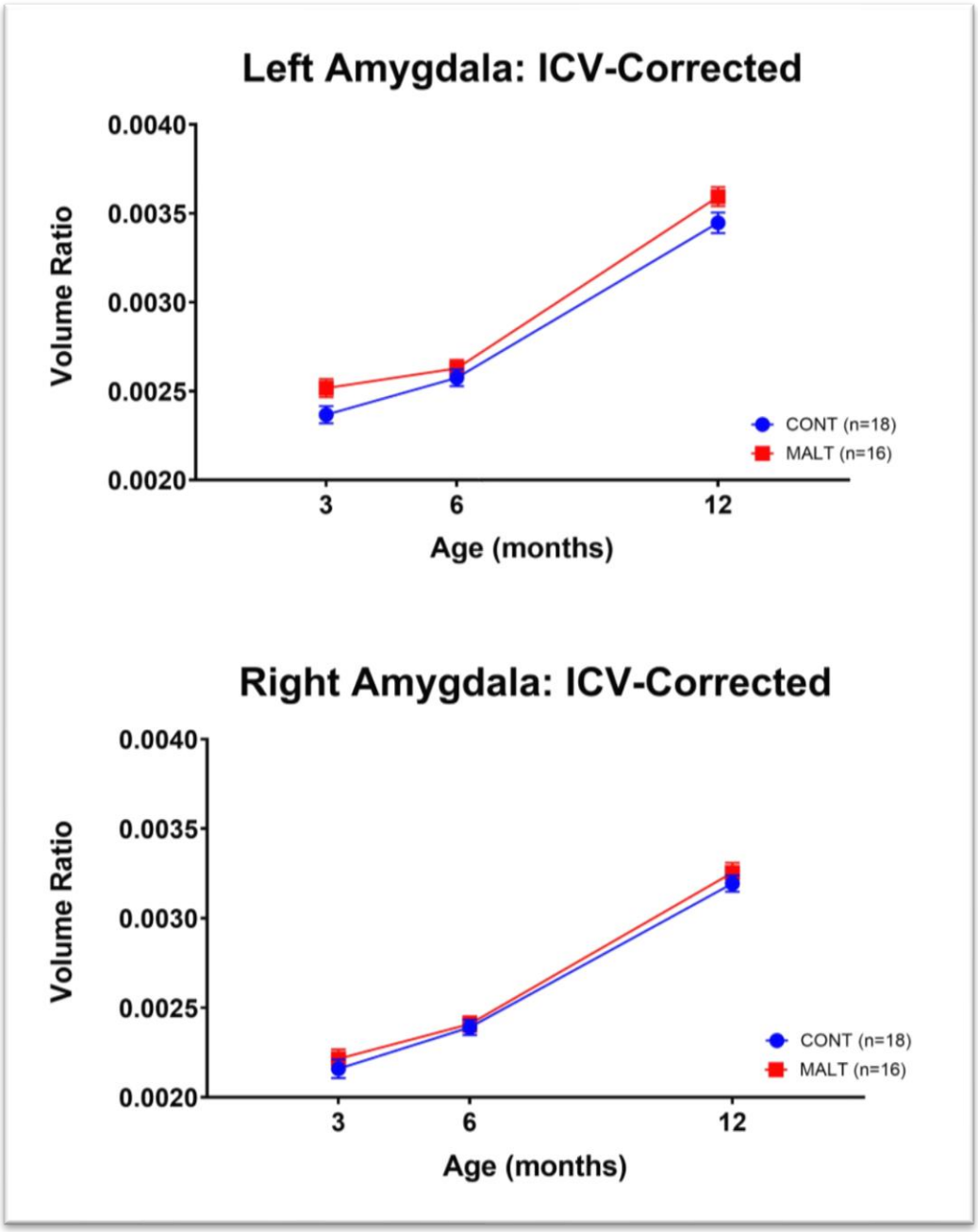


**Figure 10.** Developmental effects of infant maltreatment on ICV-corrected amygdalar volumes. The age main effect persisted after ICV-correction ($F_{1.884,56.512}$ = 1656.495, p = 2.8563E-50, $\eta^2$ = 0.982 -G-G corrected-), showing specific amygdala growth with age, independent of ICV increases. Group by laterality interaction effects were also preserved ($F_{1,30}$ = 6.646, p = 0.015, $\eta^2$ = 0.181 -G-G corrected-), and followed a linear trend ($F_{1,30}$ = 6.646, p = 0.015, $\eta^2$ = 0.181), suggesting that MALT effects on left amygdala are region-specific. Laterality ($F_{1,30}$ = 325.533, p = 1.178E-17, $\eta^2$ = 0.916 -G-G corrected-) and age by laterality interaction effects ($F_{1.634,49.021}$ = 10.722, p = 0.000340, $\eta^2$ = 0.263 -G-G corrected- following a quadratic linear trend ($F_{1,30}$ = 32.759, p = 0.000003, $\eta^2$ = 0.522)) also remained after ICV correction. Plots represent mean± SEM.

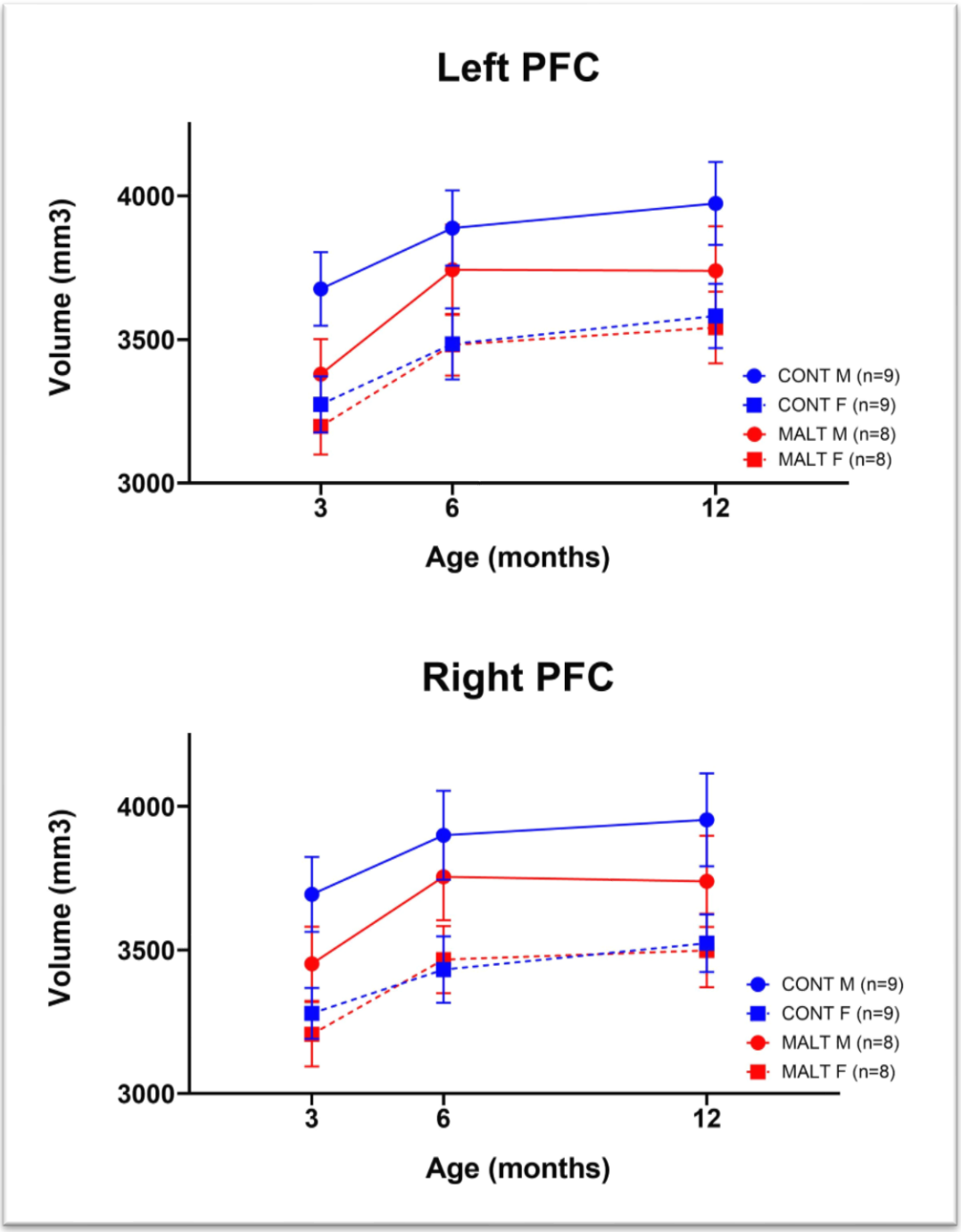


**Figure 11.** Developmental effects of infant maltreatment on total prefrontal cortex volume, separated by group and sex. A main effect of age was detected ($F_{1.366,40.966}$ = 29.882, p = 2.513E-7, $\eta^2$ = 0.499 -G-G corrected-), with PFC volumes increasing with age. An age by laterality interaction effect ($F_{1.731,51.940}$ = 14.135, p = 0.000030, $\eta^2$ = 0.320 -G-G corrected-, showing a linear trend ($F_{1,30}$ = 25.212, p = 0.000022, $\eta^2$ = 0.457) indicated that the left PFC grows faster than the right. A main effect of sex ($F_{1,30}$ = 7.517, p = 0.010, $\eta^2$ = 0.200) was also detected, with larger PFC volumes in males than females. Plots represent mean± SEM.

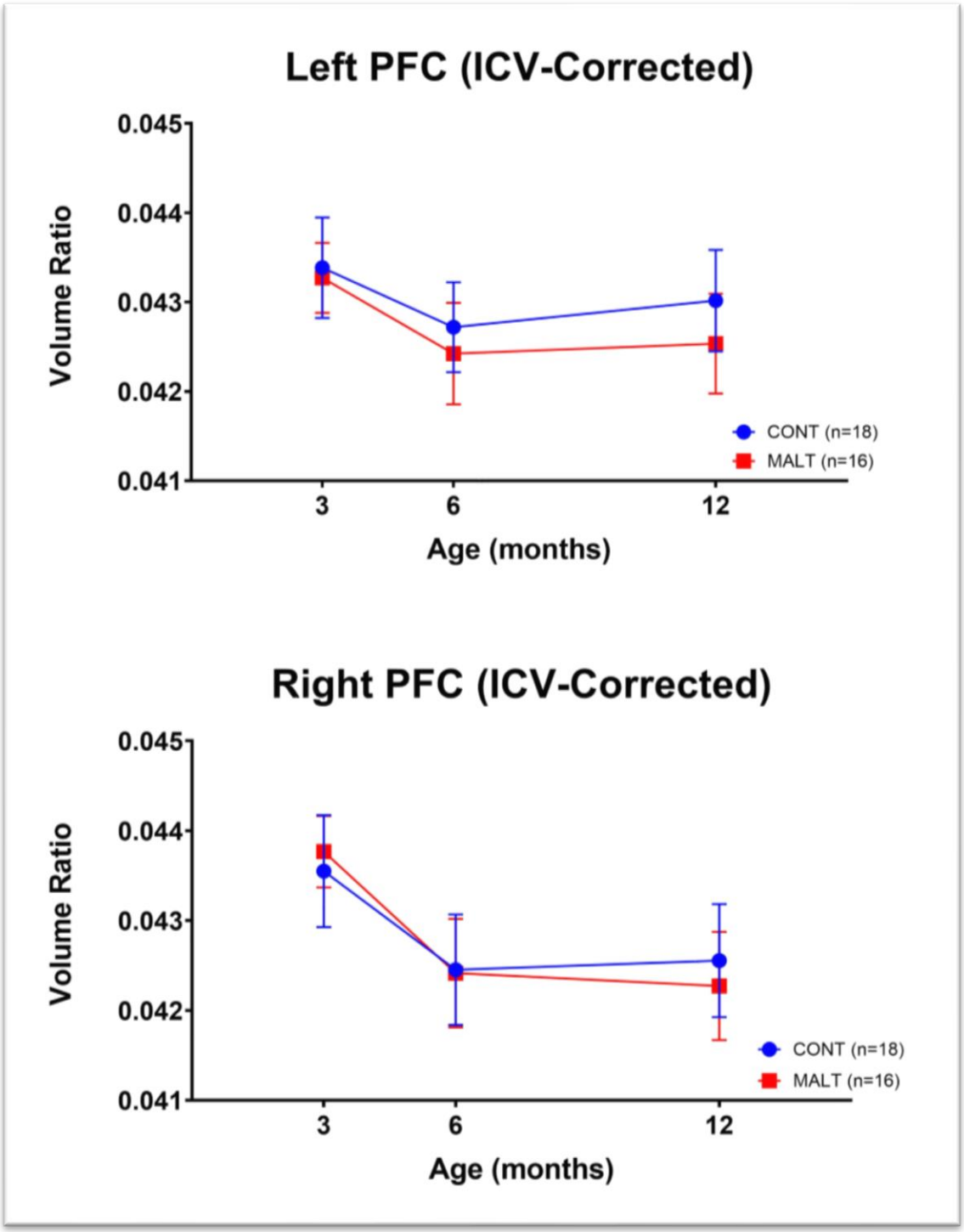


**Figure 12.** Developmental effects of infant maltreatment on ICV-Corrected prefrontal cortex volume. The main age effect persisted after correcting for brain size (ICV) ($F_{1.549,46.468}$ = 15.325, p = 0.000035, $\eta^2$ = 0.338 -G-G corrected-) (**Figure 12**). The age by laterality interaction effect also persisted after ICV correction ($F_{1.734,}$ = 14.260, p = 0.000027, $\eta^2$ = 0.322 -G-G corrected-; fitting a linear trend ($F_{1,30}$ = 23.874, p = 0.000032, $\eta^2$ = 0.443)).
Plots represent mean± SEM.

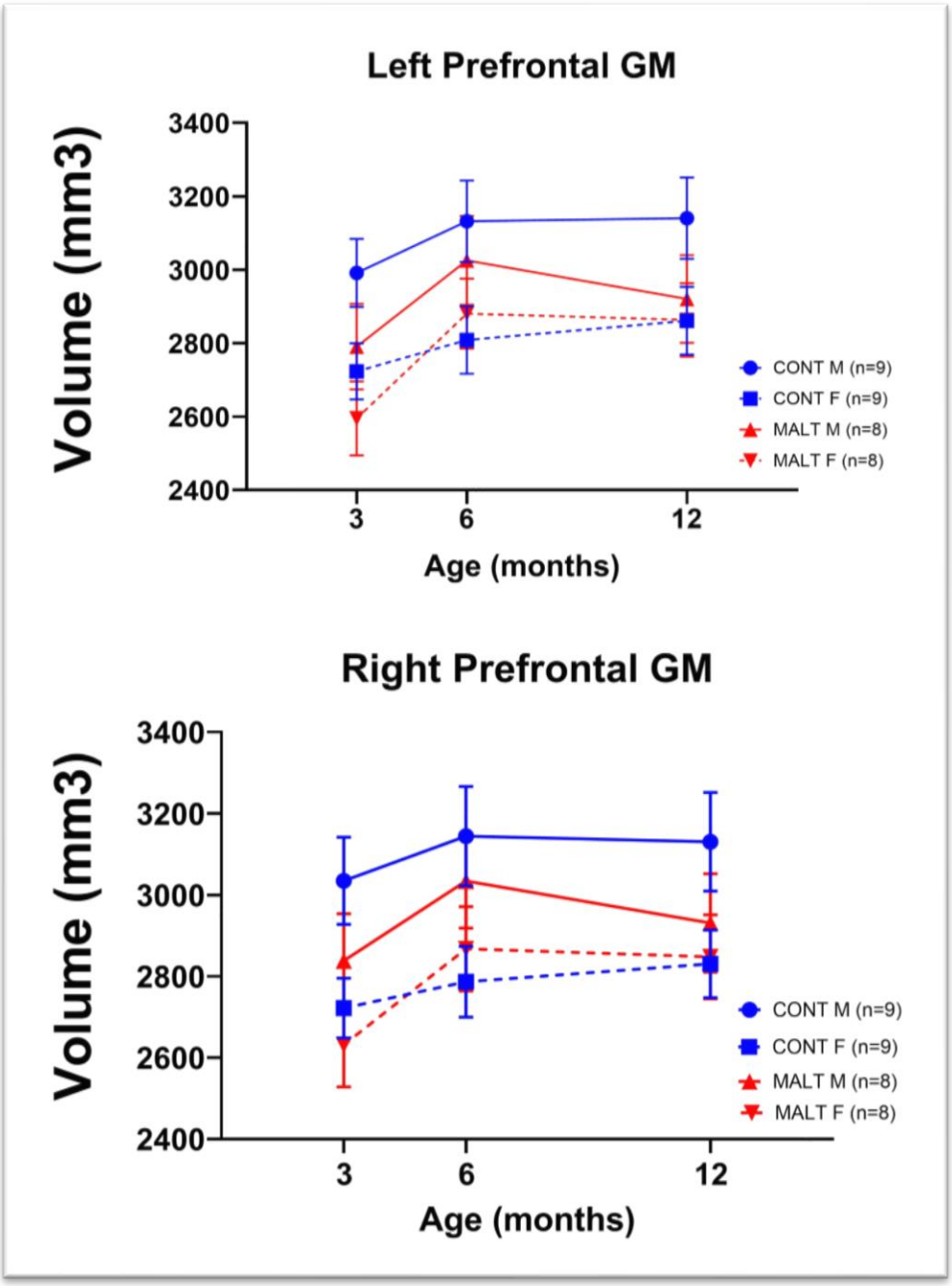


**Figure 13.** Developmental effects of infant maltreatment on total prefrontal GM volume, separated by group and sex. A main effect of age was detected ($F_{1.407,42.203} = 10.115$, $p = 0.001$, $\eta^2 = 0.252$ -G-G corrected-), so that volumes increased with age. An age by laterality interaction effect ($F_{1.914,57.428} = 13.547$, $p = 0.000020$, $\eta^2 = 0.311$ -G-G corrected-) was detected, and this interaction effect fit a linear trend ($F_{1,30} = 21.424$, $p = 0.000066$, $\eta^2 = 0.417$) indicating that the left PFC GM grows faster than the right. A main effect of sex was also detected ($F_{1,30} = 5.935$, $p = 0.021$, $\eta^2 = 0.165$), with larger PFC GM volumes in males than females. Plots represent mean± SEM.

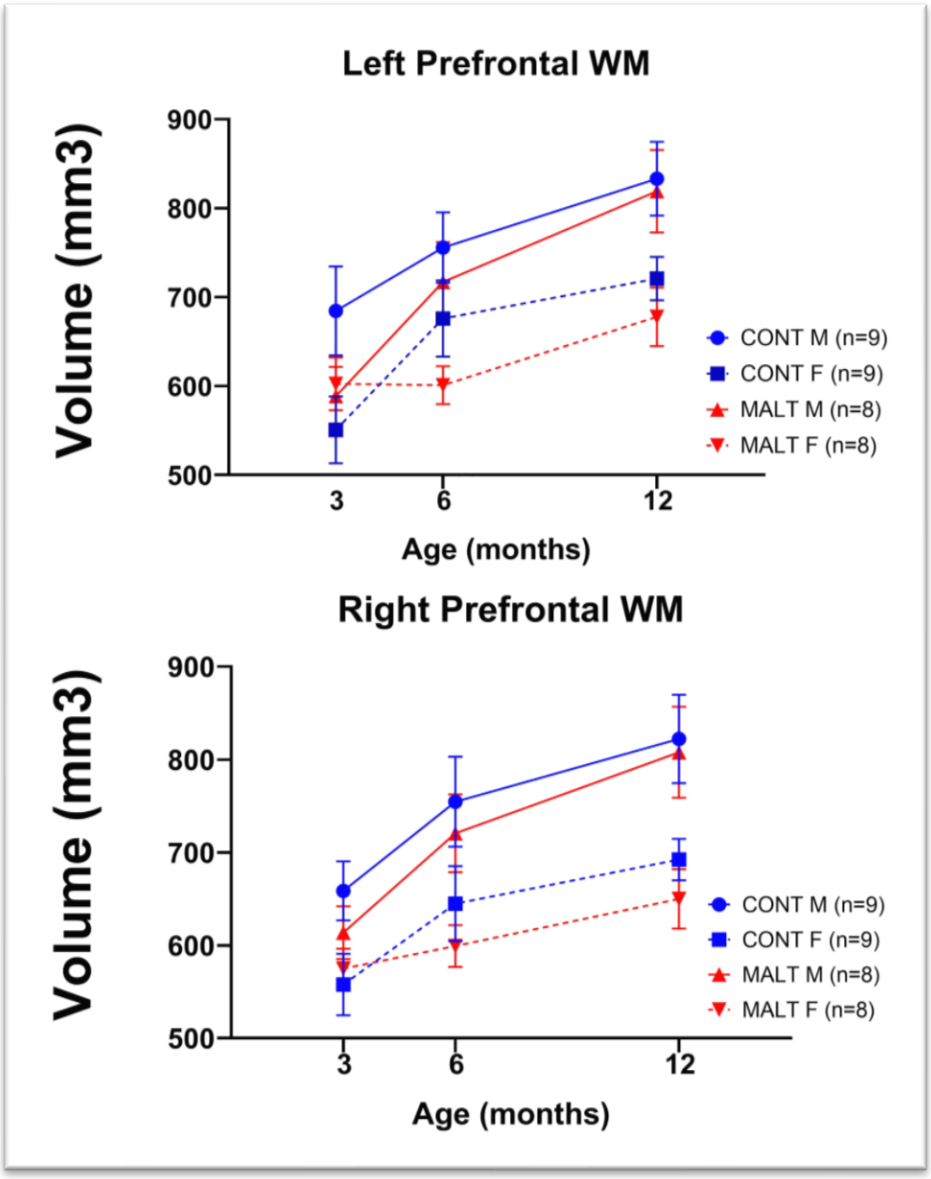


**Figure 14.** Developmental effects of infant maltreatment on total prefrontal WM volume, separated by group and sex. Main effects of age ($F_{1.881,56.433}$ = 79.016, p = 1.2402E-16, $\eta^2$ = 0.725 -G-G corrected-) and sex were detected ($F_{1,30}$ = 8.825, p = 0.006, $\eta^2$ = 0.227) with larger PFC WM volumes in males than females. Group by age by sex ($F_{1.881,56.433}$ = 5.099, p = 0.010, $\eta^2$ = 0.145 -G-G corrected-, fitting a linear trend ($F_{1,30}$ = 7.240, p = 0.012, $\eta^2$ = 0.194)) and group by sex by age by laterality interaction effects were found ($F_{1.376,41.278}$ = 5.079, p = 0.020, $\eta^2$ = 0.145, showing a quadratic linear trend ($F_{1,30}$ = 6.868, p = 0.014, $\eta^2$ = 0.186), which seem to be driven by CONT males, and indicating that the left PFC WM grows faster than the right. An age by sex interaction effect ($F_{1.881,56.433}$ = 4.452, p = 0.018, $\eta^2$ = 0.129 -G-G corrected-) was also detected, following a linear trend ($F_{1,30}$ = 8.064, p = 0.008, $\eta^2$ = 0.212). A main effect of laterality ($F_{1,30}$ = 10.140, p = 0.003, $\eta^2$ = 0.253-G-G corrected-) as well as laterality by sex interaction effect ($F_{1,30}$ = 4.890, p = 0.035, $\eta^2$ = 0.140 -G-G corrected-, fitting a linear trend ($F_{1,30}$ = 4.890, p = 0.035, $\eta^2$ = 0.140)) were detected. Plots represent mean± SEM.

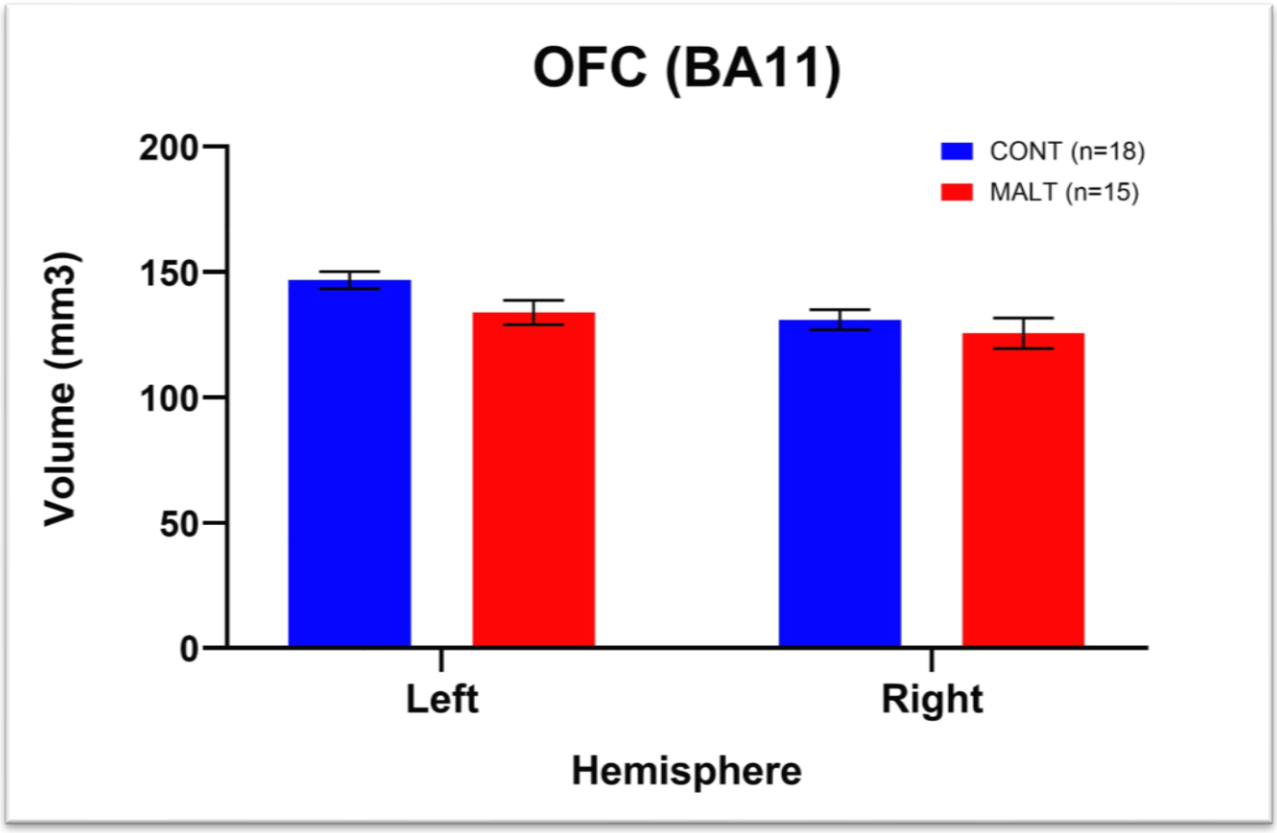


**Figure 15.** Developmental effects of infant maltreatment on OFC BA11 volume. A main laterality effect ($F_{1,29} = 22.073$, $p = 0.000059$, $\eta^2 = 0.432$) was detected, with larger left than right BA 11 volumes. Plots represent mean± SEM.

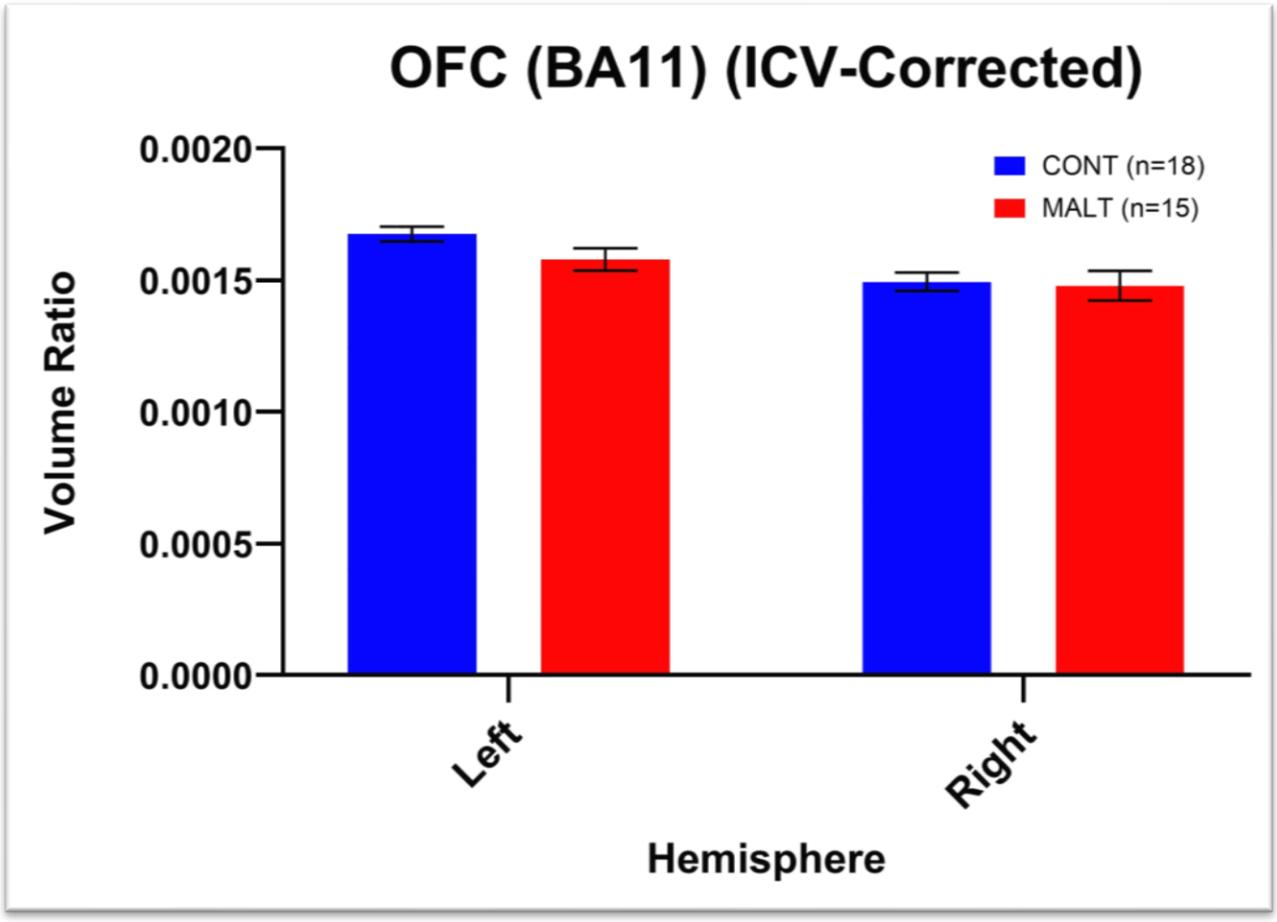


**Figure 16.** Developmental effects of infant maltreatment on ICV-Corrected OFC BA11 volume. The main effect of laterality persisted ($F_{1,29} = 21.394$, $p = 0.000072$, $\eta^2 = 0.425$), showing larger left hemisphere volumes at 12 months of age. Plots represent mean± SEM.

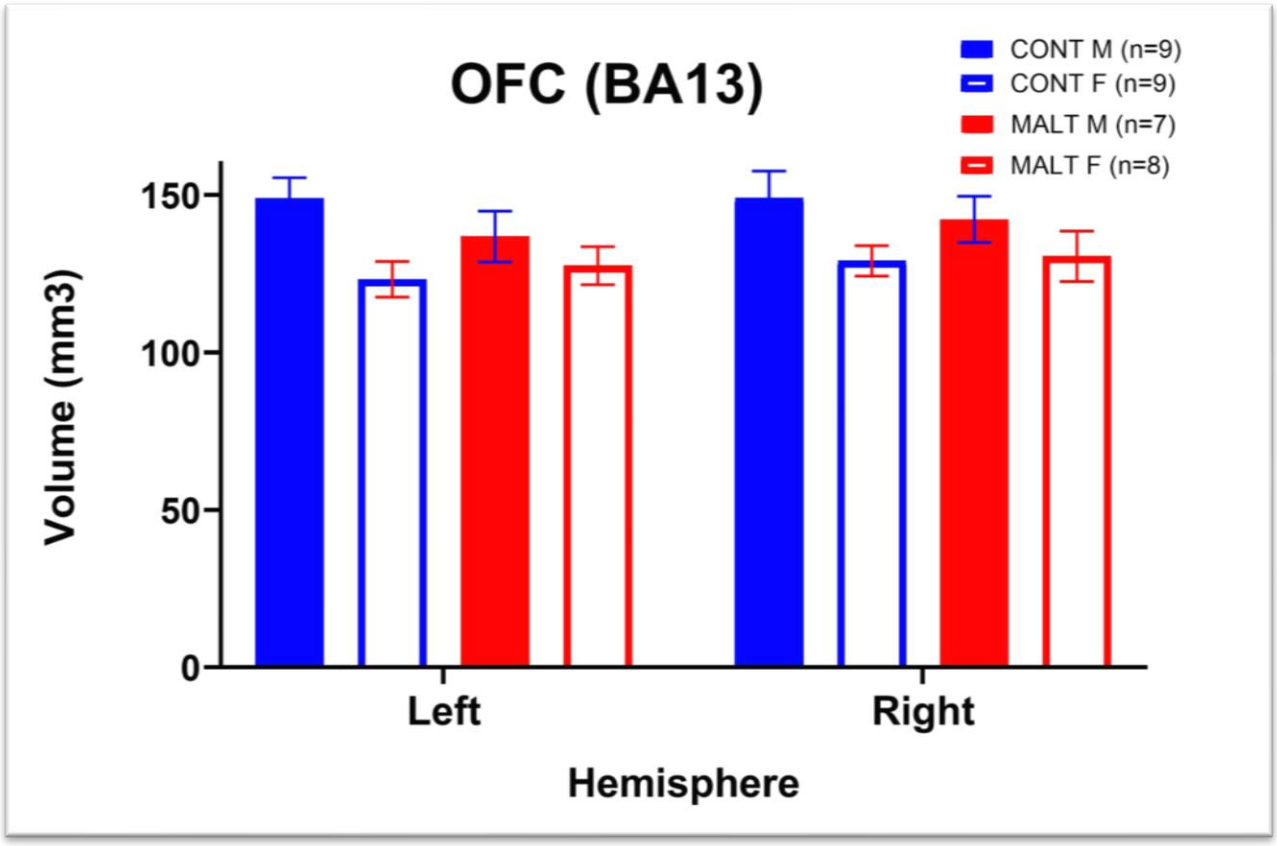


**Figure 17.** Developmental effects of infant maltreatment on total OFC BA13 volume. A main sex effect was detected ($F_{1,29} = 6.107$, $p = 0.020$, $\eta^2 = 0.174$), with larger OFC BA13 volumes in males than females. Plots represent mean± SEM.

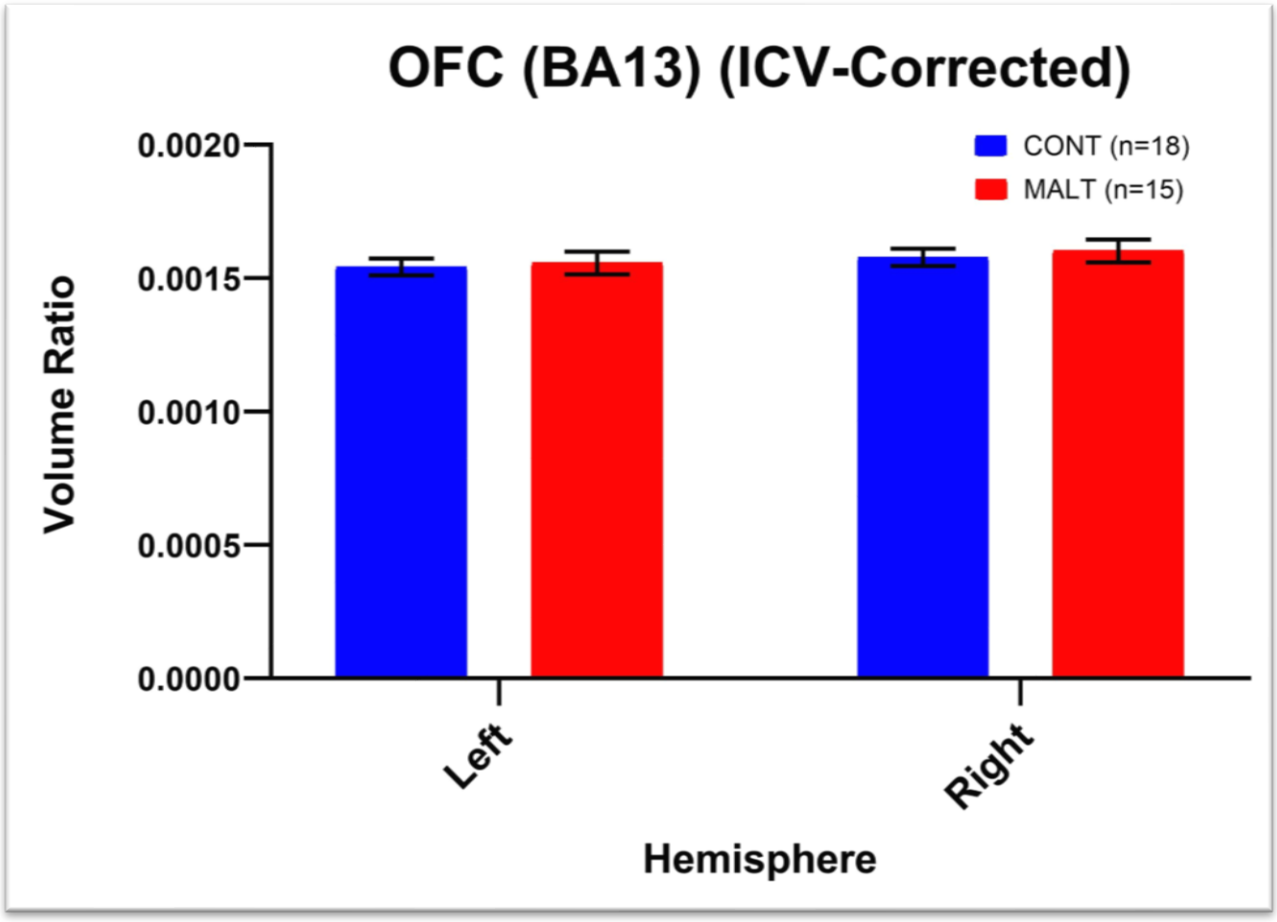


**Figure 18.** Developmental effects of infant maltreatment on ICV-Corrected OFC BA13 volume. The main effect of sex disappeared, suggesting that it was driven by sex differences in brain size. Plots represent mean± SEM.

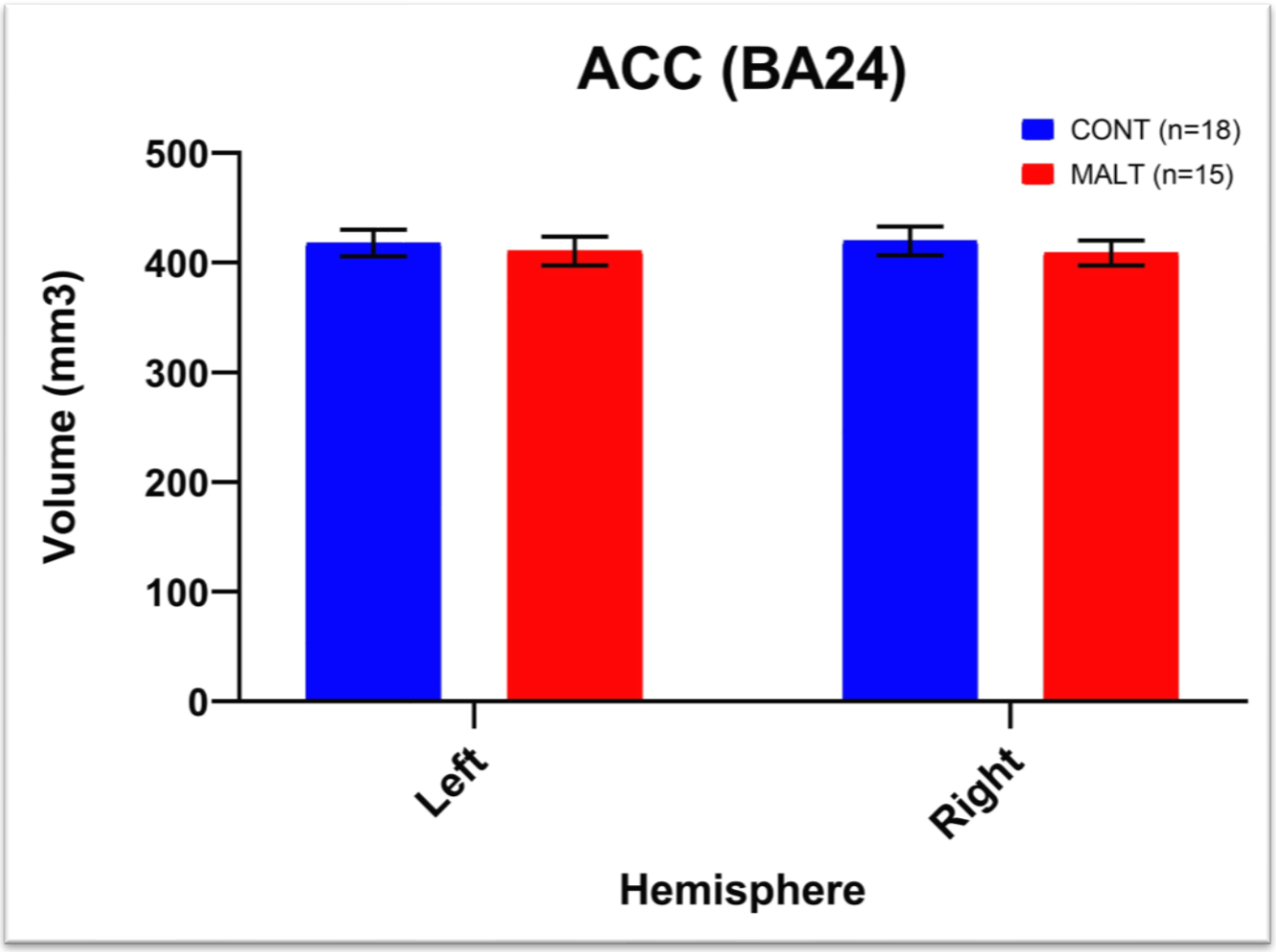


**Figure 19.** Developmental effects of infant maltreatment on total ACC BA24 volume. No significant age, group, sex or laterality main or interaction effects were detected. Plots represent mean± SEM.

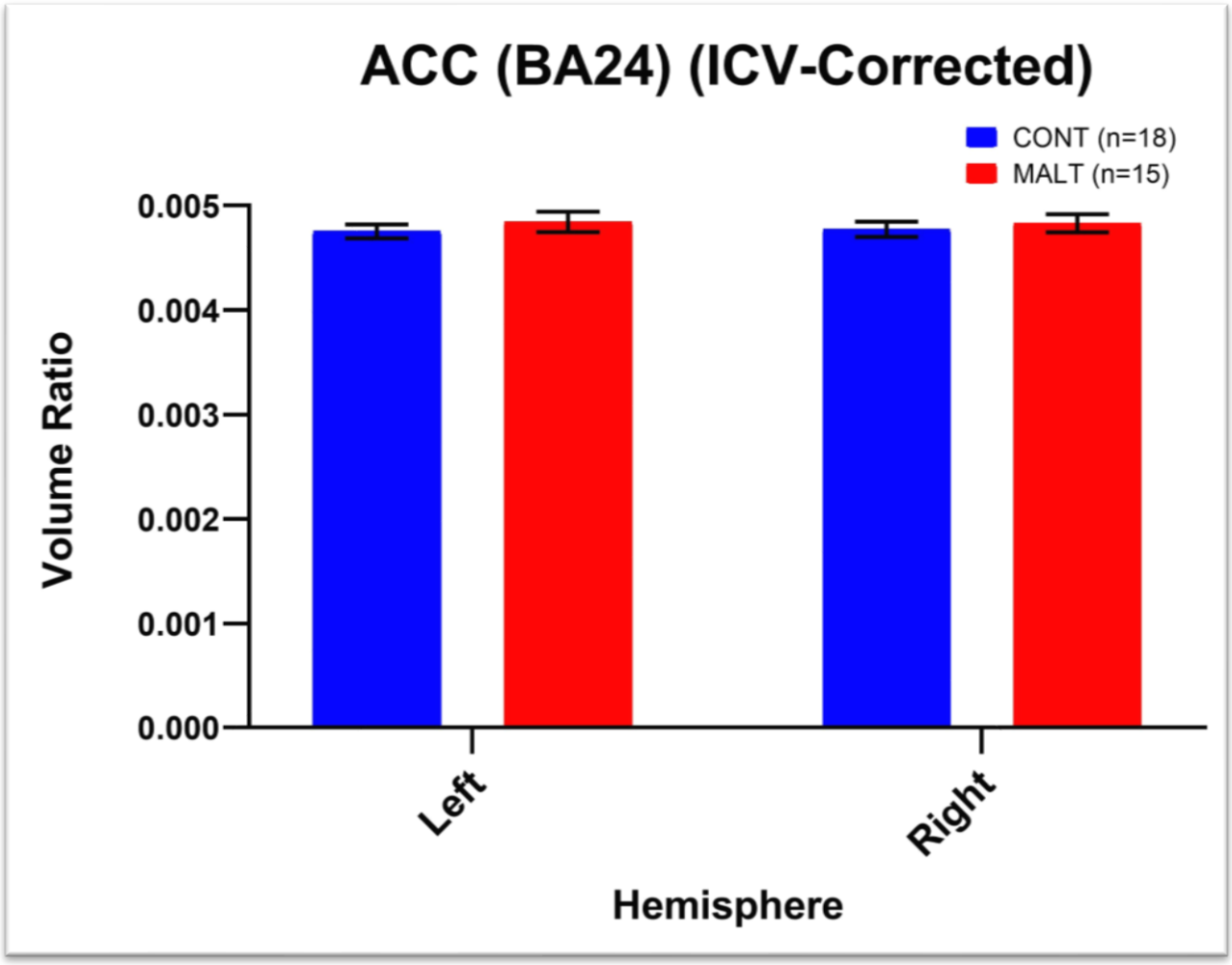


**Figure 20.** Developmental effects of infant maltreatment on ICV-Corrected ACC BA24 volume. No significant age, group, sex or laterality main or interaction effects were detected. Plots represent mean± SEM.

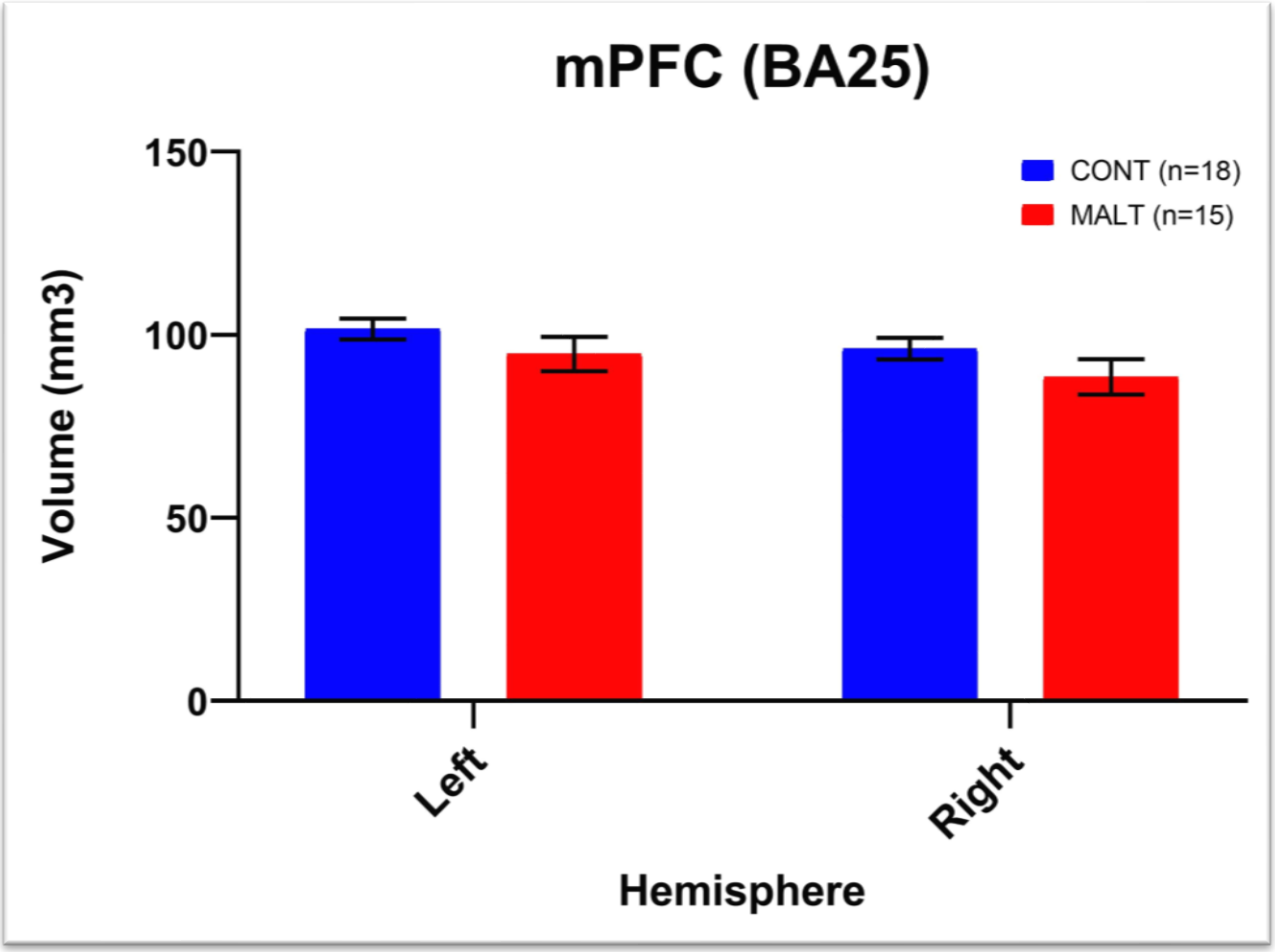


**Figure 21.** Developmental effects of infant maltreatment on total mPFC BA25 volume. A main effect of laterality ($F_{1,29}$ = 49.471, p = 9.7896E-8, $\eta^2$ = 0.630) was detected, showing larger left hemisphere volumes at 12 months of age. Plots represent mean± SEM.

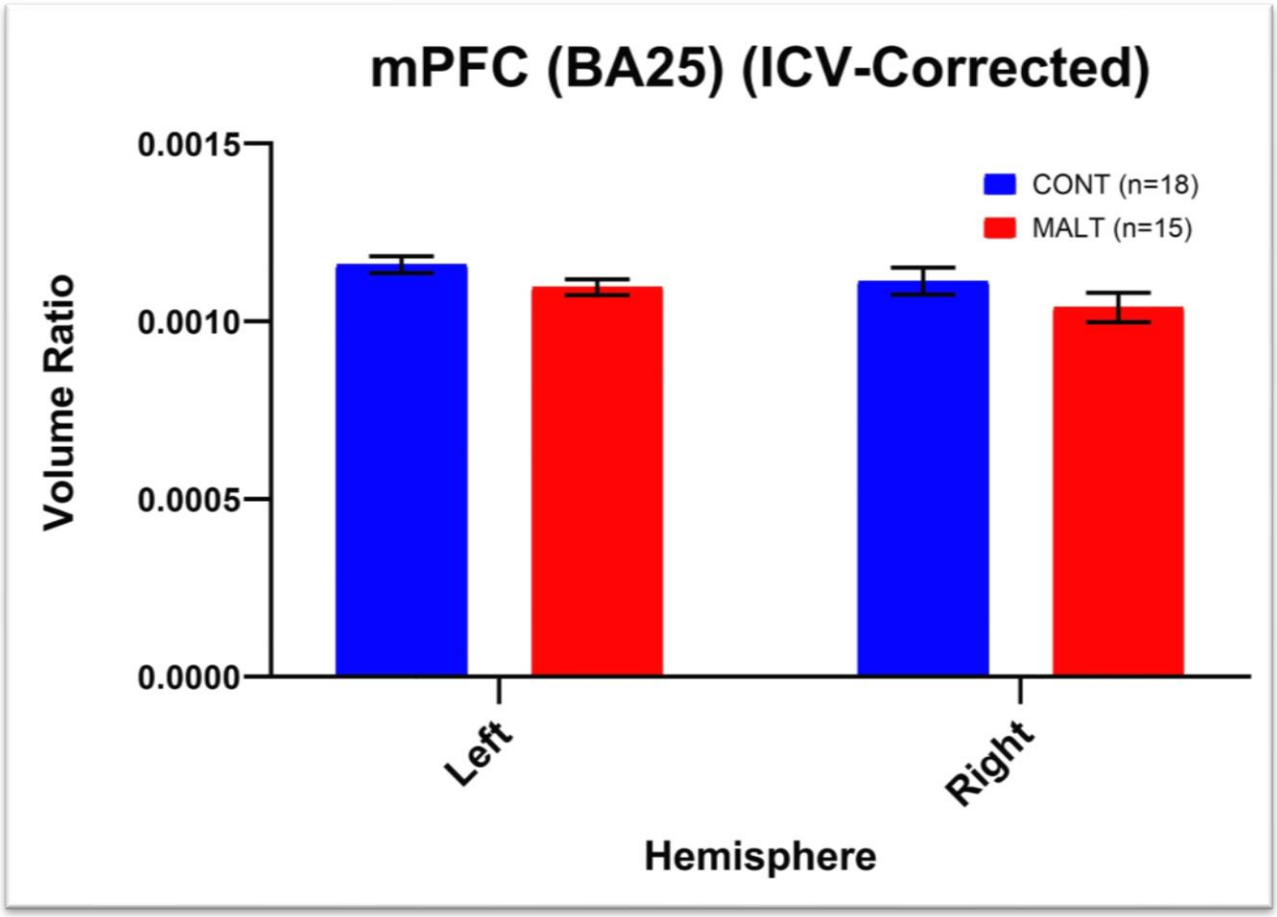


**Figure 22.** Developmental effects of infant maltreatment on ICV-Corrected mPFC BA25 volume. The main effect of laterality persisted ($F_{1,29}$ = 53.060, p = 5.0605E-8, $\eta^2$ = 0.647. Plots represent mean± SEM.

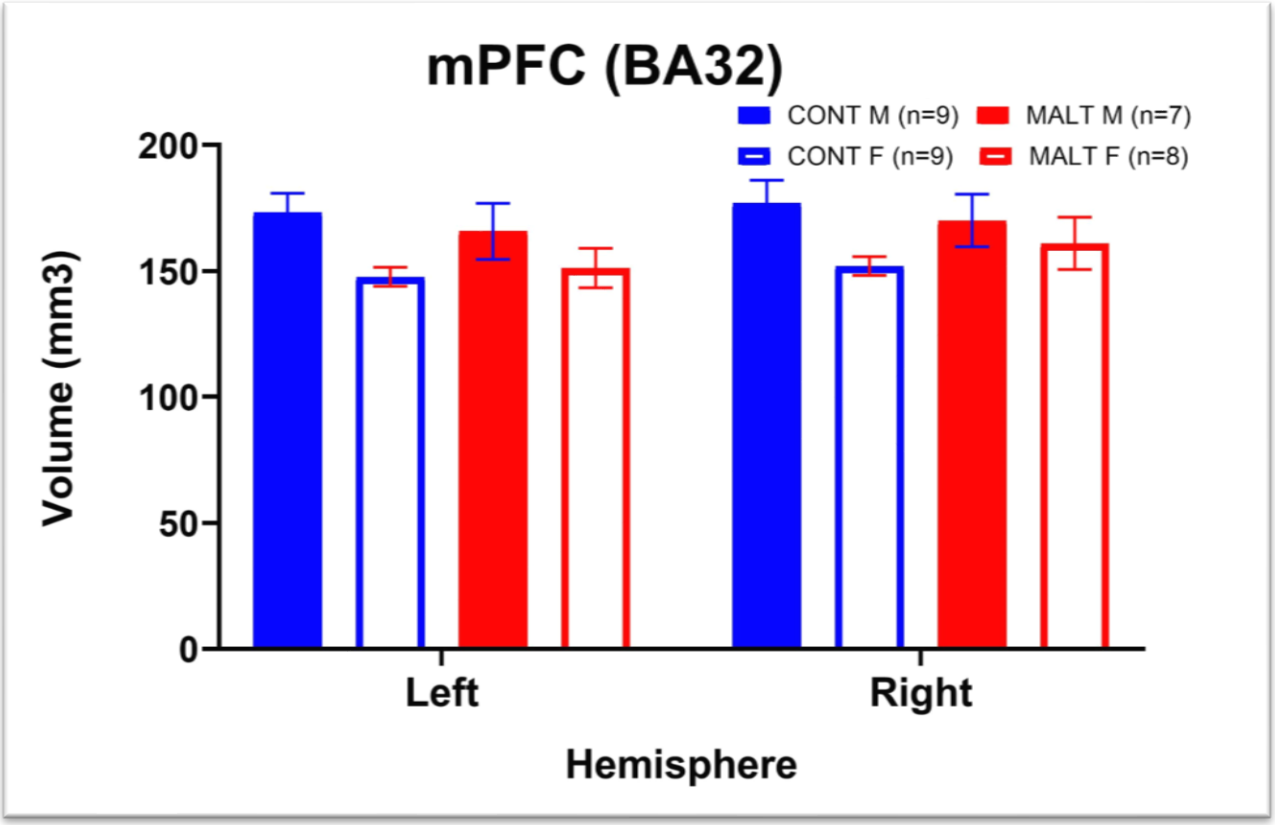


**Figure 23.** Developmental effects of infant maltreatment on total mPFC BA32 volume. A main effect of sex ($F_{1,29} = 5.526$, $p = .026$, $\eta^2 = 0.160$) was detected, showing larger volumes in males than females. A main effect of laterality ($F_{1,29} = 7.671$, $p = 0.010$, $\eta^2 = 0.209$) was also detected, showing larger volumes in the right than left hemisphere. Plots represent mean± SEM.

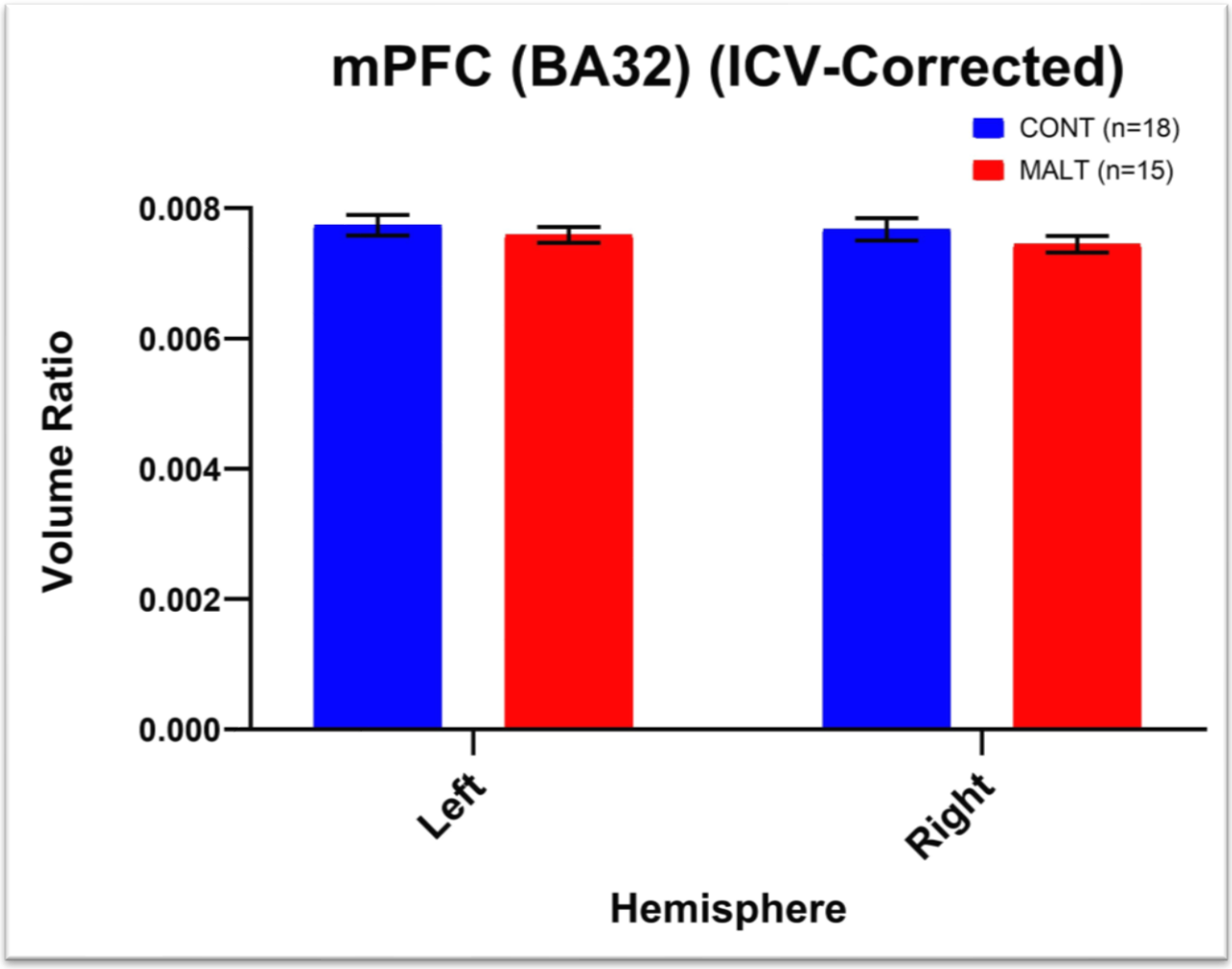


**Figure 24.** Developmental effects of infant maltreatment on ICV-Corrected mPFC BA32 volume. The main effect of laterality persisted ($F_{1,29} = 8.062$, $p = 0.008$, $\eta^2 = 0.218$). Plots represent mean± SEM.

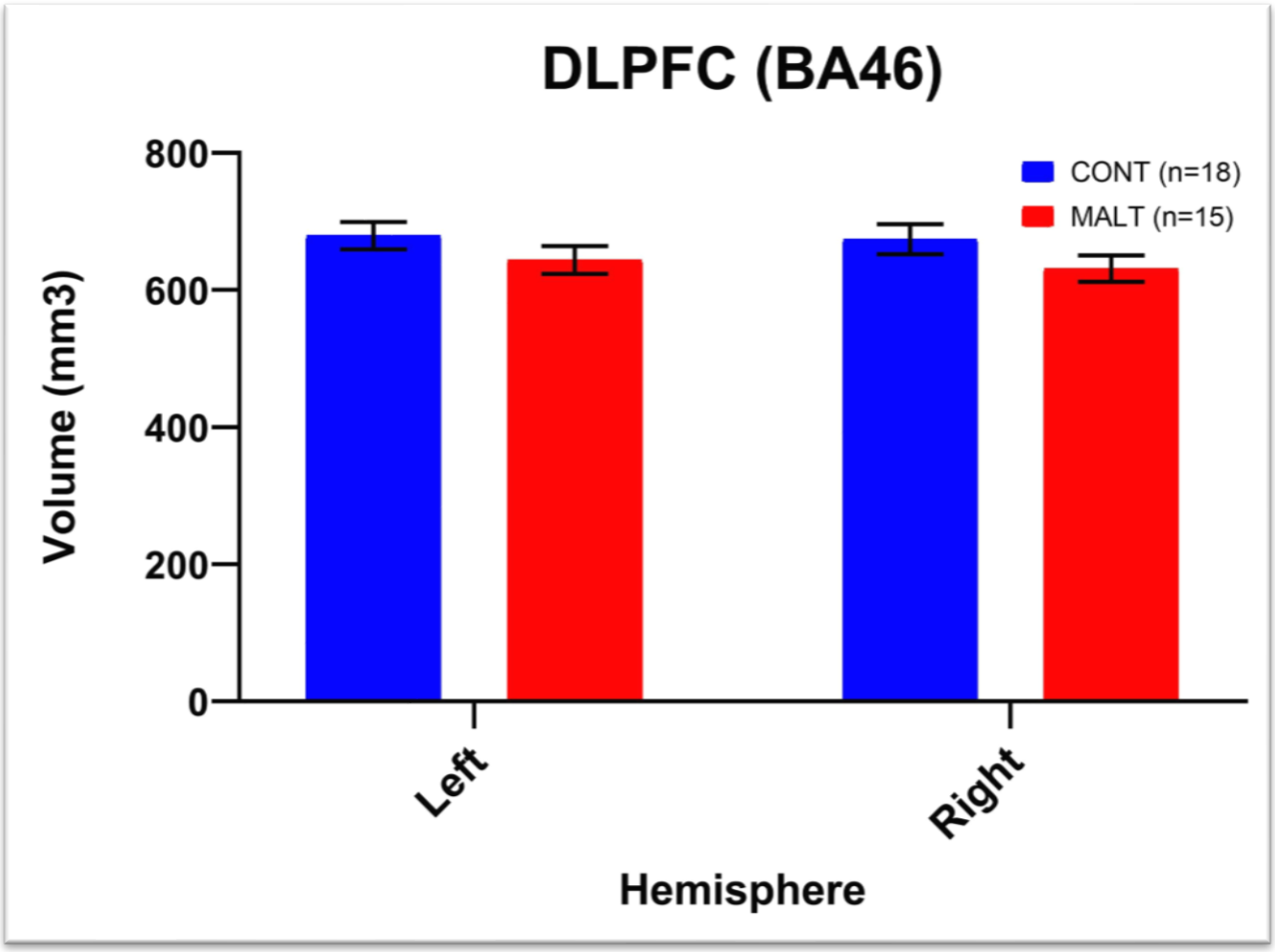


**Figure 25.** Developmental effects of infant maltreatment on total DLPFC BA46 volume. A main effect of laterality ($F_{1,29} = 4.418$, $p = 0.044$, $\eta^2 = 0.132$) was detected. Plots represent mean± SEM.

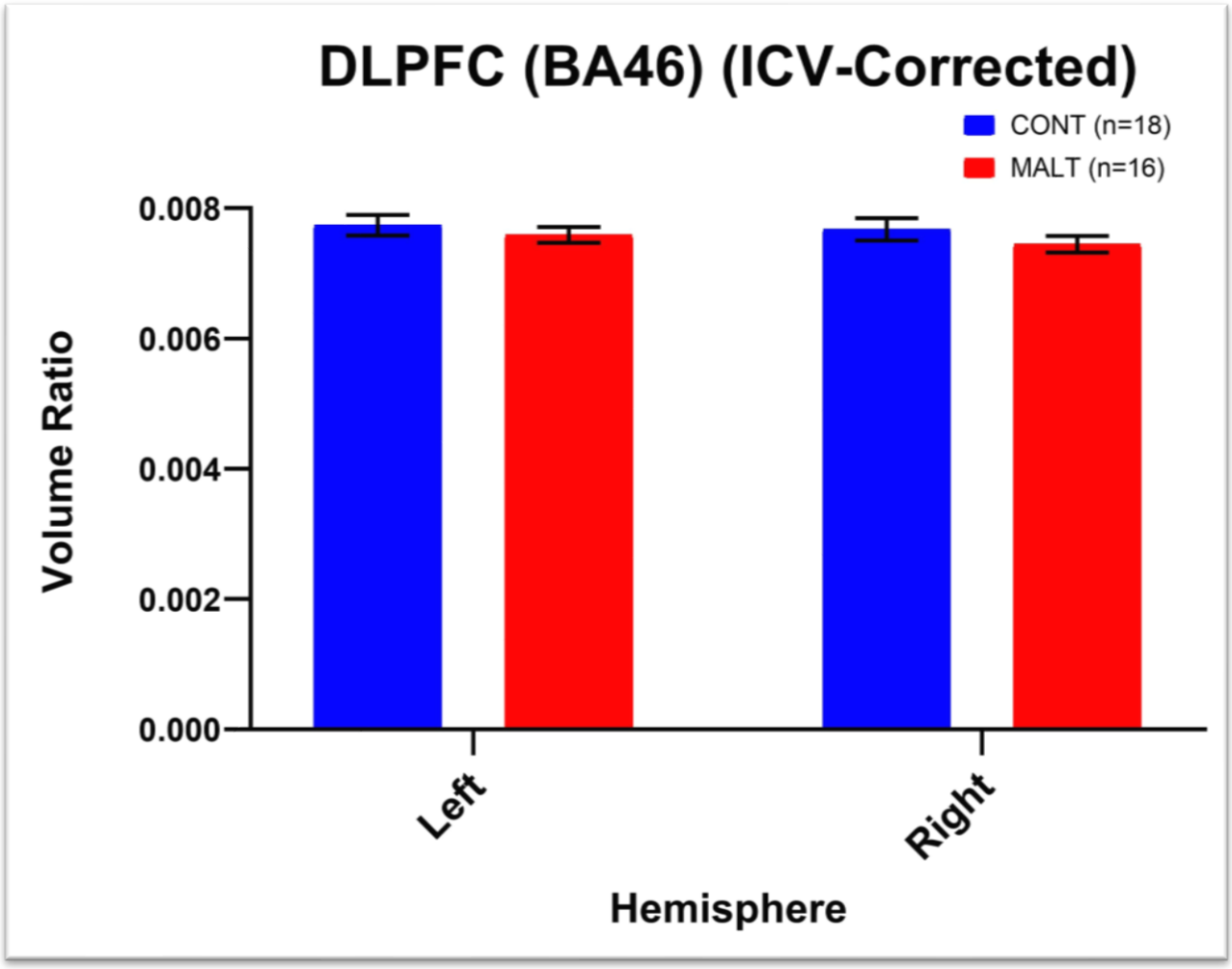


**Figure 26.** Developmental effects of infant maltreatment on ICV-Corrected DLPFC BA46 volume. The main effect of laterality persisted ($F_{1,29} = 4.777$, $p = 0.037$, $\eta^2 = 0.141$). Plots represent mean± SEM.

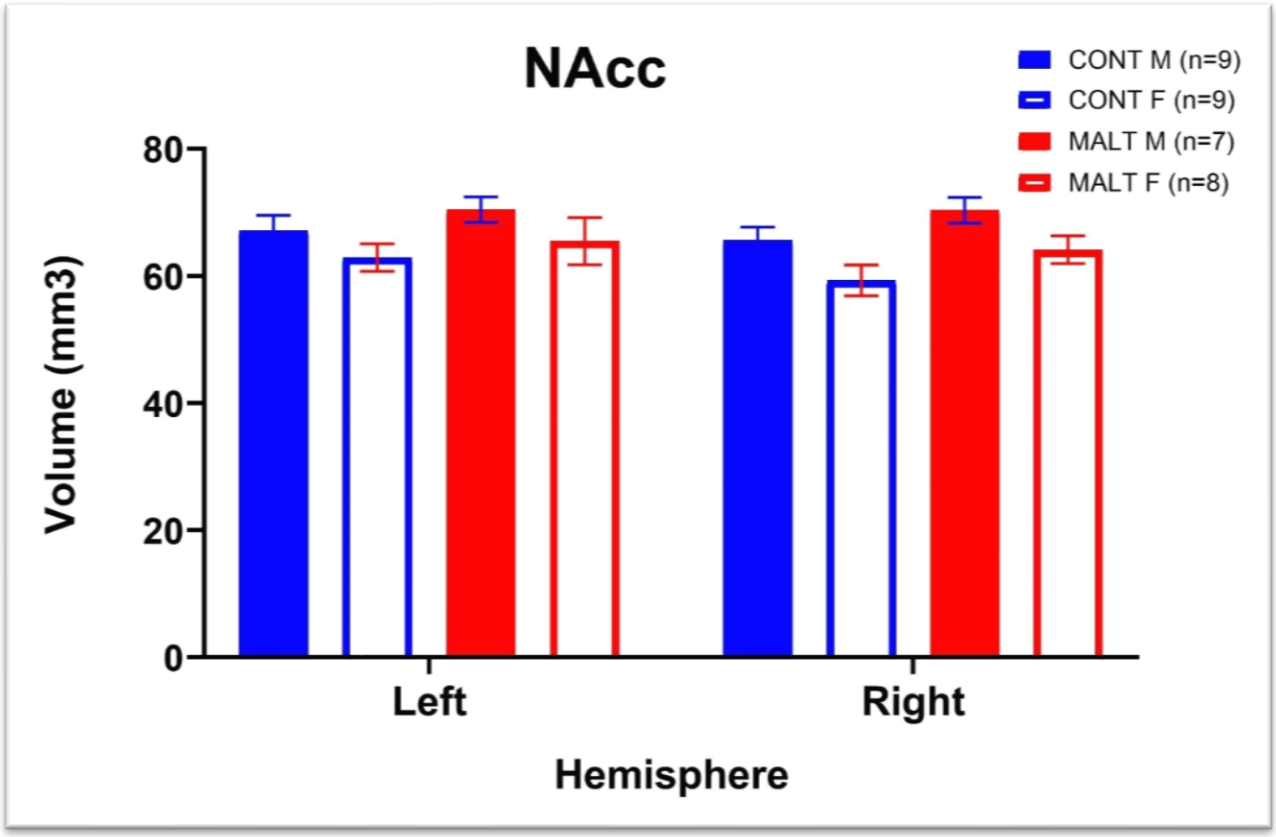


**Figure 27.** Developmental effects of infant maltreatment on total NAcc volume. A main effect of sex ($F_{1,29} = 5.643$, $p = 0.024$, $\eta^2 = 0.163$) was detected, showing larger NAcc volumes in males than females. Plots represent mean± SEM.

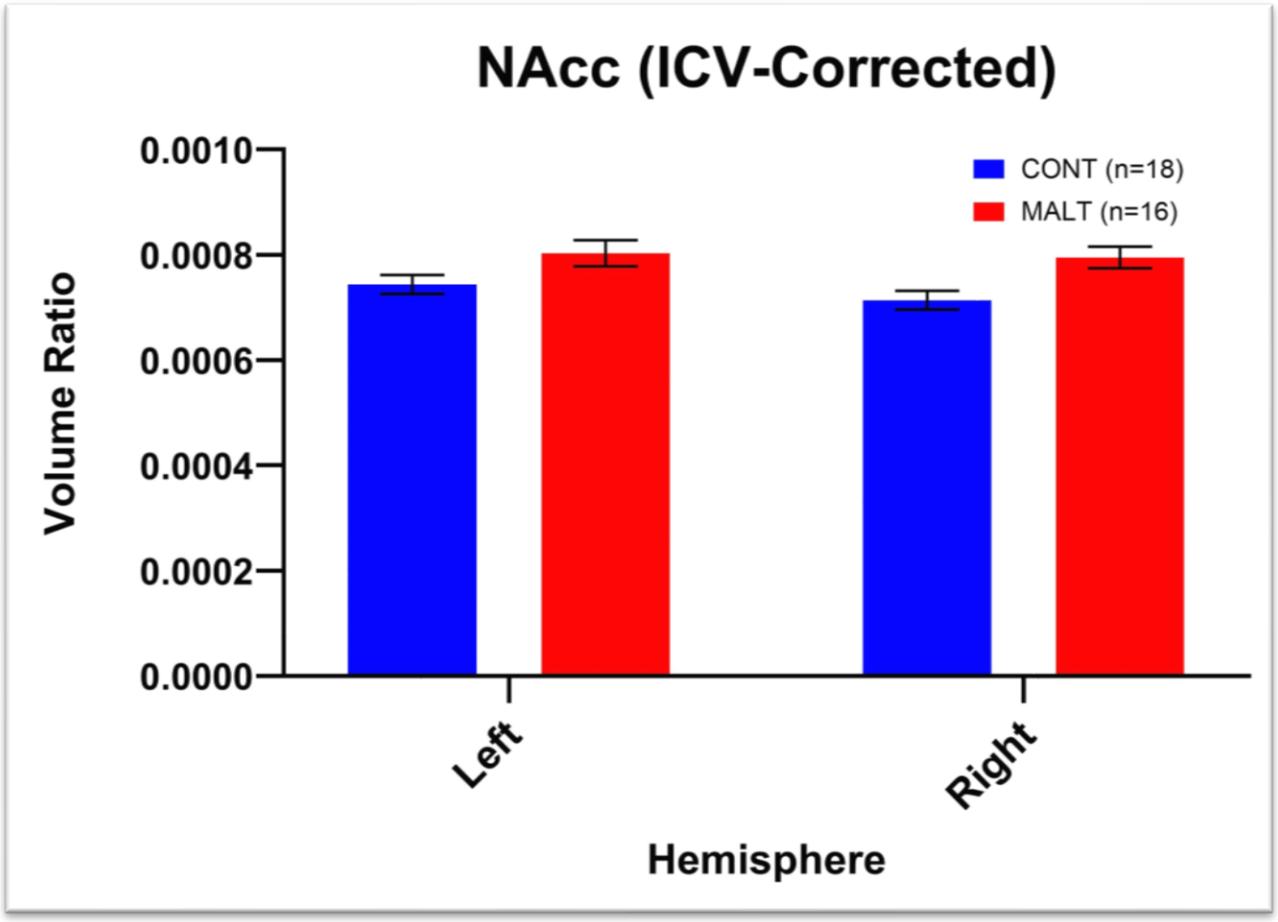


**Figure 28.** Developmental effects of infant maltreatment on ICV-Corrected NAcc volume. A main effect of group ($F_{1,29}$ =6.632, p=0.015, $\eta^2$ = 0.186) was detected, showing larger ICV-corrected NAcc ratio volumes in MALT than CONT subjects. Plots represent mean± SEM.

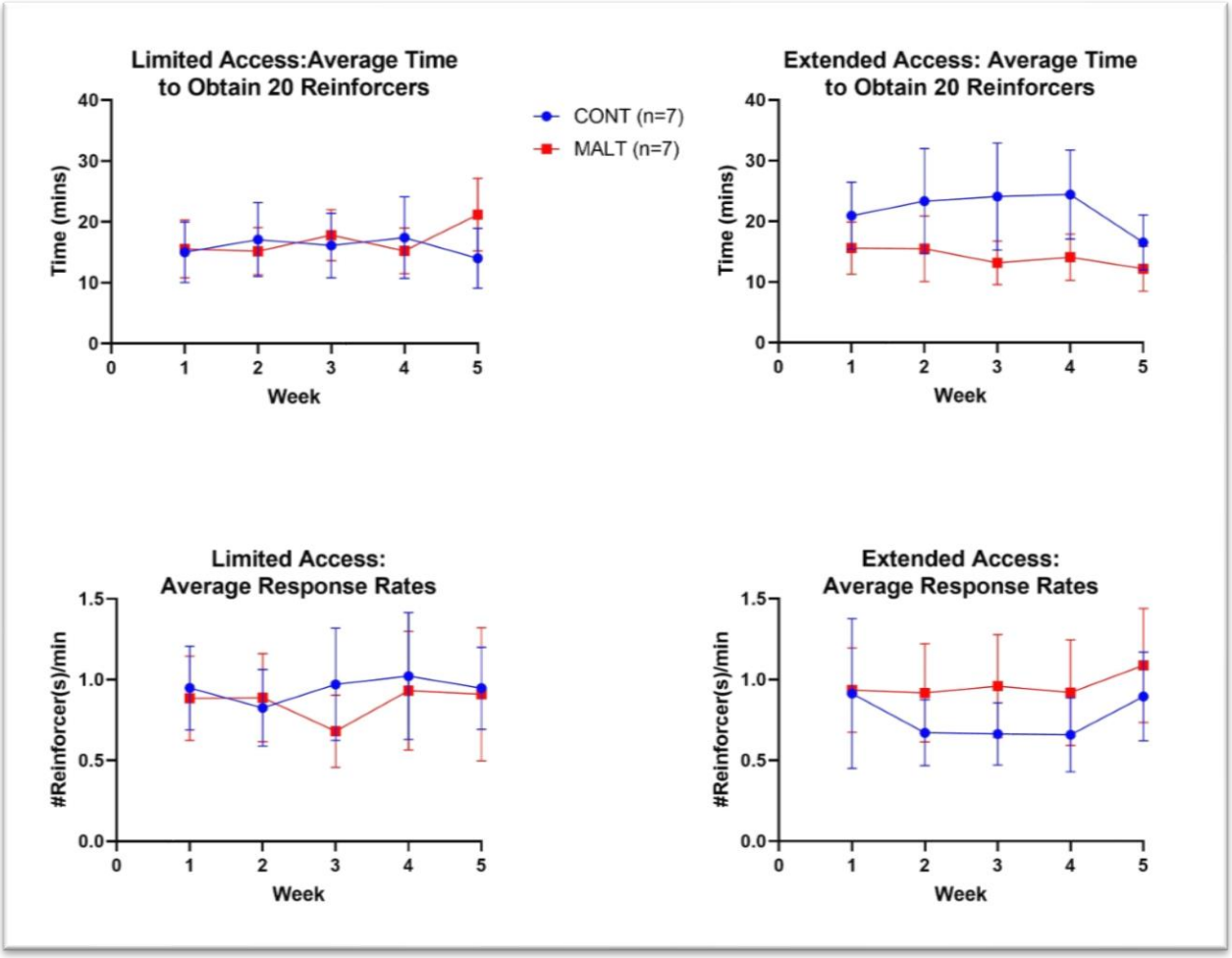


**Figure 29. (A)** Average time to obtain twenty reinforcers during limited access (top left panel) and extended access (top right panel); **(B)** rate to obtain twenty reinforcers during limited access (bottom left panel) and extended access (bottom right panel). **(A)** *Limited Access*: No main or interaction effects were found. *Extended Access*: No main or interaction effects were found. **(B)** *Limited Access*: No main or interaction effects were found. *Extended Access*: No main or interaction effects were found. Plots represent mean± SEM.

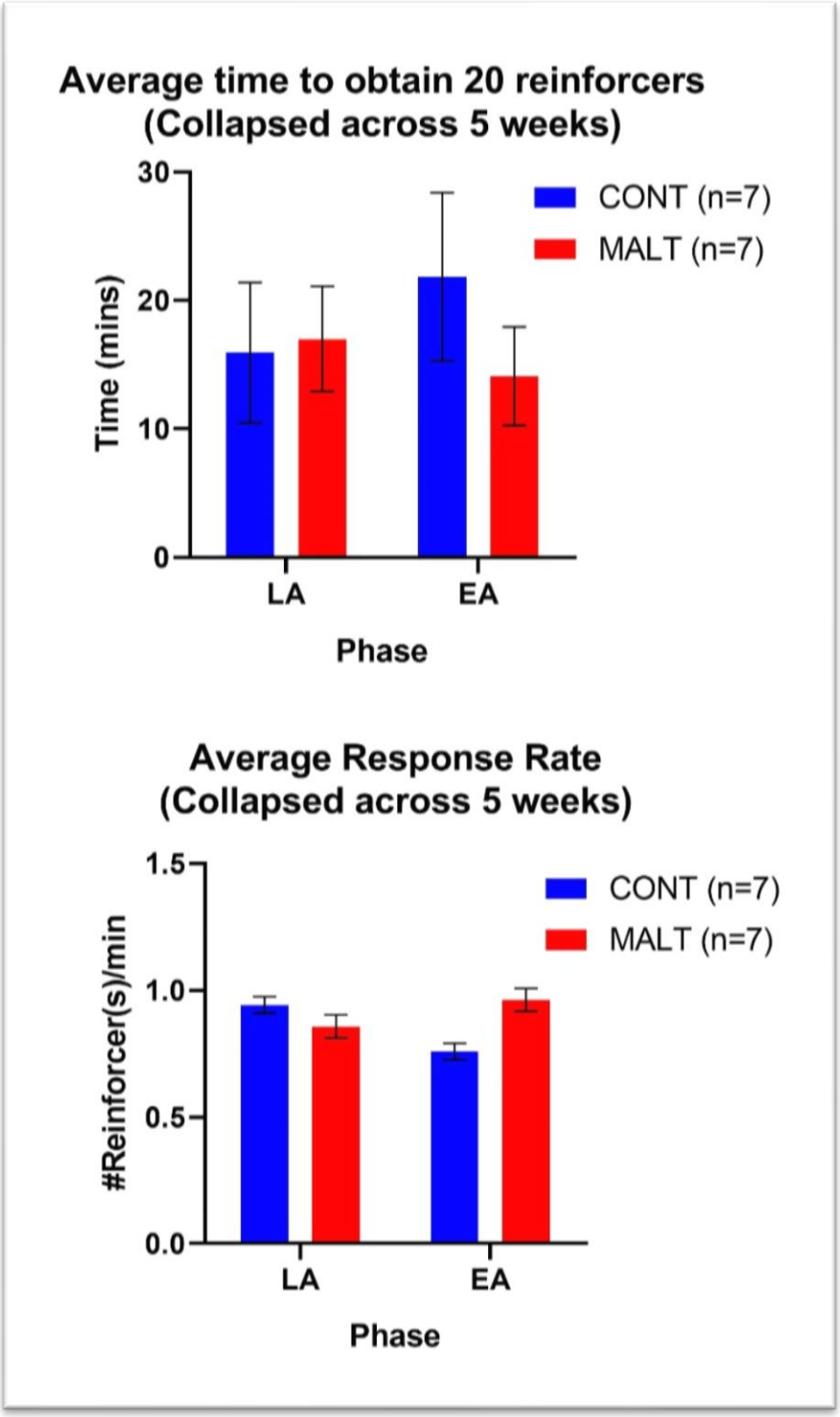


**Figure 30. (A)** Average time to obtain twenty reinforcers collapsed across five weeks; **(B)** average response rate to obtain twenty reinforcers collapsed across five weeks. **(A)** No main or interaction effects were found. **(B)** No main or interaction effects were found. Plots represent mean± SEM.

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