Development of Emotion Regulation Neural Circuitry: Anatomical Volumes and Functional Connectivity in Middle Childhood

Development of Emotion Regulation Neural Circuitry: Anatomical Volumes and Functional Connectivity in Middle Childhood

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PART ONE:

The Relative Impact of Late Gestation Maternal Cortisol and Sixth Month Postpartum Maternal Sensitivity on Offspring Amygdala and Hippocampal Volumes During Middle Childhood: Implications for the Development of Major Depressive Disorder

ABSTRACT

Background: Maternal prenatal adversity often results in changes to the hypothalamic-pituitary-adrenal axis (HPA axis) function, such as greater cortisol secretion. Recent evidence suggests that fetal exposure to elevated cortisol levels may cause structural changes to key limbic regions integral to regulation of the HPA axis such as the amydala and hippocampus in children. In the early postnatal months these same structures are particularly vulnerable to the quality of maternal care and parenting styles. However, the relative impact and interaction of such factors is still underreported. **Methods:** 24 healthy 7-8 year old children (male:female=13:11) underwent an MRI. Amygdala and hippocampal volumes were assessed and used in multiple regression models to determine the impact of prenatal cortisol and postnatal maternal sensitivity. **Results:** Larger right hippocampal volumes were associated with increases in late gestation cortisol levels (4.6 mm^3/nmol of cortisol; FDR corrected $p<0.005$). Increases in 6^{th} month maternal sensitivity predicted a decrease in right hippocampal volumes at a trend level (FDR corrected $p=0.09$). There was no interaction effect between cortisol and sensitivity. There were no significant effects on left hippocampus or bilateral amygdala volumes. No sex differences were noted. **Discussion:** Given previous work we had expected greater amygdala volume and reduced hippocampal volumes to associate with increases in cortisol and decreases in sensitivity. Our results suggest that there may indeed be a programming effect on children's hippocampi by prenatal cortisol. Findings may be reflective of a positive adaptive response or resilience to adverse prenatal environments.

INTRODUCTION

THE LIMBIC SYSTEM: A BRIEF OVERVIEW

The limbic system is comprised of a network of subcortical structures including: amygdala, hippocampus, cingulate, olfactory bulbs, mammillary bodies, and fornix. Paul MacLean first described it in its present format including prefrontal projections and role in autonomic regulation in 1952. MacLean proposed that based on its universality amongst different mammalian species it was an early evolutionary network in mammals responsible for the processing of emotional stimuli (Maclean, 1952). From his review of the literature at the time, he noted an interesting disparity among behavioural effects of bilateral limbic region ablations in different non-human mammals. In some animals such lesions caused aggressive individuals to become calm and docile, while in other animals the effect was quite the opposite. These experiments appear to lack precision in isolating anatomic regions for study; instead they report on the effects of global limbic region ablations. The findings are striking, however, considering the techniques have been available to study specific regions for some time since the work of MacLean, but the function of core limbic regions pertaining to behaviour (such as the amygdala) is still debated more than 60 years later (Palazidou, 2012).

Recently, the conventional view of the limbic system as a unified, consolidated network of regions working in concert to process emotion has been questioned (LeDoux, 2012; Rolls, 2013). Since there is a non-universal response to fear among limbic regions

(LeDoux, 2012) and there appears to be a difference in the way we process conscious and unconscious fear (LeDoux, 2014) it is argued that limbic regions contribute to *specific* rather than general emotional and cognitive circuits. Certain regions within the limbic system, such as the anterior cingulate (Bush, Luu, & Posner, 2000; Ochsner & Gross, 2005), and amygdala (Easter et al., 2005; Phelps & LeDoux, 2005; Tottenham et al., 2010) are consistently associated with emotional processing; others seem to associate independently with different neural processes. This is evidenced by strong support for the role of the hippocampus in spatial memory acquisition and consolidation (Burgess, Maguire, & O'Keefe, 2002). Moreover, functional neuroimaging studies have shown the coupling of the amygdala with anterior cingulate cortex (ACC) activity is integral to emotional regulation circuitry (Hariri, Mattay, Tessitore, Fera, & Weinberger, 2003; Herringa, Birn, & Ruttle, 2013). The formation of spatial memories for novel objects requires the recruitment of cortico-hippocampal connections, and interestingly this connection is mediated by the orbitofrontal and posterior cingulate cortex (PCC) (Ranganath, Heller, Cohen, Brozinsky, & Rissman, 2005), of which only the orbitofrontal cortex receives direct input from the amygdala (Rolls, 2013). Taken together this evidence points to a diversity of functions assigned to a small number of limbic structures, sometimes working in tandem and other times independently to form a variety of neural networks. Some of these networks, particularly involving regions in the prefrontal cortex will be further discussed in part two within the context of emotion regulation. The following document will seek to understand how environmental factors affect the development of these regions.

MAJOR DEPRESSIVE DISORDER (MDD)

Major Depressive Disorder (MDD) is a mood disorder characterized by a depressed mood or loss of interest in activities normally enjoyed for at least a 2-week period. In children, the depressed mood may be replaced by significant irritability. In addition, 4 secondary symptoms must also be experienced. Weight loss/appetite changes, difficulty concentrating or memory problems, difficulty making decisions or recurrent thoughts of suicide are examples of additional symptoms (Diagnostic and Statistical Manual of Mental Disorders - Fourth Edition Text Revision,2000).

MDD is present throughout the world with an overall point prevalence of 4.7% (CI=4.4-5.0%). Interestingly, the prevalence is relatively stable across geographic and cultural boundaries, ranging from 3.7% (CI=3.1-4.3%) in North America to 8.6% in South Asia (CI=5.2-14.0%) (Ferrari et al., 2013). In Canada, the lifetime prevalence for anyone to develop MDD is 10-12% with a point prevalence of 2% (Patten & Juby, 2008). A higher prevalence among women is one of the most reported findings in the epidemiology of depression (Grigoriadis & Robinson, 2007). In Canada women are almost twice as likely to develop the disease (Patten & Juby, 2008), a figure also reflected by most international centers (Maier et al., 1999). This sex discrepancy has been reported to begin as early as childhood with girls reported to have longer and more recurrent episodes of depression compared to boys (Birmaher et al., 2004) however, a recent review noted this dichotomy first arises around puberty, citing hormonal changes between girls and boys as a principle contributing factor (Thapar, Collishaw, Pine, &

Thapar, 2012). Another interesting and consistent finding is that depression presents in different ways and is precipitated by different factors in men and women; a finding hypothesized to be due in part to sex-specific coping strategies and social expectations (Grigoriadis & Robinson, 2007). Additionally, the disease course may be worse in women as they are 6 times more likely than men to have comorbid anxiety and depression by later life (Schoevers, Beekman, Deeg, Jonker, & van Tilburg, 2003). Numerous chronic medical conditions such as diabetes, HIV/AIDS, and cancer are also known to be frequently comorbid with MDD in both sexes. Though having a chronic medical condition is a significant risk factor for the development of depression, MDD may conversely be a contributing factor in the development of some illnesses including cardiovascular and cerebrovascular disease, and can likely worsen their prognosis (Evans et al., 2005). Ultimately, MDD is a disease that disproportionately affects females. The origin of this difference is one that has yet to be fully elucidated, however evidence to date supports an underlying cause greater than simple variations in help-seeking for mental health problems (Thapar et al., 2012).

DEVELOPMENT OF DEPRESSION

THEORIES OF DEPRESSION

Overwhelmingly, the evidence points to MDD being a disease of mixed and differing etiologies (Palazidou, 2012). The presence of one predisposing biological factor may be sufficient to cause disease but often it involves the interaction of several underlying mechanisms.

Serotonergic (5-HT) hypofunction is regarded as a principle biological theory of depression and is a fundamental target of front-line pharmaceuticals for treatment. The underlying genetic correlates of the serotonergic hypothesis include the finding that polymorphisms in the 5-HT transporter promoter region (5-HTTLPR) and 5-HT1a receptor have been associated with increases in amygdala activation to fearful faces and anxious behaviour, as well as greater amygdala activation in patients with MDD (Frodl, Möller, & Meisenzahl, 2008; Hariri et al., 2002). In addition, findings that 5-HTTLPR carriers have smaller hippocampal volumes in depressed patients (Frodl et al., 2008) further highlight the genetic basis for limbic system involvement in depression.

Chiefly, regions within the limbic system impact the development of depression through the stress diathesis model. This involves the dysregulation of the hypothalamic – pituitary – adrenal (HPA) axis leading to aberrant corticosteroid and associated stress levels typical of depression (Carroll, 1982). This HPA dysregulation is hypothesized to be due in large part to early-life experiences (Gonzalez, Jenkins, Steiner, & Fleming, 2012). The amygdala and hippocampus have strong connections to the hypothalamus,

and are involved in its regulation of hormonal output (Kasckow, Baker, & Geracioti Jr, 2001). The sensitivity of these limbic regions to pre and postnatal factors will be the focus of this document and discussed in further detail.

NEUROBIOLOGY OF EMOTION REGULATION

The amygdala is a small bilateral almond shaped structure in the medial temporal lobes with remarkable sensitivity to emotional stimuli – able to detect fear stimuli at sub-conscious thresholds (Whalen et al., 1998; Hall, Doyle, Goldberg, West, & Szatmari, 2010). Given this ability, its role in the emotional/fear response circuitry is likely to provide the initial and rapid automatic response to an environmental cue, thus assigning immediate salience to the stimulus. In the brief moments following this sub-conscious reaction, ventrolateral (N. A. Fox & Pine, 2012), dorsolateral (Koenigs & Grafman, 2009), medial (D G Gee et al., 2013), or orbitofrontal (Ongür & Price, 2000) aspects of the prefrontal cortex provide context to the stimuli. This connectivity between the amygdala and prefrontal regions, particularly the direct input from the orbitofrontal cortex is referred to as top-down modulation and is thought to allow the individual to cognitively attend to the stimuli and adjust initial emotional responses (Ochsner & Gross, 2005; Fox & Pine, 2012).

The amygdala-prefrontal circuit is integral to the fight-or-flight and stress response as well as fear related learning, both through interactions with the HPA axis (Rodrigues, LeDoux, & Sapolsky, 2009). Abnormalities within the circuit have been

implicated in disease states involving exaggerated stress responses (Akirav & Maroun, 2007).

ANTENATAL STRESS LEVELS

The HPA axis begins with hypothalamic release of corticotropin releasing hormone (CRH), which stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH acts on the adrenal cortex to release glucocorticoids, such as cortisol into the blood stream (*see Figure 1).* A hyper-activated HPA axis due to a greater perceived level of stress produces more cortisol (Carroll, 1982), and cortisol has recently been shown to be the fundamental way by which prenatal stress enacts long-term neurobiological changes in rats (Bingham, Rani, Frazer, Strong, & Morilak, 2013) and humans (Davis, Waffarn, & Sandman, 2010). Additionally, experimental use of CRH has shown similar results (Keck & Holsboer, 2001), thus further highlighting the functional importance of the HPA axis in stress reactivity.

Since the limbic system and the prefrontal cortex directly innervate the hypothalamus, and limbic-prefrontal circuits also have strong connections with the hypothalamus (Lupien, McEwen, Gunnar, & Heim, 2009; Rodrigues et al., 2009), it follows that the endocrinology of stress can be affected by mood and conversely affect mood. This may be the mechanism by which pregnant mothers communicate their environment to their fetus. If a woman is experiencing a particularly stressful event or prolonged adversity during pregnancy, recent research suggests that her elevated cortisol levels may have an impact on the development of her child's limbic system and HPA axis

with implications for affective disorder development (Claudia Buss et al., 2012; Glover, O'Connor, & O'Donnell, 2010; Lazinski, Shea, & Steiner, 2008). Particularly, effects of late gestation cortisol are associated with child temperament as early as infancy (Elysia Poggi Davis et al., 2007) and early-life temperament is in-turn a predictor of later life psychopathology (Caspi, Henry, McGee, Moffitt, & Silva, 1995).

There may be particularly vulnerable time points during gestation where fetal cortisol exposure may have the most impact; the timing of these vulnerable periods is a point of debate in the literature and is mainly divided between early and late gestation exposure.

During late gestation (third trimester), 11-beta hydroxysteroid dehydrogenase type-2 (11β-HSD2), a placental enzyme responsible for converting maternal cortisol into inactive cortisone is down regulated. This is to allow for normal maturation of fetal tissues particularly within the brain and lungs, which require sufficient corticosteroid levels for development (Meyer, 1983; Wyrwoll, Holmes, & Seckl, 2011). However, late gestation is also a period of tremendous neuroplasticity as cellular migration, dendritic arborization, and the majority of synaptogenesis are occurring (Andersen, 2003). Given that corticosteroids such as cortisol can pass through the blood brain barrier (BBB) to mature developing tissues (Uhr, Holsboer, & Müller, 2002) and female fetal placental barriers are more permeable to corticosteroids than males (Montano, Wang, & Vom Saal, 1993) it is not surprising that excess cortisol, particularly during this sensitive developmental window may have deleterious effects on the typically developing brain in a sex- specific manner.

Inhibition of 11β-HSD2 in animal models can lead to excess fetal exposure to glucocorticoids if the mother was stressed, and also greater anxiety-like behaviours in her offspring (Seckl & Holmes, 2007). Amygdala glucocorticoid receptor (GR) mRNA has been found to be up-regulated following inhibition of 11β-HSD2 and antenatal stress (Welberg, Seckl, & Holmes, 2000). This finding is consistent with earlier work showing that chronic exposure to cortisol and prenatal stress produces an up-regulation of CRH mRNA (Makino, Gold, & Schulkin, 1994) and overall CRH levels (Cratty, Ward, Johnson, Azzaro, & Birkle, 1995) within the rat amygdala. This would result in a downstream increase in offspring cortisol levels in rats (Makino et al., 1994) as well as placental CRH levels via a similar system in humans (Sandman et al., 2006). Furthermore, blocking CRH-1 receptors can have anxiolytic effects in rats (Liebsch et al., 1995) and anxiolytic drugs are known to reduce CRH levels in the brain following treatment (Owens, Bissette, & Nemeroff, 1989).

In the hippocampus, prenatal stress across pregnancy produces a decrease in hippocampal neurons in females (Zhu et al., 2004) and overall volume reductions by childhood in non-human primates (Uno et al., 1990). Prenatal stress and corticosteroid exposure are also linked to significant reductions in GRs within the hippocampus of offspring (Bingham et al., 2013). Cumulatively, this preliminary evidence underscores two main functions of the hippocampus that may be altered following maternal prenatal stress exposure: negative feedback upon the HPA axis (Lupien et al., 2009) and context dependent fear conditioning (Fanselow, 2000).

Similarly to the way elevated GR receptors in the amygdala result in greater CRH and cortisol release following glucocorticoid exposure (Welberg et al., 2000), a reduction in GRs within the hippocampus may have the same effect via reductions in negative feedback following similar exposures. Structural reductions in this region are theorized to not only lead to similar deficits in negative feedback but may also undermine the way fear is processed in the context of previous experience (Fanselow, 2000), which could lead to heightened emotional reactions to stimuli and anxious behaviour (Puliafico & Kendall, 2006).

Volume reductions as detected by neuroimaging techniques may in part reflect reductions in dendritic density (Kassem et al., 2013) and such dendritic density deficits have been reported to underlie microscopic changes to hippocampal structure following repeated stressors (Conrad, Magarinos, LeDoux, & McEwen, 1999). In humans, reduced hippocampal volumes are reported in patients with multiple episode depression (McKinnon, Yucel, Nazarov, & MacQueen, 2009) and atrophy has been related to both affective and memory problems (McEwen & Magarinos, 2001). Following MDD remission volumes are increased (MacQueen, Yucel, Taylor, Macdonald, & Joffe, 2008) further linking hippocampal structure to mood disorder pathophysiology. However, volume changes in association with prenatal stress hormone exposure in humans, particularly children, have been inconclusive to date (Buss et al., 2012; De Bellis et al., 2000). **It follows therefore, that such structural changes may be the route by which cortisol transfers risk for development of mood disorders between generations, and exacting the precise developmental window should be a goal of ongoing research**.

Increasing cortisol may permanently decrease the expression of glucocorticoid receptor genes within the offspring hypothalamus (Welberg et al., 2000). Like the hippocampus, the hypothalamus acts as an important site for cortisol-mediated negative-feedback on the HPA axis (*see figure 1,* Lupien et al., 2009) and thus a reduction of GRs could precipitate hyper-activation and release of more cortisol. In humans, the integrity of this negative-feedback system is often assessed using the dexamethasone suppression test (DST) (Carroll, 1982). Following administration of dexamethasone, an exogenous glucocorticoid, children and adolescents suffering from MDD showed greater cortisol secretion – indicating less suppression via negative feedback (Lopez-Duran, Kovacs, & George, 2009). Further highlighting the link between HPA axis integrity and mood disorders in humans - abnormal results of the DST are significantly linked to a greater prevalence of suicidality among inpatients with MDD (Coryell, Young, & Carroll, 2006) Additionally, greater sensitivity to glucocorticoids as measured by the DST has been associated with intergeneration (mother-child) transmission of risk for developing PTSD – further highlighting the mediating role of the HPA axis in vertical transmission of risk (Lehrner et al., 2014).

Antenatal administration of the synthetic glucocorticoid betamethasone is routinely given prenatally at 24-36 weeks who are at risk for preterm delivery in order to accelerate the maturation of the lungs. Following an early postnatal heel-stick, healthy full-term infants exposed to betamethasone earlier in this preterm window showed greater cortisol spikes compared to controls with no significant differences in baseline cortisol levels (Davis et al., 2010). Endogenous maternal glucocorticoids in early gestation also

predicted greater levels of cortisol during the first day of school for their children. Additionally, the mothers psychosocial anxiety of bearing a handicapped child also predicted greater cortisol responses in her children (Gutteling, de Weerth, & Buitelaar, 2005). Moreover, elevated levels of cortisol secretion is consistently reported in children and adolescents with depression (Goodyer, Herbert, Moor, & Altham, 1991; Lopez-Duran et al., 2009). These findings together highlight the potential mechanisms by which the prenatal environment, particularly a stressful one, may cause greater stress reactions in offspring with potential implications for affective disorder development.

This interaction between prenatal stress, anxiety and cortisol may also impact the child's executive functioning and cognitive development. Fetal exposure across gestation to pregnancy-specific anxiety, such as those examined in Gutteling et al. 2005, associated with lower visuospatial reasoning in boys and girls and lower inhibitory control in girls during middle-childhood (Buss, Davis, Hobel, & Sandman, 2011). Maternal cortisol levels as well as psychological stress in *early* but not late gestation were correlated with attenuated mental development scores during the first 12 months of life (Davis & Sandman, 2010) and greater anxiety scores by age 9 (Davis & Sandman, 2012). This link between the prenatal environment and cognitive function in childhood is interesting considering the established importance of cognitive control networks in emotion regulation and the etiology of mood disorders (Hare et al., 2008; Ochsner & Gross, 2005).

Betamethasone administered between 24-36 weeks gestation was associated with significant cortical thinning, most notably in the bilateral rostral anterior cingulate

(rACC) (Davis, Sandman, Buss, Wing, & Head, 2013); a region which integrates amygdala and prefrontal connections in an emotion regulation circuit known to be dysfunctional in adolescent MDD (Connolly et al., 2013). Not surprisingly, Davis et al. 2013 found that thinning in the rACC was also associated with affective problems in children. It is hypothesized that these networks regulate emotion via a top-down cognitive control mechanism whereby several regions within the prefrontal cortex moderate activity in the amygdala (Gee et al., 2013).

Prenatal maternal depression predicted reduced right amygdala microstructure but not volume in neonates (Rifkin-Graboi et al., 2013), however by middle childhood right amygdala volume mediated the pathway between *early* gestation cortisol levels and affective problems in girls (Buss et al., 2012). These volume changes of prefrontal and limbic regions are mirrored by white matter connections (Sarkar et al., 2014) and functional connectivity findings from the same regions, which were associated with greater separation anxiety (Gee et al., 2013). Moreover, increasing right amygdala size and the integrity of the functional emotional networks which center around the amygdala, have recently been shown to reliably predict anxiety severity in children as early as 7 years old (Qin et al., 2014) and adolescents (De Bellis et al., 2000). By adulthood, larger bilateral amygdala volumes are seen in first episode depression patients compared to controls (Frodl et al., 2002).

The amygdala, hippocampus, and cortical regions function in concert to detect and regulate moment-to-moment stimuli. Given their sensitivities to prenatal stress and links to the HPA-axis, These findings together could propose a preterm developmental timeline

and anatomical mechanism by which cortisol programs this neural circuitry, potentially in a sex-specific way. There has been a lack of studies addressing sex differences overall. However, sexually dimorphic findings in animal models, placental physiology, and most recently novel human studies investigating the prenatal cortisol – limbic structure connection suggest greater female susceptibility (Claudia Buss et al., 2012; Montano et al., 1993; Zagron & Weinstock, 2006; Zhu et al., 2004). Such a programming effect fits with the well established epidemiology findings concerning female rates of mood disorders (Maier et al., 1999). Still, the paucity of this literature base reflects the need for greater investigations.

EARLY LIFE ADVERSITY

It has been reported that childhood adversity accounts for up to one-third of adult cases of mood and anxiety disorders (Green et al., 2010). Childhood maltreatment; a form of adversity not necessarily involving the intentional harm of a child, is regarded as a major predictor of adverse mental health outcomes, drug abuse, risky sexual behaviour, and aggression with early and long-lasting effects (Gilbert et al., 2009; Herringa et al., 2013; Lansford et al., 2002; Thapar et al., 2012)

The first year of life is a period of marked synaptic pruning in the developing brain (Andersen, 2003). In particular, amygdala and hippocampal development is protracted with tremendous growth across the first four years of life (Uematsu et al., 2012). Given the role of these structures, this presents a critical window of significant plasticity where an adverse environment may enact lasting deleterious effects on

emotional health. Conversely, this window also offers an opportunity to forge healthy developmental trajectories.

Given the highly complex social nature of human relationships and the length of time that a child is cared for, it may be somewhat surprising that a range of species suffer very similar consequences of early-life deprivation or adverse parenting. In mice, it has been definitively shown that strong maternal care can create stable alterations in offspring genome expression in regions integral to emotion and stress regulation; importantly these changes are reversible (Weaver et al., 2004). Moreover, such alterations in DNA methylation patterns may impart particular epigenetic changes associated with psychiatric disorders (Zucchi et al., 2013). Evidently, is it not surprising that maternal deprivation may have detrimental effects on offspring cognitive function (Bredy, Humpartzoomian, Cain, & Meaney, 2003) and the development of anxious behaviour (Barna et al., 2003). These results demonstrate how mothers may continue to communicate the nature of the world they are raising their children to inhabit, a hypothesis applied to prenatal stress effects on the fetus but likely also valid in the precarious and vulnerable months that follow birth.

Mothers who are stressed during pregnancy show greater depression-like behaviour in the early postpartum period, which affects their parenting strategies (Smith, Seckl, Evans, Costall, & Smythe, 2004). This is integral considering maternal MDD during this period predicts great behavioural difficulties and internalizing symptoms in offspring (Bagner, Pettit, Lewinsohn, & Seeley, 2010). Indeed, infants are known to be well attuned to their mothers mood, particularly females (Hatzinikolaou, 2010) and

coupling of mother and child is likely mediated through syncing of mother-child HPA axes (Atkinson et al., 2013). Childhood exposure to postnatal maternal depression is correlated with hyper-cortisol secretion (Halligan, Herbert, Goodyer, & Murray, 2004) and affective disorder development by adolescence (Murray et al., 2011), as well as larger amygdala volume (Lupien et al., 2011). Together this indicates a potential programming effect on the child HPA axis and subsequently the limbic system.

Altered amygdala and hippocampal function are hallmarks of depression and other affective disorders, with elevated and sustained activation in the amygdala (Siegle, Thompson, Carter, Steinhauer, & Thase, 2007) as well as amygdala volume enlargement (Qin et al., 2014) being commonly noted. Considering these regions' sensitivity to HPA axis changes (Buss et al., 2012; Davis, Sandman, Buss, Wing, & Head, 2013; Lupien, McEwen, Gunnar, & Heim, 2009) and their integral role in emotional salience and related networks (Fanselow, 2000; LeDoux, 2012), it follows that alterations in the HPA due to the early life environment may program changes in the limbic system with consequences for mental health trajectories.

Early life adversity may come in a variety of forms and does not necessarily involve maternal psychopathology. Deprivation, a less violent but equally damaging exposure, plays a significant role in early life adversity. Deprivation mainly constitutes a lack of strong maternal attachment needed to forge long-term healthy psychological outcomes (Collishaw et al., 2007). In fact, anxiously insecure attachment in otherwise healthy adolescent females may cause attenuated cortisol awakening responses (Oskis, Loveday, Hucklebridge, Thorn, & Clow, 2010) which could have been precipitated by

insecure child attachment to the mother in the first few months of life (Roque, Veríssimo, Oliveira, & Oliveira, 2011). Insecure attachment throughout childhood significantly predicts the onset of developmental disturbances such as internalizing symptoms (Groh, Roisman, van IJzendoorn, Bakermans-Kranenburg, & Fearon, 2012). Additionally, some research indicates that insecure attachment may be associated more with internalizing symptoms in girls and externalizing symptoms, such as violent outbursts, in boys (Fearon, Bakermans-Kranenburg, van Ijzendoorn, Lapsley, & Roisman, 2010). Finally, attachment security, namely anxious types of attachment insecurity, are associated with hyper-vigilance and hyper-reactivity to threatening stimuli in adults (Mikulincer, Shaver, & Pereg, 2003). Such hyper-reactions to threats have been further linked to changes in amygdala sensitivity in mood disorders (Siegle et al., 2007).

Following institutional rearing with very high caregiver-child ratios, children who were adopted later had significantly larger amygdala than children adopted earlier (Mehta et al., 2009; Tottenham et al., 2010) with little to no change in hippocampal volumes. This was mirrored by findings that severity of preadolescent emotional neglect predicted increasing right amygdala volumes in a dose-response fashion (Pechtel, Lyons-Ruth, Anderson, & Teicher, 2014). These findings are in contrast to another study finding smaller amygdala and smaller hippocampi (Hanson et al., 2014). Smaller hippocampal volumes are in line with the expected results, considering reductions in GRs and HPA negative feedback problems associated with mood disorders (Liu, 1997). This discrepancy in anatomic sensitivity is puzzling considering hippocampi have greater dendritic spine recovery following cessation of adversity (Vyas, Pillai, & Chattarji, 2004)

and lifetime cellular regeneration (Lugert et al., 2010), which could in-part explain the lack of significant findings. However, the amygdala has a longer period of development overall (Uematsu et al., 2012), therefore beneficial plastic changes following adversity cessation in early life would be plausible. This could indicate that the amygdala and hippocampus have different sensitivities, which are critical at different times – something that has yet to be worked-out.

Beyond neglect, maltreatment may also predict alterations in amygdala-ACC connectivity and internalizing behaviour – a cognitive control circuit previously discussed in-terms of its sensitivity to prenatal stress levels (Herringa et al., 2013).

In short, the neurobiology of pre and postnatal stress appears to be similar. This may help explain why the postnatal environment may influence effects caused during gestation. More specifically, strong maternal sensitivity, predictive of an eventual strong mother-child attachment could mitigate the effects of prenatal cortisol and anxiety on infant cognitive development - an important faculty in emotion regulation (Bergman, Sarkar, Glover, & O'Connor, 2010; Grant, McMahon, Reilly, & Austin, 2010) with a notable moderation of hippocampal atrophy (Buss et al., 2007). There is an apparent lack of evidence regarding the interactive effects of pre and post-natal maternal factors on neurobiological outcomes in children, particularly within the amygdala. This highlights the need for longitudinal studies to investigate this link.

The present study will begin to address the discrepancy and paucity regarding both hippocampal and amygdala volumes following fetal-life cortisol exposure; shedding light on vulnerable prenatal periods. In addition, it will attempt to highlight a postnatal

window where interventions designed to strengthen parenting would be most advantageous. Finally, this study will add to the literature by investigating the interaction between prenatal stress levels and postnatal maternal sensitivity (parenting) on amygdala and hippocampal volumes in childhood for the first time.

We predict that prenatal maternal cortisol and postnatal sensitivity will have opposing influences on offspring amygdala and hippocampal volumes. Moreover, we expect increased cortisol in late gestation to predict greater amygdala and smaller hippocampal volumes and strong postnatal sensitivity to have a moderating effect on the direction of such effects.

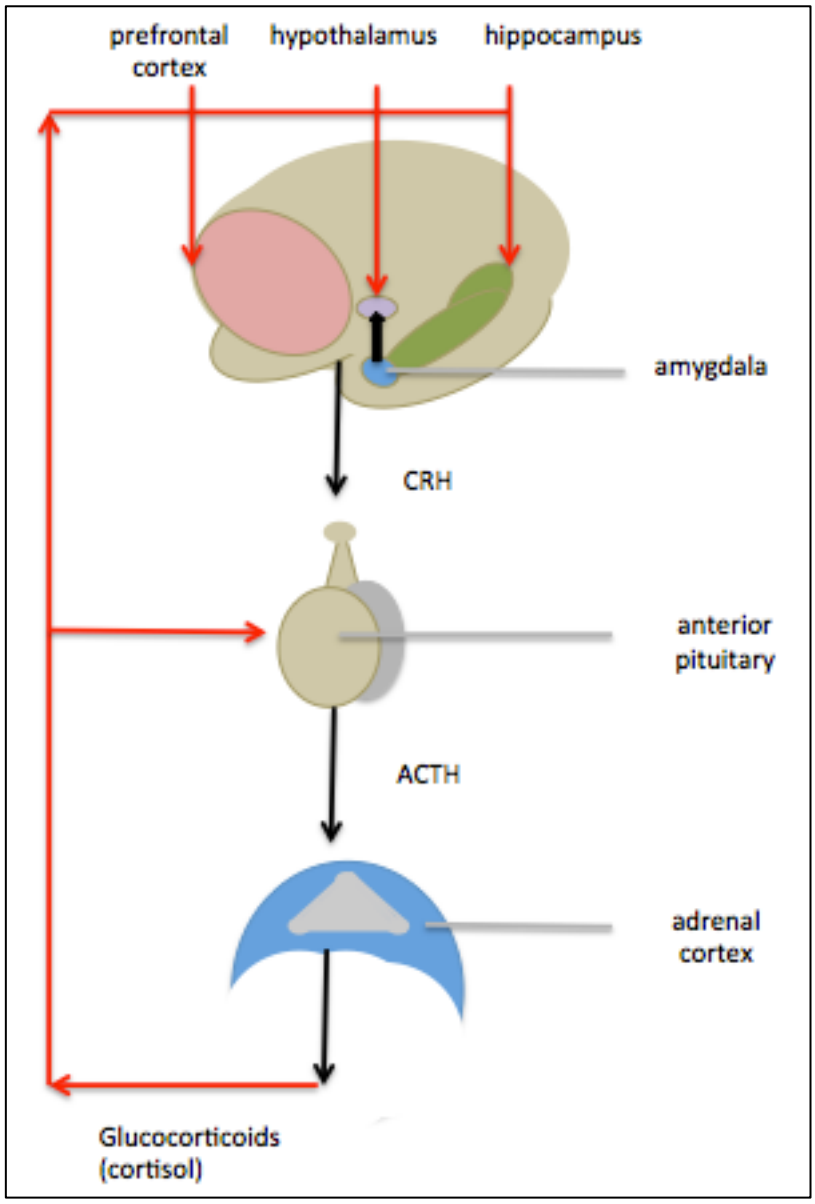

Figure 1: representation of the HPA axis with cortical and subcortical projections. Negative feedback by cortisol is indicated by red arrows.

METHODOLOGY

MATERNAL ADVERSITY VULNERABILITY AND NEURODEVELOPMENT (MAVAN)

The maternal adversity vulnerability and neurodevelopment (MAVAN) study is a longitudinal follow-up cohort consisting of mother-child dyads at two geographic sites: Montréal, Québec, Canada, and Hamilton, Ontario, Canada. MAVAN was designed to study the impact of prenatal and postnatal environmental variables and genetic factors on the health and developmental outcomes of offspring. To accomplish this, the MAVAN study collects a large number of genetic and physiological markers as well as an extensive battery of behavioural measures from the mother and her child on a regularly scheduled basis. Pregnant women experiencing adversity due to prenatal maternal depression, anxiety or low socioeconomic status during pregnancy were recruited into the study during the prenatal period after being referred to the Women's Health Concerns Clinic (WHCC) at St. Joseph's Healthcare in Hamilton, Ontario for treatment. In addition to this adversity group, a group of healthy control pregnant women were recruited. All women included in the study had a singleton gestation, were at least 18 years of age at the date of delivery, and were fluent in English or French. Exclusion criteria included: severe chronic illness, placenta previa, diagnosis of an incompetent cervix from a previous pregnancy, or expected delivery of an infant with a major anomaly.

PARTICIPANTS

7 and 8 year old children from the MAVAN study were recruited to participate in a neuroimaging study at the Imaging Research Centre (IRC) at St. Joseph's Healthcare approved by the Hamilton integrative research ethics board (HIREB). Informed parental consent and child assent was given after an explanation of the study protocol.

Children with a formal diagnosis of a neurodevelopmental or psychiatric disorder were excluded from participation in this study. Screening for the child's compatibility with an MRI scan was performed with the MRI screening form provided by the IRC. Scanning was done during a 1-hour session with approximately 50 minutes of scan time broken into 3 task-based functional scans, an anatomical, resting-state functional, and diffusion tensor imaging (DTI). Pre-training was done a separate day as close to the scan date as possible. Final training on the task paradigm was conducted immediately before scanning. Participants were reimbursed for travel expenses and parking, and were given 25 dollars remuneration for their participation.

MATERNAL PRENATAL CORTISOL

Biologically active cortisol unbound to corticosteroid-binding-globulin (CBG), is free in blood plasma, and can be accurately measured in saliva. Salivary cortisol levels were obtained from the mother at 24-36 weeks gestation using salivary pipettes. Saliva was collected over two consecutive days at 6 time points each day (0800 h, 0830 h, 1000 h, 1600h, 1800 h, 2100 h) to assess the mothers diurnal cortisol rhythm. Samples were

stored in a -20°C freezer prior to being assayed using a high-sensitivity salivary cortisol enzyme immunoassay (Salimetrics, State College, PA).

Average area under the curve with respect to ground (*AUCg, see figure **2***) was analyzed using the trapezoid method (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Using this method the area under a line drawn connecting the 6 daily collection time points is divided into 5 trapezoids and the area of these trapezoids is then calculated and summed to give a single estimated value of total daily cortisol secretion in nmol/L of saliva.

The mean AUCg across the two consecutive days was calculated and used in subsequent analysis. Pruessner et al. note an advantage to this approach is in the creation of a single estimate of a variable - thus reducing the likelihood of a type-one error (erroneous rejection of the null hypothesis) often attributed to a repeatedly sampled variable, such as salivary cortisol levels.

In order to prevent contamination of the saliva samples, the women were asked to refrain from brushing their teeth, smoking, eating, consuming alcohol, or caffeine immediately (60 mins) prior to sampling.

$$AUC_g = \sum_{i=1}^{n-1} \frac{(M_{(i+1)} + M_i)t}{2}$$

Figure 2 AUCg: Equation describing the trapezoid method, area under the curve with respect to ground (AUCg) as presented in *Pruessner et al. 2003, Psychoneuroendocrinology*. n = the number of measures; i = the time interval between measures; M_i = individual measure. t = time difference between samples

MATERNAL SENSITIVITY

Maternal sensitivity was measured at 6-months postpartum using the maternal behaviour Q-sort (MBQS) (Pederson & Moran, 1995). The MBQS is a validated behavioural measure of the quality of maternal caregiving; more specifically it is a measure of a mother's sensitivity towards her child's needs. A recent study noted the validity of the MBQS in predicting attachment security in infants and those in early childhood (Moss et al., 2011). The MBQS was assessed by raters watching a videotape of a mother-child interaction conducted in the home environment, and consists of 90-items of maternal behaviour. During observation a rater assigns cards to 1 of 3 piles: most like the mothers behaviour, neutral, or most unlike the mothers behaviour. Subsequently, the rater then divides each pile into 3 new piles creating a total of 9 piles. The first of nine piles describes the behavioural characteristics that the rater determines to be most like the mother – this pile is given a score of 9. Each of the subsequent piles are given scores of 8, 7, 6 etc… all the way to 1. As the pile numbers decrease the perceived applicability of those behaviours to the mother decreases. Inter-rater reliability is calculated between both observers of the task and a prototypically sensitive mother. The final global score reflects the correlation between our ratings and the prototypically sensitive mother and thus ranges from -1 to 1, the least prototypically sensitive mother to the most sensitive, respectively. The present study made use of a shortened video recorded mother-child interaction in the home environment. This modified format uses a validated 25-item battery instead of the full 90 items (Tarabulsy et al., 2009). The intra-class correlation using the 25-item format on the MAVAN data is greater than .85.

NEUROIMAGING

IMAGE ACQUISITION

Before proceeding to the MRI scan, children were screened with the IRC screening form for MRI compatibility. Subsequently, children were allowed to experience the environment of a typical MRI machine using a full-scale model of the real machine including exposure to sights, sounds and procedures associated with the MRI. This has been found to be a beneficial way to prepare children for the experience and to reduce motion artifacts and participant drop-out (Raschle et al., 2009).

3-dimentional high-resolution anatomical images (T1-weighted) were acquired in the sagittal plane in a 3-tesla GE short-bore MRI scanner (General Electric Healthcare, Milwaukee, WI). The scan parameters were: Spoiled Gradient Recalled T1 weighted acquisition with a repetition time (TR)=10.8 ms, echo time (TE)=2 ms, flip angle of 20^{o}, field of view (FOV) 24 cm and slice thickness of 1 mm.

ANATOMICAL SEGMENTATION

Subcortical segmentation was performed using Freesurfer (Harvard University, Boston, MA). T1-weighted images were motion corrected, averaged and subsequently converted into a single compressed anatomical file in native space. Variation in voxel intensity was then normalized and the skull and meningeal surfaces were removed leaving only white matter, grey matter, intraventricular cerebral spinal fluid (CSF) and the intimately associated pial layer. During the registration step a transform matrix was

applied to co-register the brain with the atlas (Fischl et al., 2002) to be used for subcortical structure labeling. Using the co-registered atlas and the voxel intensities of neighbouring voxels, regions were assigned 1 of 37 sub-cortical structural identities.

Freesurfer is known to consistently overestimate the volumes of the amygdala, hippocampus, and putamen. Moreover, the boundary distinction between the hippocampus and amygdala is bilaterally problematic (Dewey et al., 2010). Following the recommendations by Dewey et al. 2010 to ensure validity of results, manual corrections were made to Freesurfer's automated segmentations where necessary. Manual tracing was performed in the coronal plane and verified in the sagittal plane. Particular attention was paid to the amygdala and hippocampal segmentations

Amygdalae tracing followed boundaries outlined in Entis et al. 2012**.** The amygdala is bordered by the parahippocampal white matter at the inferior aspects and by CSF at the medial aspects near the midpoint of the structure. At the most rostral points, the amygdala is adjacent to temporal lobe white matter at the lateral aspects and entorhinal cortex grey matter at the medial aspects. More caudally, the amygdala encompasses more of the medial temporal lobe grey matter and is bordered by the alveus at the medial-inferior aspects, and inferior horn of the lateral ventricle as well as temporal lobe white matter at the lateral and superior aspects. At the most caudal point of the structure, the amygdala reduces in volume and migrates in the superior direction with the horn of the lateral ventricle giving way to the hippocampal grey matter (Entis, Doerga, Barrett, & Dickerson, 2012).

The hippocampi tracing followed the anatomical boundaries outlined in Appendix 2 of Boccardi et al, 2011**.** Hippocampal boundaries begin posterior to the amygdala, inferior to the alveus and superior to the parahippocampal white matter. Moving caudally, the hippocampus constitutes more of the medial temporal lobe grey matter as amygdala volume decreases while remaining inferior to the alveus white matter. Throughout the length of the structure, it remains bordered by the temporal horn at lateral aspects and by the white matter of the parahippocamapal gyrus at the inferior aspects. At the caudal regions it is bordered by the entorhinal cortex and the isthmus grey matter and its most superior aspect terminates at the pulvinar and as the fimbria white matter begins to thicken becoming the fornix (Boccardi et al., 2011)**.**

ANALYSIS

With power set at 0.8, alpha at 0.05, 2 continuous and 2 categorical predictors, and a small to medium effect size (ρ=0.2) our required sample size was 60 children. When we add the AUC*MBQS variable it rises to 64. At our present sample size of 24, we have a detection power of 34%.

Raw anatomical region of interest (ROI) volumes (mm^3) were adjusted for total intracranial volume (ICV) by presenting the ROI as a proportion of ICV (O'Brien et al., 2011). This method was preferred over using ICV as a covariate in-order to minimize loss of statistical power associated with multiple covariate regression models. Ordinary least squares regression models with simultaneous entry were created for each bilateral ROI. AUCg and MBQS were considered main regressors with child's sex and family

SES included as covariates. Missing data points due to absent or inadequate saliva among the 6 daily collection times in addition to missing MBQS scores were estimated using multiple imputation methods similar to Buss et al 2012 since variables were missing at random. A bootstrapping algorithm was applied to the raw data using the AMELIA 2 package in R to create 45 complete datasets (R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/). Left Amygdala volume was not normally distributed so a post-imputation logarithm transformation was performed on this data and the imputation procedure was re-run. Post-imputation transformations were also performed on preliminary models in-order to apply interaction effects between AUCg and sex, and MBQS and sex. The regression models were subsequently run on each complete dataset and the results were pooled using the ZELIG extension (Matt Owen, Kosuke Imai, Gary King and Olivia Lau (2013). Zelig: Everyone's Statistical Software. R package version 4.2-1. http://CRAN.R-project.org/package=Zelig). In-order to control for experimentwise error, a step-down false discovery rate (FDR) technique was applied to correct raw p-values (Benjamini & Hochberg, 1995).

RESULTS

PARTICIPANTS

Demographic and general characteristics of the 24 children included in this study are summarized in table 1. Mean age from both the adversity and control groups varied very little (mean age: 7.55 ± 0.53, and 7 years for the adversity and control groups, respectively.) reflecting the recruitment strategy designed to test this particular developmental window. Socioeconomic status (SES) was stratified into 3 ascending ordinal levels based on family income. In accordance with group assignment based on experiencing an adverse environment during pregnancy, group differences in socioeconomic status (SES) were approaching significance ($X^2 = 4.7$, $p<0.1$). However, to increase statistical power in subsequent analysis the adversity and control groups were collapsed and each variable's relative contribution to volume variance was investigated on a continuous rather than group level.

BRAIN MORPHOMETRY

The anatomical volume measurements of the amygdala and hippocampus are presented by sex in table 2 in uncorrected raw volumes (mm^3) and percentage of total ICV. There were no volume differences between adversity and control groups (left amygdala (LA): p=0.78; right amygdala (RA): p=0.47; left hippocampus (LH): p=0.23; right hippocampus (RH): p=0.22). These results in addition to the relatively small adversity group (n=10) prompted the collapsing of groups previously mentioned.

On average boys had larger amygdalae and hippocampi bilaterally than females (see table 2), however these differences did not meet statistical significance.

In addition, no significant variation in volume was found within each sex for left and right amygdala or hippocampus. Volumes are presented graphically in figure 3.

Characteristic	Group		Totals
	Adversity (n=10)	Control (n=14)	n=24
age (years), mean (SD)	7.55 (0.53)	7 (0)	7.21 (0.41)
sex ratio, M:F	6:3	7:8	13:11
socioeconomic status (1=lowest; 3=highest)	1 (n=1); 2 (n=3); 3 (n=5)*	2 (n=1); 3 (n=13)	1 (n=0); 2 (n=4); 3 (n=19)
ethnicity	White/Caucasian (n=7)	White/Caucasian (n=15)	White/Caucasian (n=22)
	Asian/South Asian (n=2)		Asian/South Asian (n=2)

Table 1 characteristics: general characteristics of the participants separated by mother-child dyad group. *Denotes a participant in the adversity group from whom a socioeconomic (SES) score was not obtained.

ROI: mean (SD)	boys			girls		
	unadjusted (mm^3)	adjusted for % ICV		unadjusted (mm^3)	adjusted for % ICV	p-value
right amygdala	1679.5 (256.3)*	0.11 (0.016)		1484.4 (104.2)	0.1 (6.9e-3)	0.42
left amygdala	1567.8 (185)	0.1 (0.011)		1425.2 (175.4)	0.09 (0.011)	0.5
right hippocampus	4696.7 (254.8)	0.3 (0.015)		4281.1 (301.04)	0.29 (0.02)	0.24
left hippocampus	4635.3 (343.5)	0.3 (0.025)		4186.6 (489.1)	0.28 (0.03)	0.34

Table 2 ROI volumes: Raw and adjusted measurements by sex for the mean left and right amygdalae and hippocampal volumes [*mean (standard deviation)]*. Volumes were adjusted for total intracranial volume (ICV) by presenting each ROI as a proportion of individual ICV. In this figure adjusted volumes are shown as a percentage of ICV. P-values depict hypothesis tests comparing volume differences between boys and girls. *Denotes an unadjusted non-parametric distribution.

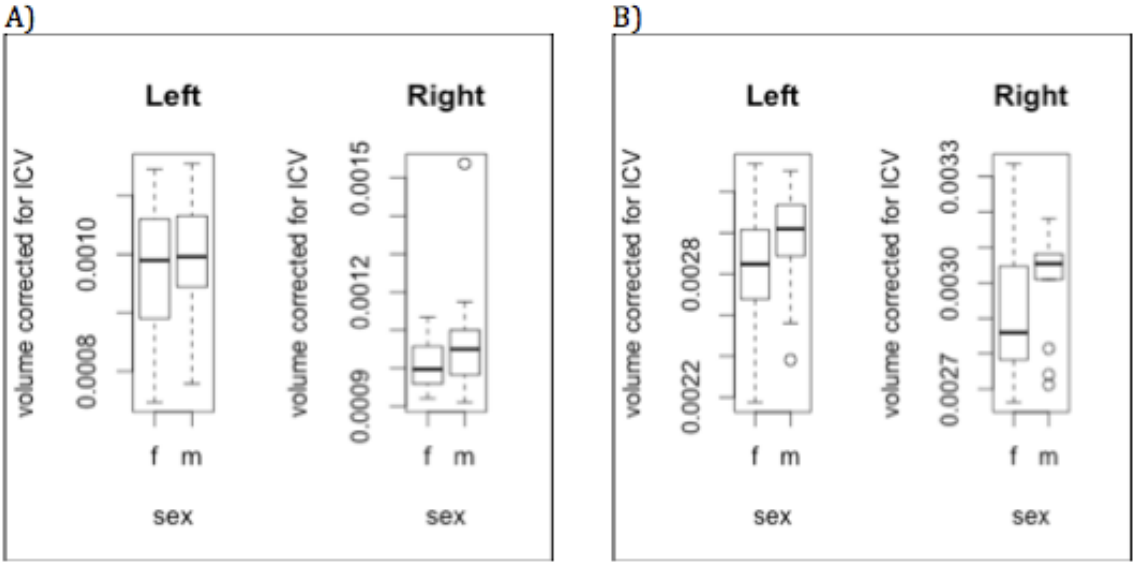

Figure 3 ROI volumes: A) represents amygdala volumes by sex B) represents hippocampal volumes by sex. Overall, only amygdala volumes showed a left-right volume difference approaching significance (P=0.1), and there were no significant differences by sex. ROI volumes are presented here as a dimensionless proportion of total ICV

REGRESSION RESULTS

Four simultaneous-entry least squares multiple regression models were performed, one for each dependent variable (ROI) under investigation, and are summarized in table 3. The first main regressor, cortisol, did not differ in quantity between consecutive sampling days (p=0.64, 95% CI [-16.6, 26.6]), however it did have a large range of approximately 94 nmol/L across participants on both days of collection. After controlling for the effects of postnatal maternal sensitivity, child sex, and SES levels during late pregnancy, every 1 nmol/L increase of maternal cortisol during the 24-36 week gestational period predicted a 0.0003 ± 0.0014 % increase in right hippocampal proportion of intracranial volume in children (FDR corrected p<0.005). Given that the mean ICV in the sample was 1.5 x 10^6 mm^3 (95% CI = 1.48 x10^6, 1.57 x 10^6) each nmol/L of cortisol equated to 4.6 mm^3 of additional volume.

Maternal sensitivity at 6-months postpartum also predicted right hippocampal volumes at a trend level. A 1-unit increase on the MBQS scale predicts offspring right hippocampal volume proportion decreases of 0.02 ± 0.008 % (FDR corrected p=0.09) which equates to a 304.6 mm^3 decrease in volume. Maternal sensitivity was not found to moderate the effect of cortisol on hippocampal volumes (p=0.9). The four regression models were also run on the original, non-imputed dataset with similar results thus providing confirmation of the relationships identified through the imputation and pooled regression method utilized.

		beta-coefficent	standard error (SE)	p-value
RH	cortisol	3.0×10^{-6}	1.4×10^{-6}	***0.003 (q=0.004)***
	MBQS	-2.0×10^{-4}	8.0×10^{-4}	***0.07 (q=0.09)***
LH	cortisol	4.5×10^{-6}	2.6×10^{-6}	0.09
	MBQS	-2.1×10^{-4}	1.9×10^{-4}	0.28
RA	cortisol	-1.27×10^{-6}	1.28×10^{-6}	0.3
	MBQS	-5.7×10^{-5}	8.0×10^{-5}	0.47
LA	cortisol	3.7×10^{-7}	1.2×10^{-6}	0.75
	MBQS	3.61×10^{-5}	6.37×10^{-5}	0.57

Table 3 regression results: main effects for the four regression models as identified by their ROI. RH=right hippocampus; LH=left hippocampus; RA=right amygdala; LA=left amygdala. Highlighted are the main effects from the RH model and their FDR-corrected p-values (q-values). Sex did not contribute to any models.

A) B)

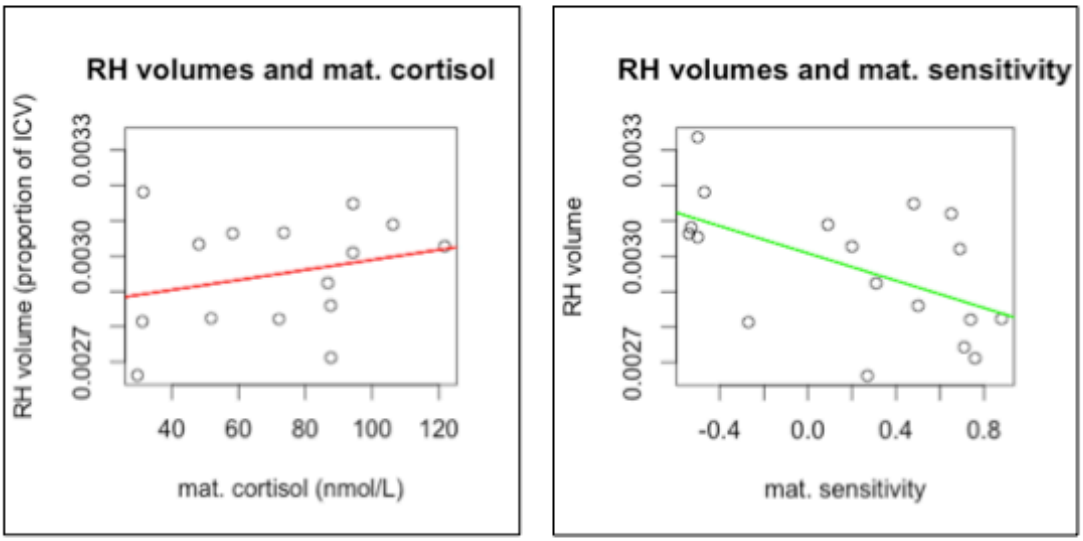

Figure 4 Mat. Cortisol and sensitivity on right hippocampal vol.: A) right hippocampal volumes increase with prenatal maternal cortisol B) right hippocampal volumes decrease with maternal sensitivity (MBQS). These charts were created with the raw data demonstrating the same effect without imputation (β= 3.13 x10^{-6} and -1.0 x10^{-4}, respectively). Formula : RH volumes= $\beta_0 + \beta_{(AUC)} + \beta_{(MBQS)} + \beta_{(sex)} + \beta_{(SES)}$. ($R^2$= 0.77; p<0.01)

DISCUSSION

This study was conducted to increase the understanding of how mothers communicate their prenatal and postnatal environments to program brain regions integral to emotion regulation in their offspring. There has been a paucity in the human literature regarding the influence of the mothers HPA axis during pregnancy on the development of neural regions integral to HPA regulation. Parenting style and sensitivity have been repeatedly shown to affect similar regions and strongly predict future psychopathology, however the interaction of these pre and postnatal variables has not been previously investigated and is therefore a novel question being assessed by this study.

Findings from structural magnetic resonance imaging conducted on 7-8 year old children in this study found that only the right hippocampus was sensitive to the main effects of prenatal maternal cortisol levels and to a lesser extent postnatal maternal sensitivity. Maternal HPA axis function during late gestation was measured using a salivary proxy of her circulating unbound cortisol. Across the sample, salivary levels varied by as much as 94 nmol and each additional nmol of saliva predicted a 4.6 mm^3 increase in right hippocampal volume when children were approximately 7 years old. This effect did not differ by sex, however there was an overall trend for larger hippocampal volumes and amygdala volumes in boys. This amygdala finding is consistent with previous work showing larger amygdala but inconsistent in regard to the hippocampus in 7-11 year old boys (Caviness, Kennedy, Richelme, Rademacher, & Filipek, 1996). A more recent study, however, found greater hippocampal volumes in 9-

11 year old boys and noted girls tend to reach mature levels 1.5 years earlier, indicating that our results may in part be explained by this developmental difference (Uematsu et al., 2012). The clinical significance of this increase is difficult to determine as the majority of psychopathology research reports reduced hippocampal volumes, particularly in affective disorders (Videbech & Ravnkilde, 2004). Volume reduction makes intuitive sense in the context of affective dysfunction considering that smaller volumes are likely accompanied by GC receptor decreases (Bingham et al., 2013) leading to attenuated regulation of the HPA axis. To this end, such findings also support the cortisol mediated programming hypothesis whereby maternal stress caused by illnesses such as anxiety predispose her offspring to the same mental health outcomes. However, the inconsistencies in the few studies that have specifically investigated maternal cortisol on offspring brain volume in humans suggest that there are particular developmental windows during which cortisol may have such effects. In the present study we used late-gestation cortisol levels (24-36 weeks). This timing fits well with the expected rise in cortisol throughout gestation, the normal reduction in 11β-HSD2 function and the protracted developmental time-course of both the hippocampus and amygdala. A similar study in 2012, and the first to directly assess prenatal cortisol on volume changes found no effect on hippocampal volumes across three gestation points but did find larger right amygdala volumes in girls due to *early* gestation exposure (Buss et al., 2012). This may in part explain the negative findings regarding amygdala volumes in the present sample. Prenatal cortisol levels were not collected at this early gestational time-point as many mothers had yet to be recruited. This represents a potential shortfall in our longitudinal

cohort, limiting how extensively we can explore the impact of prenatal cortisol. However, our negative findings from 24-36 weeks on variance in amygdala size in childhood (see table 3) do mirror those of Buss et al examining a similar preadolescent age group. Keeping in mind the pool of literature regarding these particular endocrinological effects in humans is tremendously small, it is in agreement thus far that the amygdala's prenatal developmentally sensitive window is not during late gestation, as predicted in this study. This is somewhat surprising considering that relatively less cortisol is available in the fetal space during early gestation (Wyrwoll et al., 2011), indicating that some protective factors in late gestation or moderating effects in the postnatal period could be at play. Considering the breadth of knowledge accumulated on early childhood experience and neural structure and disease development it seems likely that such moderating factors exist in the early post-natal years. Indeed, results from the present study indicate that hippocampal volume changes in childhood may also be predicted by maternal sensitivity. We found that maternal sensitivity and prenatal cortisol had opposing influences on volume, however this was only at a trend level and the effect may benefit from increased statistical power through greater sample sizes. Despite the opposing influence and previous findings that maternal care can moderate the hippocampal effects of prenatal adversity (Buss et al., 2007) the present study failed to find a significant interaction between cortisol and MBQS scores. Interestingly, both cortisol and MBQS influenced right hippocampal volumes in the opposite directions than expected. We had anticipated greater MBQS would predict greater hippocampal volumes and reduced amygdala volumes in childhood given previous results from early life

environment studies (Luby et al., 2012; M. A. Mehta et al., 2009; Pechtel et al., 2014). The literature on amygdala sensitivities to early life stress or adverse parenting is more equivocal than the hippocampus, however results are generally not negative in regard to the amygdala, which further highlights the peculiar MBQS-amygdala negative findings. Speculatively, the approximate 7-year period between MBQS assessment and imaging data collection presents a large window whereby a multitude of factors beyond the reasonable scope of statistical control in an observational study could have had a significant impact on our sample. Some recent studies have noted reduced hippocampal volumes (Hanson et al., 2014) while others have reported no effects following early life stress (M. A. Mehta et al., 2009)or increased volumes following quality parenting (Luby et al., 2012), thus it is difficult to explain the MBQS-hippocampus effects which only approach significance in our sample.

From an evolutionary perspective increased right hippocampus size following elevated cortisol levels prenatally may reflect the same effect demonstrated in Luby 2012. If a pregnant women communicates an adverse environment to her fetus in-order to prepare her child, than the child's fitness in such an environment would be improved with better HPA-axis regulation and enhanced memory and cognitive flexibilities, both linked to larger hippocampi and healthier mental trajectories (Gilbertson et al., 2002). A recent review noted the importance of glucocorticoids in emotional memory consolidation tied to the hippocampus. It was noted that increases in GRs as well as other pro-growth molecular changes respond as part of an adaptation mechanism following stress to ensure the organism is adequately prepared the next time such an event occurs. The authors

noted, however, that this effect is reversed following chronic or extreme glucocorticoid exposure (Finsterwald & Alberini, 2014). Additionally, moderate elevations in late gestation cortisol have been linked to improved cognitive abilities in children - a result likely involving elevated hippocampal volumes (Burgess et al., 2002; Davis & Sandman, 2010). In the present study, we looked at cortisol levels at one prenatal time-point and on a spectrum, not stratifying cortisol exposure levels. It is possible, therefore, that we encapsulated a non-chronic, relatively moderate exposure level and our hippocampal findings reflect the biological effect previously described. Since reduced hippocampal volumes are mainly associated with disease states (McKinnon, Yucel, Nazarov, & MacQueen, 2009; Palazidou, 2012) it is possible that our findings in 24 typically developing children reflect a healthy adaptation to adverse environments. It is also possible, however, that genetic predisposition, additional factors during adolescent development, or an interaction of both could shift this trajectory to one of adverse mental health outcomes. This possibility certainly presents a case for the continued following of such cohorts and the future incorporation of genetic factors into these analyses.

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PART TWO:

A cross-sectional look at the developmental state of cognitive neural networks and how they interact with limbic networks to resolve a mixed cognitive and emotional task in 7-8 year old children

ABSTRACT

Introduction: Emotion regulation (ER) is an integral component to mental health. ER is thought to incorporate limbic as well prefrontal regions in several cognitive top-down circuits to utilize higher-order executive functions to adequately monitor and inhibit emotion when necessary. However, only recently has research targeted the developmental trajectories of these circuits from childhood. **Methods:** 29 healthy children aged 7-8 years (mean 7.34 ± 0.48) underwent functional magnetic resonance imaging (fMRI) with an implicit emotion go/nogo cognitive task to assess the developmental state and interaction between cognitive and emotional circuitry using functional connectivity (FC) in this age group. **Results:** Central executive networks (CEN) and salience networks (SN) showed more diffuse FC than mature networks, with greater inter-network connectivity. During exposure to fearful stimuli, there was greater connectivity within CEN and SN during go trials. Nogo trials were associated with more limbic-cognitive network interaction during concurrent exposure to fearful stimuli than neutral stimuli, Connectivity with the dACC was found to be common between limbic and CEN seeded networks. **Discussion:** Results indicate that cognitive networks are present but generally less mature than previous results from adult populations. Particularly, diffuse connectivity between the insula and PCC was negatively correlated indicating a developing switch between resting and salience networks. Additionally, greater connectivity for response inhibition tasks (nogo) during fearful stimuli exposure in the dACC, amygdala, anterior prefrontal, and DLPFC, suggests a maturing emotion regulation network, capable of managing cognitive tasks during emotional stimuli presentation.

INTRODUCTION

FUNCTIONAL MAGNETIC RESONANCE IMAGING (FMRI)

Functional magnetic resonance imaging (fMRI) is a unique tool in that it provides high temporal acuity of neural physiology while subjecting the participant to far fewer discomforts associated with other imaging modalities. As such, there has been growing widespread use since it's inception in the early 1990's, particularly within medical and psychological research disciplines. fMRI has been employed in psychiatric research with great success as it has provided novel insights regarding the association between neurophysiology and behaviour.

fMRI and general MRI take advantage of the ubiquitous presence of hydrogen throughout the body in-order to acquire detailed images of organs. Hydrogen atoms have a resonant resting frequency of oscillation, and when a magnetic field is applied the a proportion of H+ atoms align in a common direction. During MRI, a pulse of radiofrequency energy is applied which is absorbed by the atoms and released during a relaxation period as they transition back towards their resting equilibrium. By varying the time between successive pulses (repetition time, TR) it is possible to vary a signal detected by the receiver. At short TRs hydrogen atoms predominantly relax at a characteristic time called spin-lattice relaxation (T1). At longer TRs the relaxation time is called spin-spin (T2). Gray and white matter have significantly different T1 times and thus a high contrast ratio can be achieved which is ideal for structural images. The T1 relaxation times are almost the same for deoxygenated vs. oxygenated blood but are very

different for T2. T2-weighted images are therefore used for detecting changes in blood flow.

Blood oxygen level dependent (BOLD) signal relies on the differences between magnetic properties of deoxygenated and oxygenated hemoglobin. An intrinsic property of neurons is that they do not have stored energy deposits, and thus are entirely reliant on vascular perfusion for energy needs. When neuronal activity increases so does the metabolic requirement of oxygen. As such, more oxygenated blood is delivered than required metabolically (vascular overshooot) during a process called the hemodynamic response (HDR). Following the HDR there is an increase in deoxygenated hemoglobin in the venous system leaving activated regions. A high amount of deoxygenated hemoglobin, which is paramagnetic, produces an attenuation of signal from the hydrogen atoms by altering their magnetic fields. Through the hemodynamic response, more oxygenated blood than is required is delivered, thus creating more deoxygenated blood, and the change in overall magnetic properties of a region on a T2-weighted image can be detected.

An fMRI scan consists of at least 2 types of image acquisitions: T1-weighted structural images, and T2 or T2*-weighted functional images. Structural images are obtained initially; first a rapid image is taken with very limited acuity in-order to obtain orientations of where the participant's head is in the scanner space. Subsequently, a high resolution T1 image with clear boundaries of white and gray matter is taken. T2*-weighted images of BOLD signal across the brain are taken and then projected onto the

structural images during post-processing. The timing of BOLD signal in particular regions of interest is associated to the presentation of stimuli to the participant and a temporal association is deduced.

Perhaps the most defining human characteristic is our capacity for rationale thought and foresight. Mounting evidence suggests that the same cognitive capacities that are involved in planning and reasoning are also involved in our ability to regulate moment-to-moment reactions to stimuli in the environment. Some stimuli may elicit programmed or learned emotional responses and the cognitive capacity that facilitates appropriate regulation of these stimuli is termed cognitive control (Ochsner & Gross, 2005).

THE ORIGINS OF EMOTION

Reactions to emotionally salient images are widely accepted to originate from subcortical structures, particularly limbic regions such as the amygdala (LeDoux, 2012). Such visceral reactions can be the starting points of our behaviour, and the structures from which they originate act as gatekeepers to the selection of stimuli that will enter conscious thought and guide behaviour and actions. The process from stimulus to

behaviour likely begins by assigning stimuli some relevant association: often to food, mate selection, or fear avoidance. The latter category is of particular interest to emotional circuitry and consequently to the development of affective disorders (Puliafico & Kendall, 2006). The amygdala and hippocampus play crucial roles in assigning associations to stimuli and are not surprisingly strongly tied to affective disorder pathophysiology (McKinnon et al., 2009; Siegle et al., 2007).

Acting through the hippocampus, fearful reactions may be conditioned via associations with objects or discrete episodic memories of aversive stimuli in the environment (Burgess et al., 2002), or through unconditioned programmed evolutionary aversions (Dellarosa Cummins & Cummins, 1999). The amygdala appears to then play a role in orchestrating the physiological reaction in conjunction, or independently of the hippocampus (Labar, Spencer, & Phelps, 1995; LeDoux, 2014). The amygdala is well suited to this role as it can react on the order of approximately 30 milliseconds, even to human emotion, which likely requires the prior recruitment of conditioned associations (Hall et al., 2010; Phelps & LeDoux, 2005). Additionally, such reactions are greater among children with anxiety disorders (Thomas et al., 2001) and adults with depression (Drevets, 1998; Siegle et al., 2007). Perhaps more inline with more common fear acquisition in humans is the development of emotional reactions by witnessing others experiencing pain or suffering. Recent work found increased amygdala, as well as anterior cingulate (ACC) and insula activity when viewing, not experiencing aversive stimuli, implicating the same systems in direct and indirect emotion and fear acquisition (Olsson, Nearing, & Phelps, 2007). Through many afferent connections to the

hypothalamus, a consequence of an overactive amygdala is abnormal HPA function (Rodrigues et al., 2009). Abnormal HPA axis (Carroll, 1982), specifically cortisol secretion are consistently linked to psychopathology(Lopez-Duran et al., 2009).

Cumulatively, this indicates the potential of these emotional systems to underlie the progression of affective disorders beginning in childhood. With this in mind it becomes apparent that a regulatory mechanism, providing context to the stimuli must be present in tandem with rapid emotion response systems in-order to maintain physiological homeostasis and a healthy demeanor.

REGULATION OF EMOTION

Recent work summarizing emotion regulation circuitry highlights two key strategies by which humans utilize cognitive skills to manage sub-cortical reactions to stimuli: attentional control, and cognitive manipulation (K. Ochsner & Gross, 2005).

Attentional control is taken to mean the conscious decision to attend to the stimuli at hand or to attempt to ignore it. When a stimuli happens to be one of learned or programmed negative valence, focusing cognitive faculties anchored in the prefrontal cortex have been shown to diminish the initial subcortical reaction from the amygdala (Hariri et al., 2003). This effect has been achieved in previous work by asking participants to rate the emotional experience they had when viewing negative stimuli. Compared to passive viewing, active cognitive engagement to the stimuli resulted in

reduced activity in the right amygdala and increases in the dorsal medial prefrontal cortex (DMPFC) (Taylor, Phan, Decker, & Liberzon, 2003). In addition, consciously repressing the emotion can increase activity in the dorsal lateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) (Carew, Milne, Tatham, MacQueen, & Hall, 2013) and exert parallel physiological changes, such as facial muscle tonicity associated with affective responses (Jackson, Malmstadt, Larson, & Davidson, 2000).

Beyond simply repressing or qualifying emotional impulses, cognitive manipulation involves higher-level analysis of a stimulus or even premeditated response to expectation of negative stimuli. This type of cognitive dynamic often constitutes mentally altering the connotation of a stimulus. In an approach known as reappraisal, subjects are asked to take an optimistic view of a given affectively charged stimulus. Performing such a task recruits prefrontal regions including dorsal ACC, DMPFC, DLPFC, and the orbito-frontal cortex (OFC) and reduces activation in limbic regions (Ochsner, Bunge, Gross, & Gabrieli, 2002; Phan et al., 2005). Moreover, when simply passively viewing affective images activation was mainly confined to the amygdala, however, when neutral images were displayed and participants viewed them in a negative way, amygdala activity was maintained and ACC, dorsal and lateral PFC was engaged (Ochsner & Gross, 2003). Given that increased amygdala activity is tied to affective disorders (Siegle et al., 2007; Stein, Simmons, Feinstein, & Paulus, 2007) it is not surprising that reductions in PFC recruitment, have also been associated with generalized anxiety disorder (Greenberg, Carlson, Cha, Hajcak, & Mujica-Parodi, 2013; Shin & Liberzon, 2010).

In addition to this voluntary regulation via predominately dorsal prefrontal cortex regions, there is evidence that emotion regulation may also be achieved automatically through a ventral route including: the medial prefrontal (mPFC), orbitofrontal (OFC), and subgenual anterior cingulate (Gottfried & Dolan, 2004; Phillips, Ladouceur, & Drevets, 2008). More so than other prefrontal regions, the amygdala has direct connections with the OFC, and such direct connections are thought to underlie an automatic emotional reaction system through rapid salience perception (Phillips et al., 2008; Rolls, 2013).

The Dorsal stream is thought to contribute more cognitively than the ventral stream, which has been found to be more attuned to emotional salience (Yamasaki, LaBar, & McCarthy, 2002). Considering that many of the same regions have been implicated in both dorsal and ventral circuits it is likely that both pathways work in tandem to effectively regulate emotion (Banks, Eddy, Angstadt, Nathan, & Phan, 2007). Moreover, there are findings that the dACC may facilitate a cross-talk between these networks, facilitating integration creating a more consolidated reciprocal network (Milad et al., 2007; Yamasaki et al., 2002).

Cumulatively, these findings suggest several bidirectional (top-down, bottom-up) ways in which emotion is processed. From these results it is clear that when cognitive resources are focused on the stimulus at hand, either consciously by attempting to suppress or mentally manipulating the perception, or by automatic reactions, effective regulation of subcortical emotional reactions are possible. However, what isn't clear is whether the presentation of implicit affect simultaneously with an independent cognitive task will facilitate a recruitment of necessary cognitive networks or intrusion by bottom-

up reactivity will occur. Emerging evidence suggests that such cognitive flexibilities are possible (Lamm, White, McDermott, & Fox, 2012; Pessoa, McKenna, Gutierrez, & Ungerleider, 2002). However, it may be deficient or delayed in children with anxiety as such children demonstrate greater attentional bias towards fearful stimuli (Eschenbeck, Kohlmann, Heim-Dreger, Koller, & Leser, 2004).

NEURAL COMMUNICATION AND FUNCTIONAL CONNECTIVITY

In-order to facilitate cognitive control, different anatomical regions must cooperate and communicate; when doing so, such regions are considered to be functionally connected if their time-course of neural activity are correlated (Friston, 1994). The BOLD signal, reflecting the hemodynamic response of vasculature within a region to metabolic demands is a proxy measure of the neural activity of the region (Fox & Raichle, 1986). Though the actual neuronal activity has a higher signal-to-noise ratio than the BOLD effect, owing to greater variability in hemodynamic responses, BOLD is considered to be significantly correlated with the activation patterns of a region (Logothetis, 2002). The electrical activity of neurons or groups of neurons is known to oscillate at varying frequencies and levels of excitability and can be specifically recorded using intracranial probes in animal models (Csicsvari, Jamieson, Wise, & Buzsáki, 2003) and less focally precise, with EEG in humans (Teipel et al., 2009). It has been proposed that when separate groups of neurons begin to oscillate in sync they have the opportunity to communicate, but given the cycling nature of these frequencies there may only be a

small window of opportunity (Fries, 2005). Additionally, this syncing of excitability is hypothesized to be of practical significance when the regions in question are also physically linked by white matter tracts, reflecting direct neurotransmission between regions (Koch, Norris, & Hund-Georgiadis, 2002). It is also likely that during the fine-tuning of neural networks that occurs with age (Hoff, Van den Heuvel, Benders, Kersbergen, & De Vries, 2013), regions that are frequently excited for the same stimuli recruit more myelinated tracts between them, increasing the strength and likelihood of coherence and transmission (Lenroot & Giedd, 2006). This may be achieved by the selective recruitment of appropriate brain regions by certain stimuli to work in tandem to elicit a greater overall reaction in the target region (Dmochowski, Datta, Bikson, Su, & Parra, 2011).

Recent work demonstrated acquired cellular firing frequency between the mPFC and amygdala during learning and fear extinction in rats (Likhtik, Stujenske, Topiwala, Harris, & Gordon, 2014). Adding direct evidence, stimulation of the mPFC by microelectrode resulted in attenuated amygdala projection response to conditioned fear, highlighting the importance of the mPFC-amygdala system in the expression and thus regulation of acquired fear (Quirk, Likhtik, Pelletier, & Paré, 2003). This corroborates human studies, providing cellular level support for findings of negative coupling between the mPFC and amygdala activity in association with diminished anxiety levels in kids (Dylan G Gee et al., 2013) and reductions in mPFC firing in adults with GAD (Greenberg et al., 2013).

With the recent use of diffusion tensor imaging (DTI) techniques, the underlying white matter connectivity relating functionally associated regions has begun to be elucidated. The majority of this work, however, has focused on correlating the functional brain at rest with physical connections (Damoiseaux & Greicius, 2009) and only recently begun investigating the same associations with task-based connectivity (Hermundstad et al., 2013). This is an important step considering task-based functional MRI is the tool by which we study the strategies used to regulated emotion and their underlying neural networks (Phan et al., 2005).

FUNCTIONAL NEURAL NETWORKS

Through neural coherence, functionally separate sets of neural networks (circuitry) can be determined. Cognitive neuroscience to date has shown many such networks exist that are oscillating between levels of excitability (Bressler & Menon, 2010). These include but are not limited to: the default mode network, Salience network, and central executive network. Research is emerging regarding how these networks regulate and evaluate the generation of subcortical impulses that eventually contribute to emotion.

DEFAULT MODE NETWORK

Networks made up by functionally connected regions are tasked with managing external tasks, which change from moment to moment; the resting brain is also capable of

such dynamic fluctuations (Fox et al., 2005). There is evidence of several different resting state networks which arise at different times throughout development. Visual and sensorimotor networks develop first, with the default-mode network (DMN) showing a more protracted timeline (Hoff et al., 2013). The DMN is primarily composed of the mPFC, posterior cingulate cortex (PCC) and the parietal lobes, particularly the angular gyrus (AG) (Supekar et al., 2010). The mPFC has been repeatedly implicated in cognitive processes, particular with regard to emotion regulation (Ochsner et al., 2002; Phan et al., 2005) and is clearly implicated in diseases states such as GAD (Greenberg et al., 2013). The PCC-prefrontal axis along with the parietal lobes has been shown to be involved in episodic memory retrieval and future planning (Buckner & Carroll, 2007). Together, the function of these DMN sub-components suggest that this particular resting state network may play a role in internal behavioural processes, as well as emotion regulation and generation.

The functional development of this network, like most networks, proceeds from strong associations only with relatively adjacent structures (e.g. PCC and angular gyrus) towards strengthened long-range connections like PCC-mPFC (Fair et al., 2009) and follow white matter maturation patterns (Supekar et al., 2010). Recent evidence suggests that maturation of these networks continues well into adolescence (Jolles, van Buchem, Crone, & Rombouts, 2011) and that immature networks show greater interconnection compared to their more mature and functionally independent counterparts (Stevens, Pearlson, & Calhoun, 2009). Moreover, during resting-state greater fronto-parietal network strength has been associated with greater working memory performance (Song et

al., 2008). Given that the DMN is downregulated when other non-resting network are engaged in response to the perception of salient internally or externally generated stimuli (Sridharan, Levitin, & Menon, 2008), it follows that immature functional connectivity with frontal lobes in the DMN and other cycling resting networks may extend to salience or central executive networks as well. Such a finding would have clear implications for the development of cognitive faculties and cognitive control of emotion in children (Bunge, Dudukovic, Thomason, Vaidya, & Gabrieli, 2002). However, to our knowledge no such finding has been reported.

SALIENCE NETWORK AND CENTRAL EXECUTIVE NETWORK

Though extrinsic stimuli are continuously being processed, the majority do not make it into conscious perception. It is at this time that resting state networks, including the DMN, are utilized to process subconscious or *intrinsic* thought processes (Fox et al., 2005). When a stimulus with a learned or inherent associated meaning is presented it becomes salient as aberrant stimuli in the environment are detected by the dACC and insula (Blasi et al., 2006). Subsequently, resting state networks are deactivated with concurrent activation of the salience network (SN) (Fox et al., 2005; Sridharan et al., 2008). The salience network is made up of hubs in the dorsal anterior cingulate cortex (dACC), the ventral lateral prefrontal cortex (VLPFC) and the anterior insula (AI). Emerging evidence points to the AI as being the switch that is responsible for triggering alternations between the DMN and SN (Naqvi, Rudrauf, Damasio, & Bechara, 2007; Sridharan et al., 2008) following salient stimuli presentation.

When a salient stimulus is perceived, cognitive faculties are recruited to mentally attend to it and formulate a plan of action, and as stated such attentional demands are orchestrated by the dACC and AI (Ham, Leff, de Boissezon, Joffe, & Sharp, 2013). On top of SN activation for stimuli detection, dorsal lateral prefrontal cortex (DLPFC) as well as posterior parietal cortex (PPC), regions known to be integral in working memory and response inhibition are activated (Curtis & D'Esposito, 2003; Quintana & Fuster, 1999) and may have been primed by similar neural activity at rest (Song et al., 2008). Together these regions are hubs in a functionally important network known as the central executive network (CEN) (Seeley et al., 2007). Indeed, working memory improvements are tied to greater activation within the DLPFC and posterior parietal regions (Olesen, Westerberg, & Klingberg, 2004) as well as greater structural white matter connectivity between said regions (Takeuchi et al., 2010).

A recent large meta-analysis of over 2000 individuals across the developmental spectrum found the underlying network subserving cognitive control is rooted in the dACC, DLPFC, and PPC (Niendam et al., 2012). This corroborates the importance of the SN and CEN and suggests a complimentary relationship among structures across the umbrella of cognitive control, which includes: working memory, selective attention and response inhibition. Following detection by dACC and insula in the SN and cognitive appraisal by prefrontal-parietal networks, an additional control mechanism of the CEN, termed response inhibition may be required if the stimuli is aversive or not conducive to a predetermined goal (Dempster, 1992). Response inhibition is seated in the DLPFC and PPC like other higher order functions such as working memory, and also has been shown

to include the ventrolateral prefrontal cortex (VLPFC) (Blasi et al., 2006). Studies have found that this system also shows particular developmental changes. Using a Go/No-Go paradigm to create pre-potent responses that then requires inhibition of such responses, younger participants performed poorer on this task and had greater event-related potentials (ERPs) in the orbitofrontal cortex (OFC) (Lamm, Zelazo, & Lewis, 2006).

Given findings that the OFC preferentially responds to emotional images or events as part of the emotionally salient ventral stream (Hartikainen, Ogawa, & Knight, 2012), a follow-up study from Lamm et al. using the same Go/No-Go format with affectively charged pictures, either of scary or neutral animals found greater DLPFC activation but no differences in OFC in children when required to inhibit responses in the presence of scary animals vs. neutral animals (Lamm et al., 2012). These seemingly contradictory findings may underscore the importance of both the dorsal and ventral streams to effectively perform cognitive functions with conflicting emotional stimuli, and children may require a greater contribution from dorsal executive networks. Indeed, this effect on prefrontal regions was shown to be affected by age, as children recruited less ventral prefrontal region activity (including OFC) as they developed (Lamm & Lewis, 2010). In children, differences in response inhibition predict ability in regulating emotion (Carlson & Wang, 2007). The authors suggest that as cognitive systems regulating emotion mature there is a reduced dependence on overall prefrontal faculties, particularly within the ventral prefrontal regions (Lamm & Lewis, 2010). However, others have shown steady OFC activation is integral to inhibiting responses from the amygdala in children (Ameis et al., 2014) and affectively valenced NoGo tasks in healthy adults in a similar OFC-

amygdala circuit (Goldstein et al., 2007). To this end, reduced VMPFC activity, lesions to the OFC, and reduced OFC activity and size have been shown to underlie emotional regulation deficiencies and impulsivity in conditions ranging from GAD to borderline personality disorder as well as behavioural deviant behaviour in children (Ameis et al., 2014; Berlin et al., 2005; Greenberg et al., 2013; Shamay-Tsoory, Aharon-Peretz, & Perry, 2009).

In summary, many regions throughout the cortex have been shown to play a role in cognitive control. These include the rACC, VLPFC, and AI for salient stimuli detection and the inclusion of DLPFC and PPC regions for executive functions. These regions along with other ventral prefrontal structures such as the OFC interact during emotion regulation. However, very little has been done to elucidate their developmental trajectories by studying this effect in children, and such findings have not been consistent. Of particular interest is how the allocation of cognitive resources is affected by multiple competing stimuli, and how this ability changes across development.

The goal of this study is to demonstrate using functional connectivity analysis, the developmental stage of cognitive control networks and how they interact with emotional stimuli in middle childhood. We predicted that during this developmental time-point there will be an immaturity in the CEN and SN networks characterized by functionally diffuse connections as well as stronger connections to anatomically adjacent rather than

distant regions. Additionally, we anticipate there to be greater functional connectivity between the amygdala and prefrontal regions during the cognitively demanding inhibition task when paired with a fearful-valenced image.

METHODOLOGY

FUNCTIONAL TASK

The present study consists of each participant performing three runs of an implicit affective go/no-go task. Generally, during a typical task the participant is asked to respond as quickly as possible to a certain stimuli and refrain from responding when presented with a specified different stimulus (eg. respond to "O" and withhold responses for "X"). When the go stimulus is presented far more frequently than the no-go stimulus this creates a pre-potent response towards responding, and thus requires cognitive control to withhold a response. This response inhibition effect, as well as stimulus interference suppression together make-up executive functions that have been previously assessed using a variety of methods (like go nogo; stop task) (Bunge et al., 2002; Goldstein et al., 2007). Additionally, the concept has been extended to an aspect of cognitive control called emotion regulation by utilizing fearful and neutral animal images as the go and no-go stimuli respectively in a ratio of 3:1. This extension of the typical task is undertaken

to probe neural regions thought to underlie how emotional stimuli are actively regulated by executive capacities (Lamm et al., 2012).

The present study used a mixed block-event design consisting of 6 blocks based on emotional valence of either neutral or fearful images. There were 3 blocks of neutral valenced animals (e.g. deer, pandas, ducks) and 3 blocks of fear valenced animals (e.g. sharks, large spiders, snarling wolves) with 3 rest blocks spaced throughout. Rest blocks, consisting only of a central fixation cross on a gray screen, were included to assess a baseline BOLD signal. Within each block there were 14 go/no-go trials each superimposed on an animal image that was either neutral or fearful, depending on the block. Block order was randomized between runs and trials were randomized within blocks to ensure each animal only appeared a maximum 3 times throughout an entire run. The go stimuli were Black squares and the no-go stimuli were black circles, which appeared superimposed on the animal image in a ratio of 2.5:1 (go:nogo). The animal image appeared first and following a very brief delay, the shape (circle or square) appeared. After the shape appears the participant has 1000ms to respond by pressing a button on a response box they are holding in the scanner. If they were correct in their choice to respond the circle turned gray. However, if the child responded to a circle, the circle then turned red to signal the error and a beep was heard through headphones. The delay before the shape appears, as well as the inter stimulus interval (ISI) was jittered, varying from 250ms-1750ms to prevent the participant from falling into a rhythm. This task is unique in that the affective stimuli are processed implicitly rather than explicitly perceived, as was the case in the studies by Goldstein (2007) and Lamm (2012). The

advantage of this design is that it provides a method of assessing how affectively charged stimuli not being explicitly processed interfere with executive functions.

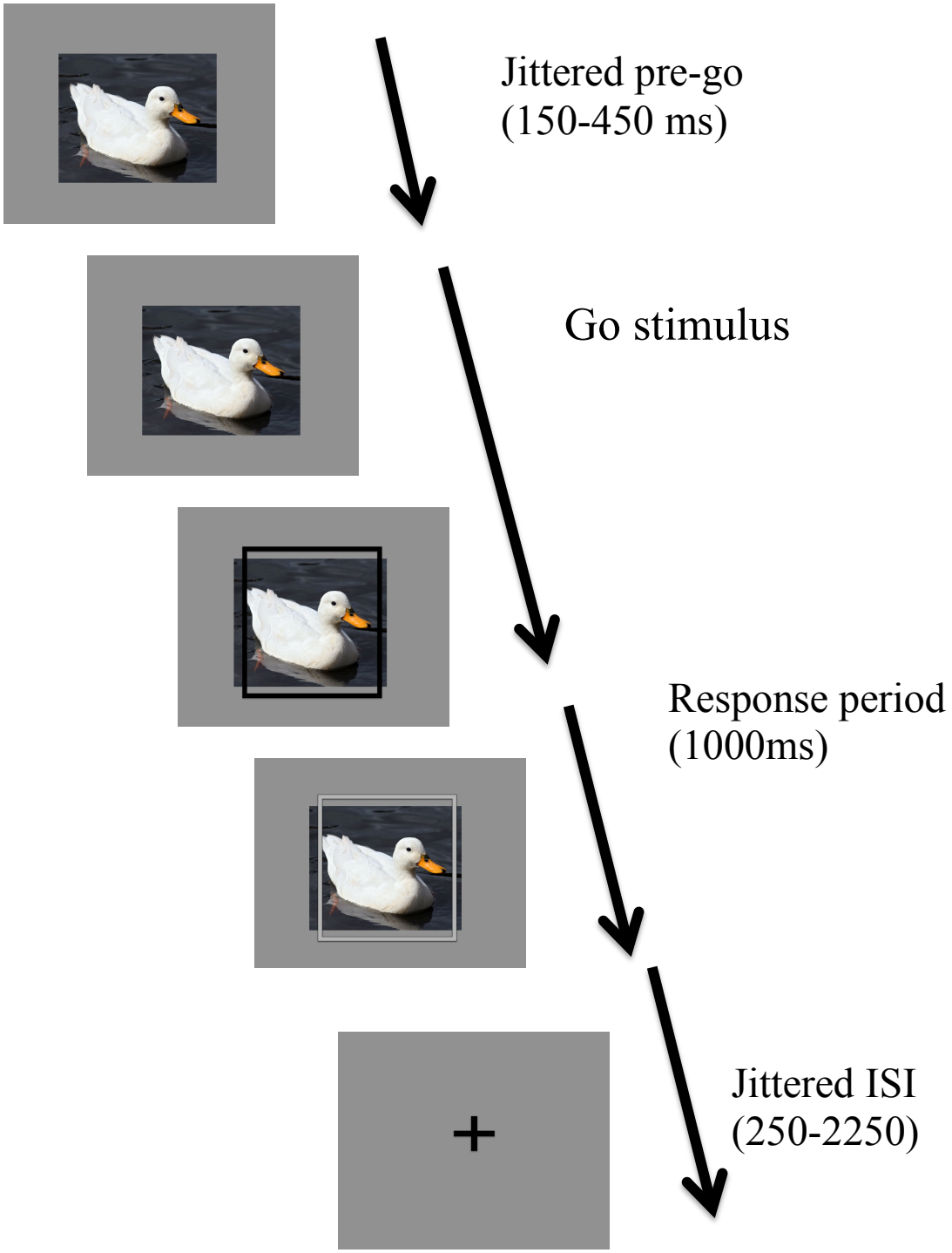
Immediately prior to entering the scanner, the participant was trained on the go/no-go task with the same conditions except the animals were replaced by solid shapes in order to prevent any expectation bias from forming prior to the experiment.

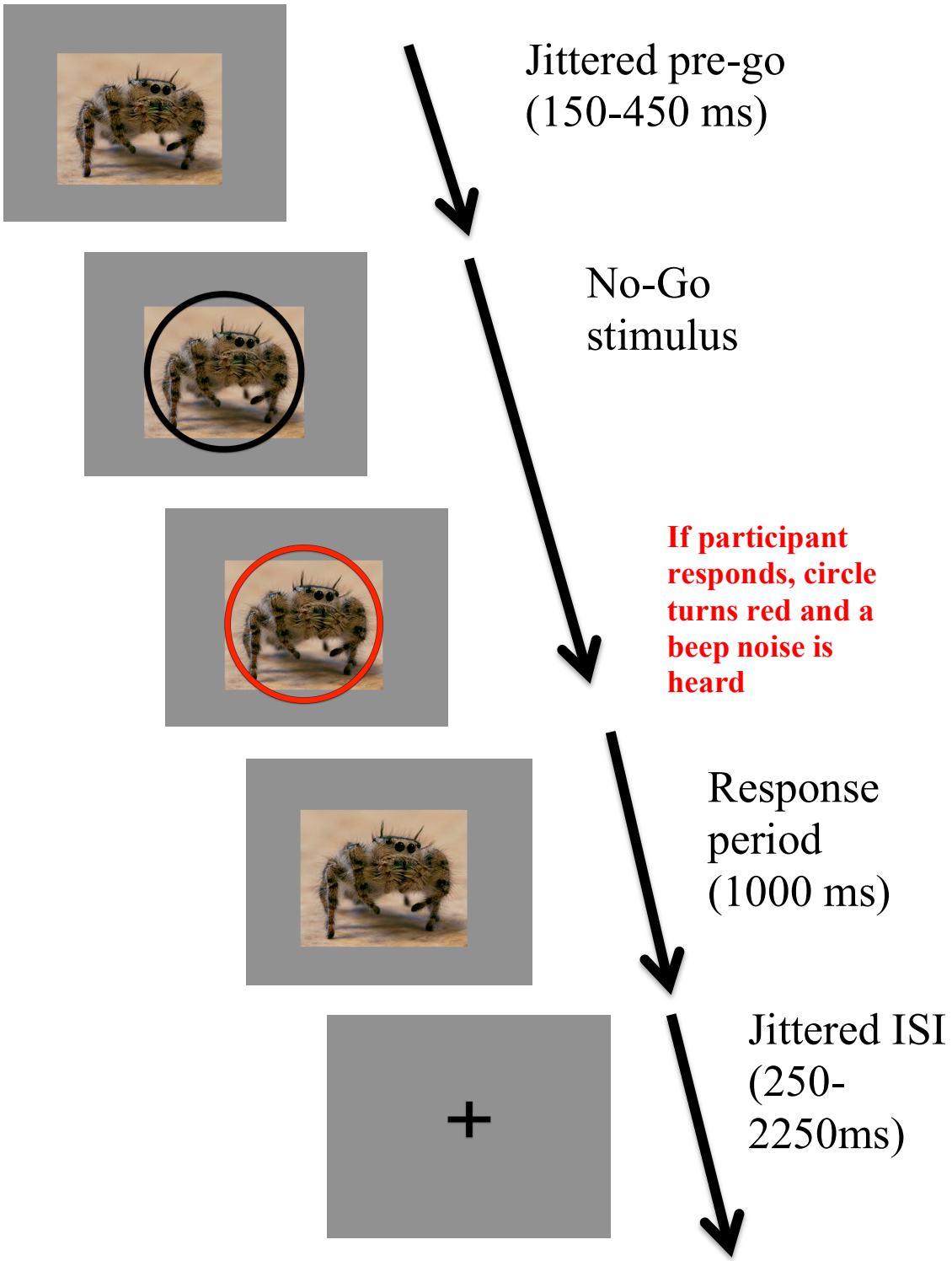
IMAGE PROCESSING AND FUNCTIONAL CONNECTIVITY

MRI images were pre-processed using SPM8 (*statistical parametric mapping, Wellcome Trust Centre for Neuroimaging, London England*). A voxel size of 2 mm^3 was set for all functional images. Functional T2*-weighted files are realigned, normalized into Talairach space, spatially smoothed using a gaussian filter set at full width half max (FWHM) of 6 mm to reduce the effect of high frequency noise; increasing the signal to noise ratio. T1-weighted images were segmented into white and gray matter, and normalized into talairach coordinates so that they occupy the same standard space and can thus be compared to one another more effectively. After this step there is still anatomic variation among cortical and sub-cortical structures, however, the spatially smoothed functional images are more readily generalized across small anatomic variations, permitting data from different subjects to be collapsed and an average BOLD signal time-course from each voxel obtained. The final preprocessing step was co-registering the functional series to the normalized structural image so region of interest (ROI) and functional connectivity analysis could take place.

Functional connectivity (FC) analysis was done using the Conn toolbox in SPM. FC analyses involved setting an aproiri defined seed region and correlating the temporal BOLD signal fluctuations (time-course) of that region with the time-course of other regions of interest. A band-pass filter was applied (low-pass=0.09 Hz, high-pass=0.8 Hz). The band-pass filter removes physiological noise caused by respiratory and cardiovascular oscillations and scanner/coil induced fluctuations. In addition to covarying for each participant's intra-scan motion, the band-pass filter increases the signal-to-noise ratio. In the present study, subjects' functional series were divided into 4 groups based on affective valence block and event: *Go-fearful, NoGo-fearful, Go-neutral, NoGo-neutral.* From this division it was possible to contrast the FC of emotional processing, cognitive function including response inhibition, and how they interact. Voxels of significant activity compared between groups were corrected for multiple comparisons (experiment-wise error) using false-discovery rate (FDR) techniques.

Figure 1 functional paradigm (following pages): mixed block-event related implicit affective Go/No-Go paradigm. Participants are shown 4 blocks of neutral and 4 blocks of affectively charged animal images. In a ratio of 2.5:1, either go (square) or no-go (circle) is superimposed for 100 ms. If the participant responds (goes) for a circle it then turns red instead of gray, as for a squre, and they hear a "beep" indicating their error


Jittered pre-go
(150-450 ms)
Go stimulus
Response period
(1000ms)
Jittered ISI
(250-2250)


Jittered pre-go
(150-450 ms)
No-Go
stimulus
If participant responds, circle turns red and a beep noise is heard
Response
period
(1000 ms)
Jittered ISI
(250-
2250ms)
+

RESULTS

PARTICIPANTS

Twenty-four children aged 7 years were recruited from the MAVAN cohort (see part 1 for earlier description of cohort) In addition a further 5 typically developing 8 year-old children were added from the McMaster Psychology department cohort. The full group characteristics for the 29 participants are summarized in Table 1. Two subjects were excluded from final analysis due to excessive motion and spatial normalization error. Therefore, the following results reflect functional MRI findings from 27 subjects.

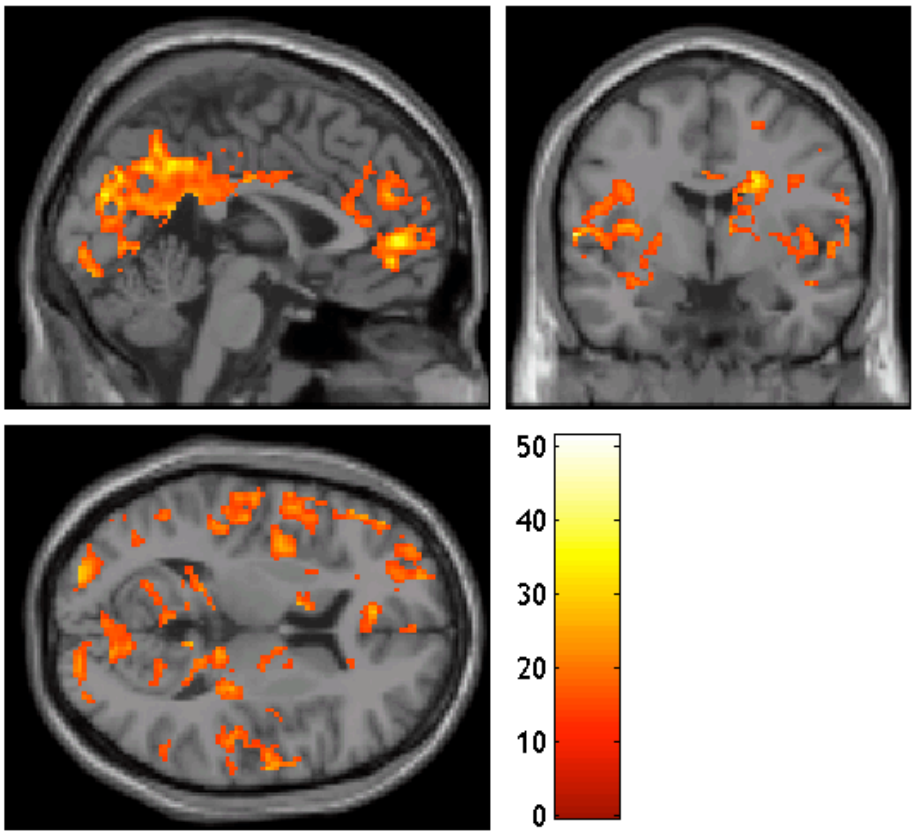
Characteristic	Totals
	n=29
age (years), mean (SD)	7.34 (0.48)
sex ratio, M:F	17:12
ethnicity	White/Caucasian (n=27)
	Asian/South Asian (n=2)

Table 1 characteristics: Summary of 29 participating children. MAVAN participant groups were collapsed as 5 additional children from a different cohort were added.

IMAGING RESULTS

FEAR NETWORKS

Figure 2 displays the results for the fear and neutral block design. Results demonstrate that when fearful images were contrasted against neutral images there was greater activation for fearful images in the anterior cingulate (ACC) ($p<0.001$) and the posterior cingulate (PCC) as well as regions within the right parietal, left superior temporal gyrus, and bilateral cuneus ($p<0.001$). During functional connectivity analysis (FC), the amygdala was seeded and was functionally connected to the subgenual ACC (BA 25) when viewing fearful images in contrast to neutral images (uncorrected $p=0.002$). Together these regions are part of a maturing paralimbic network.



Region	Brodmann area (BA)	region side	(X,Y,Z)	p-value (uncorrected)
superior temporal gyrus	22	L	(-54, 6, 6)	<0.001
posterior cingulate (PCC)	23	L	(-4, -30, 18)	<0.001
cuneus	19	R	(6, -82, 28)	<0.001
cuneus	19	L	(-6,-82,34)	<0.001
postcentral gyrus	3	R	(50, -14, 44)	<0.001
middle occipital gyrus	19	R	(50, -76, -4)	<0.001
anterior cingulate	32	R	(10, 38, 22)	0.001

Figure 2 fear vs. neutral: greater activation for fearful compared to neutral images in the ACC and PCC. Voxel coordinates (x,y,z) reflect the peak voxel in a cluster. Results did not survive correction by false discovery rate (FDR) at alpha 0.05. Images are in neurologic space, left = left hemisphere.

SALIENCE NETWORK (SN)

The salience network is comprised of the insula, dACC, and inferior aspects of the prefrontal cortex. Figure 3A depicts functional connectivity findings for the SN when the insula (BA 13) is seeded bilaterally. Figure 3B depicts the SN seeded in the dACC (BA32). All contrasts were initially collapsed to assess the overall developmental state of the network. Results demonstrate that a relatively mature salience network is present in children in middle childhood (n=29; mean age 7.34 ± 0.48). Network nodes are generally all present with a noted variation - the DLPFC is bilaterally connected to both the insula and the dACC. However, this connection is rather weak compared to the stronger dACC-insula connectivity (right: $\beta=7.29$, $q<0.001$; left: $\beta=6.44$, $q<0.001$). Inferior aspects of the prefrontal cortex were not functionally connected to either the insula or the dACC.

The functional time-course of both insula are also negatively correlated to that of the left posterior parietal cortex (PPC) (right: $\beta=-3.72$, $q=0.0018$; left: $\beta=-2.66$, $q=0.022$) and the posterior cingulate cortex PCC ($\beta=-4.11$, $q<0.001$). The PPC is a hub in the central executive network (see figure 4A) and the PCC is integral to DMN function during non-task external related neural activity (resting-state) (Supekar et al., 2010).

CENTRAL EXECUTIVE NETWORK (CEN)

The CEN is comprised of the DLPFC and the posterior parietal cortex. Figure 4A represents the CEN seeded bilaterally at the inferior PPC and figure 4B depicts the network seeded at the DLPFC. Results suggest that there may be a developmental difference by brain hemisphere. The left inferior PPC and DLPFC appear functionally connected to one another relatively equally (β=3.14 & 3.14, q=0.01 & 0.03, respectively). Both the left and right inferior PPC are well connected (β>12.0, q<0.001) as are the left and right DLPFC (β>15.0, q<0.001). These cross-hemispheric connections to bilateral structures appear to be the strongest of this CEN network, however the intra-hemispheric connections between the PPC and DLPFC is only present on the left side and is also weaker than the inter-hemispheric connections.

As in the SN, there appears to be residual connectivity with other networks. When seeded at the inferior PPC, there is negative connectivity with the ACC (β=-3.17, q=0.01) and insula (Left: β=-2.66, q=0.03 & Right: β=-3.73, q=0.003) as well as stronger connectivity with the more anatomically adjacent PCC (β=9.19, q>0.000). In-fact seeding at the PPC shows much stronger connectivity with closer structures such as the precuneus and PCC as compared to the anatomically distant frontal structures.

Overall, these cognitive networks appear to be diffuse with connections to anatomical regions tied to other functional networks.

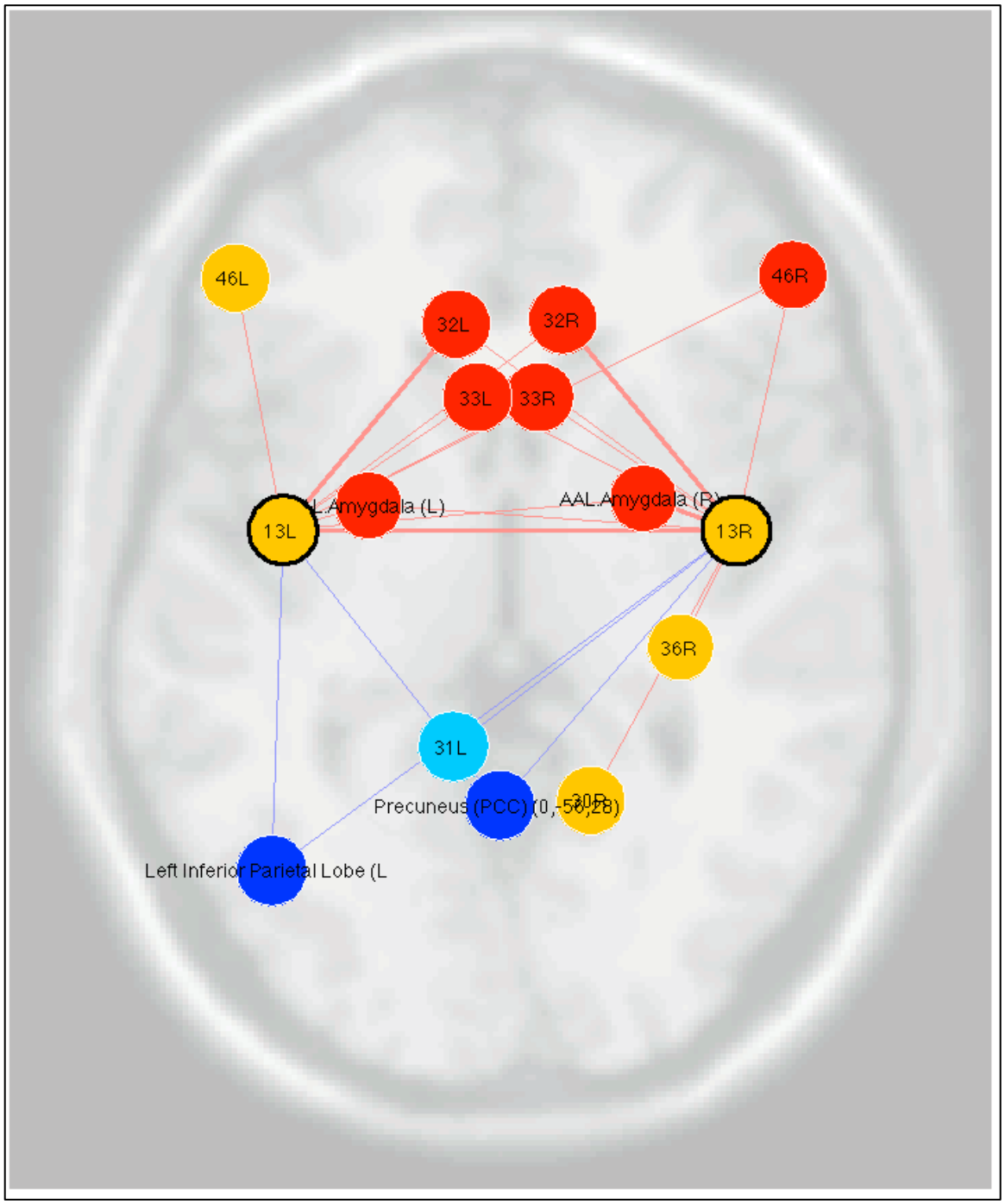


Figure 3A SN: Salience network seeded bilaterally in the insula. Heavier connections reflect greater beta-values (adjusted correlation coefficients) between regions. Red = positive correlation; blue = negative correlation. Yellow = positive correlation to only one side. Images are in neurologic space. Numbers correspond to brodmann areas (BA).

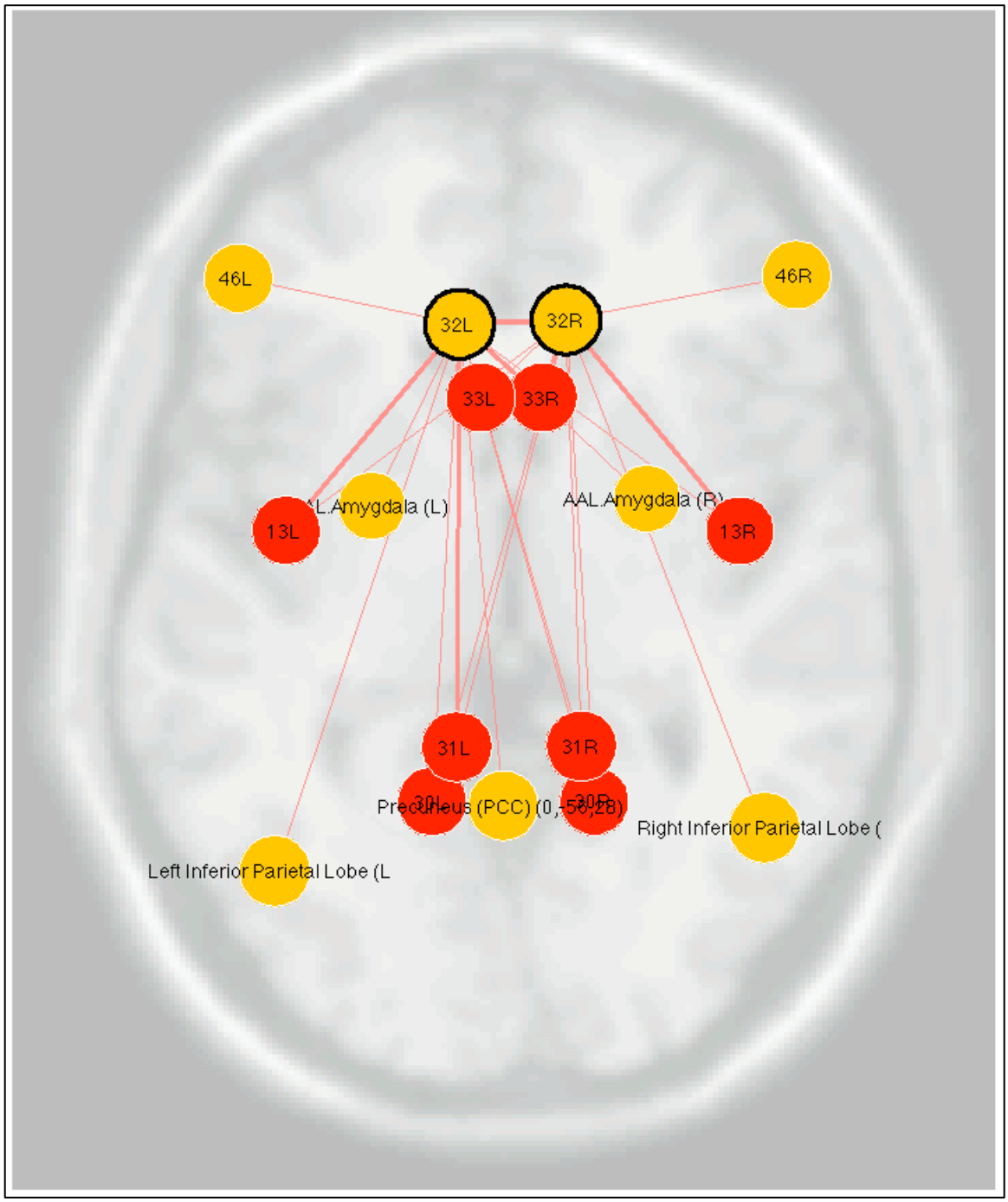


Figure 3B SN: Salience network seeded bilaterally in the dorsal anterior cingulate cortex (dACC)

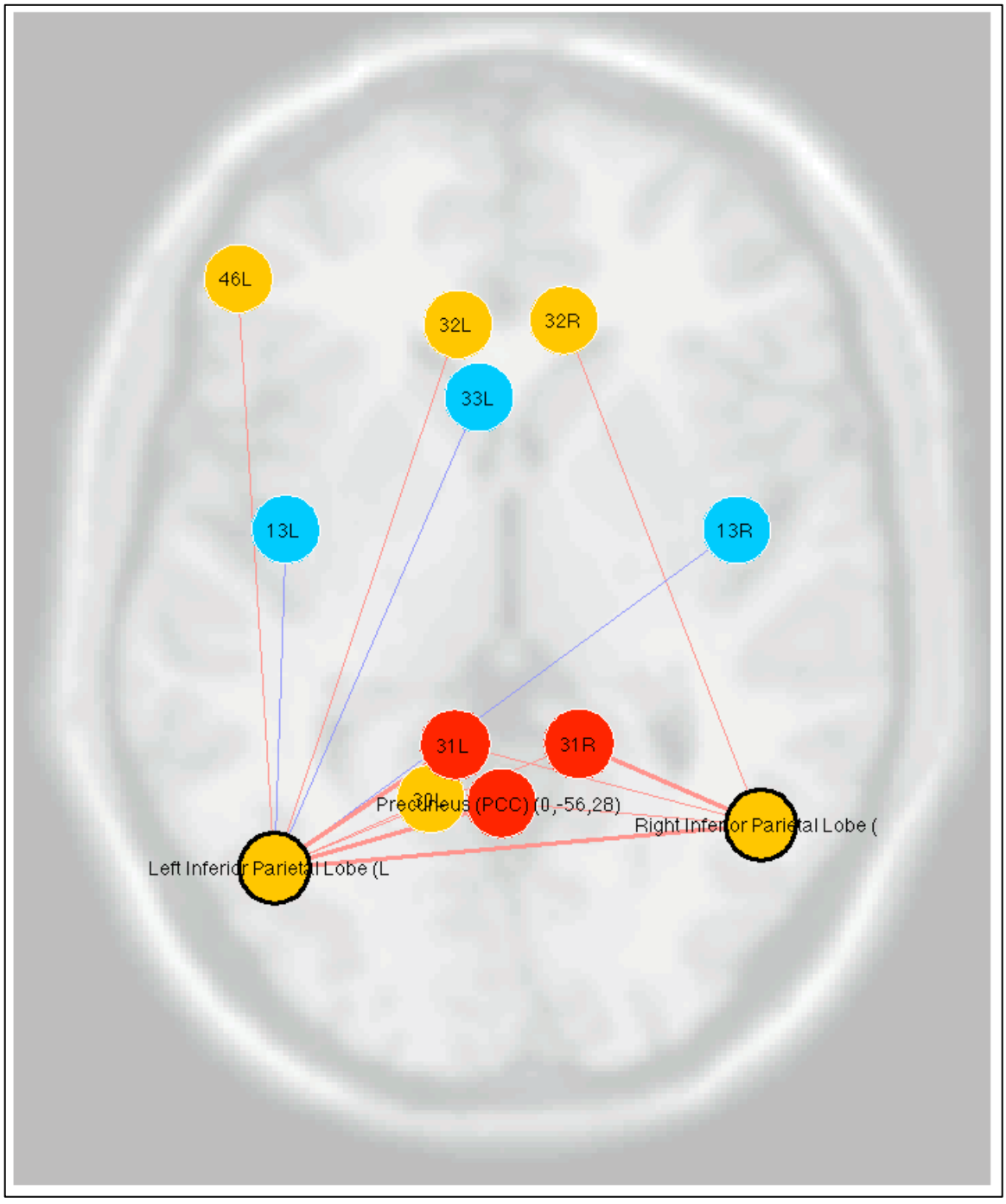


Figure 4A CEN: Central executive network seeded bilaterally in the inferior parietal lobes.

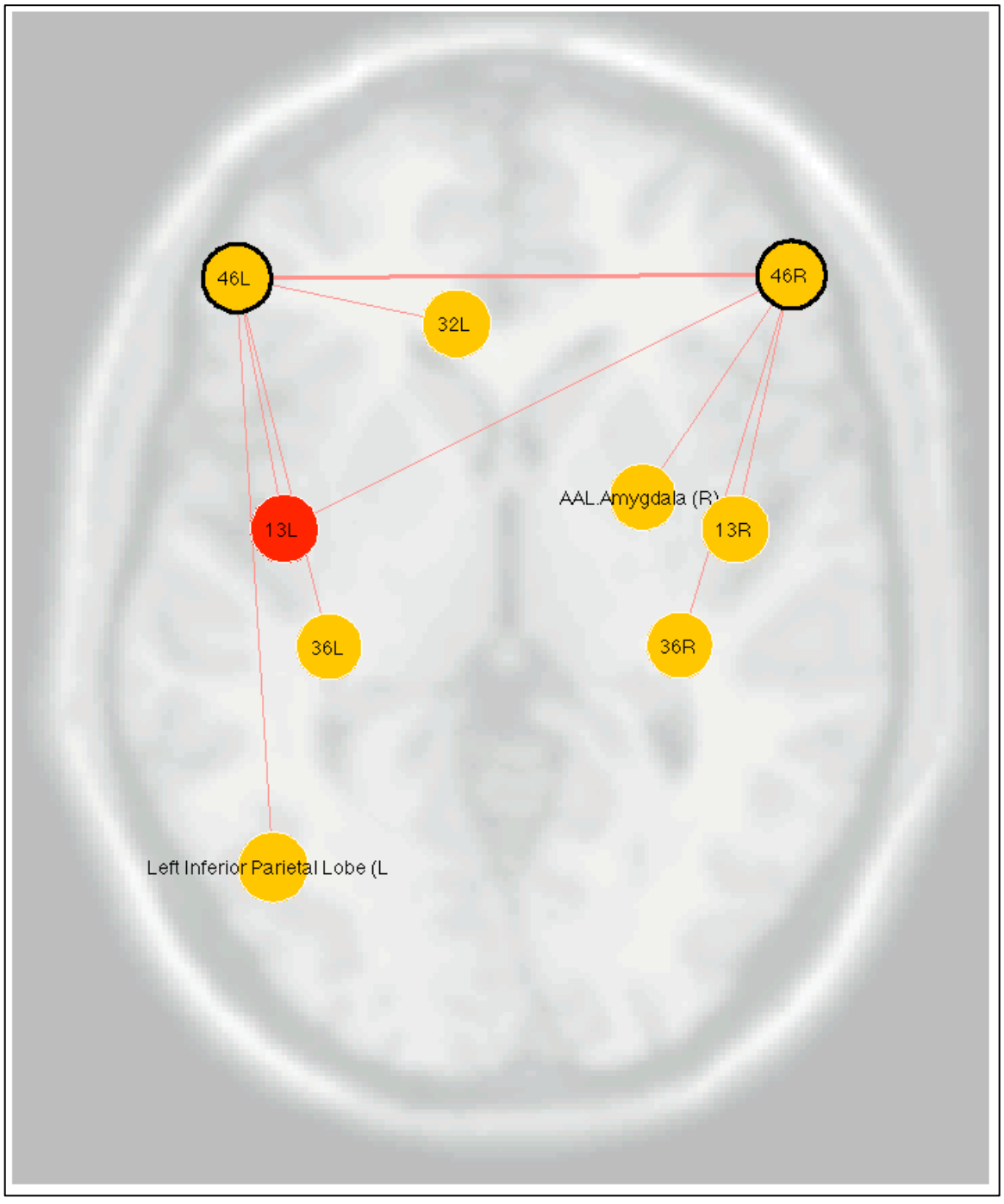


Figure 4B CEN: Central executive network seeded bilaterally in the dorsal lateral prefrontal cortex (DLPFC).

COGNITIVE CONTROL

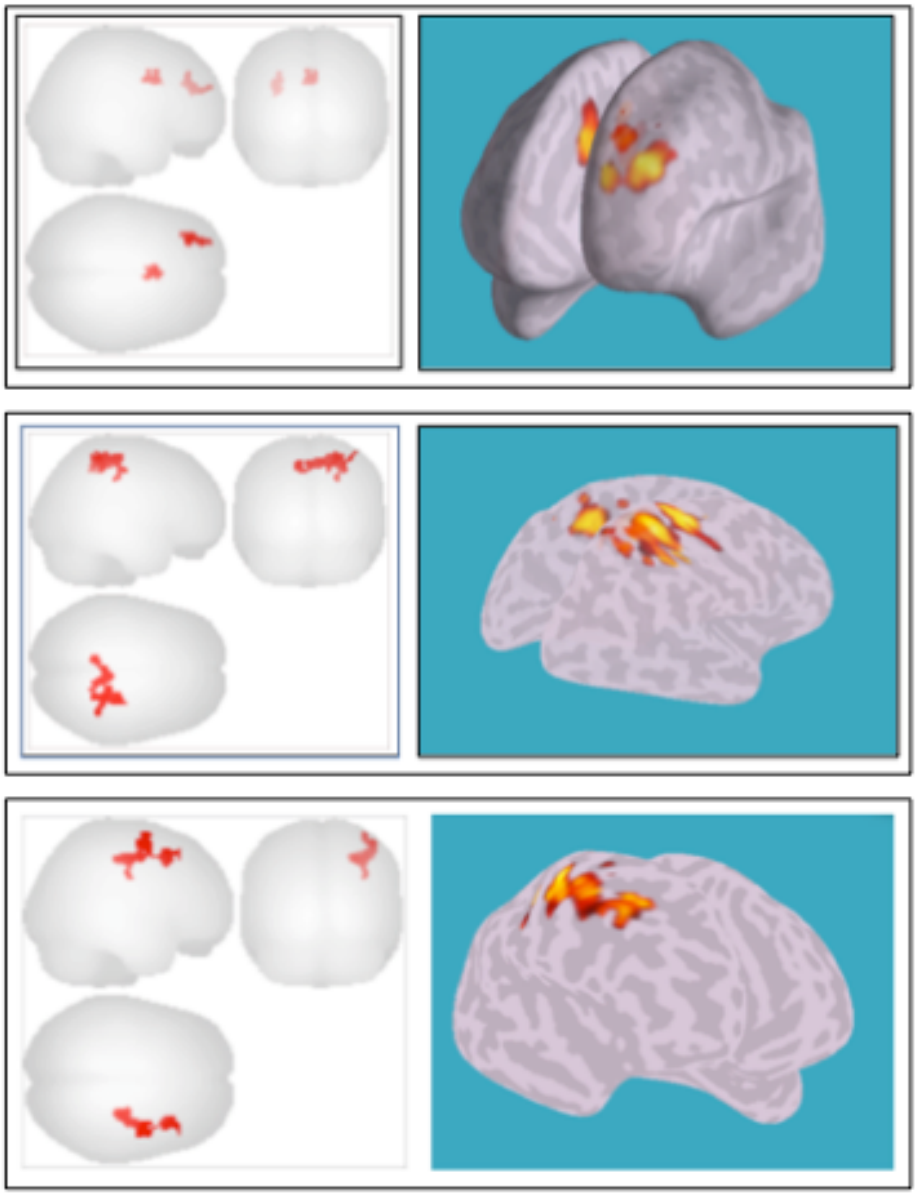


Figure 5: CEN seeded in the inferior PPC. Functional connectivity from the PPC to the ACC and left DLPFC *go* > *nogo* (top). CEN seeded in the DLPFC. FC from the DLPFC to regions within the dorsal parietal and dorsal PCC *go* > *nogo* (middle). SN connectivity from the insula to primary motor and somatosensory cortex greater for *go* than *nogo* ($q<0.05$).

As depicted in Figure 5 above, results after contrasting *go* and *nogo* from the in-scanner cognitive task irrespective of emotional valence revealed there was significantly more activation within the CEN when actively responding to the *go* cue compared to inhibiting responses to the *nogo* cue. Following results from the overall functional structure of the CEN network (figure 4A and B, above) the PPC had significantly greater FC with the left DLPFC (FDR $q=0.005$), and bilateral ACC ($q=0.007$) when actively responding. Conversely, the DLPFC had greater connectivity with the right dorsal parietal regions, including the somatosensory cortex (BA 7) and primary motor region (BA 4) and right PCC when the participant was responding to *go* cues ($q=0.004$).

Salience network connectivity seeded in the insula was greater for *go* cues compared to *nogo* cues with the majority of intra-network connectivity to the right somatosensory and right motor cortex ($q<0.05$).

COGNITIVE AND EMOTION INTERACTION

The functional connectivity (FC) of a limbic network rooted in the amygdala, as well as an emotion regulation network based in the dorsal prefrontal cortex was assessed. FC differences between the *go* and *nogo* task was assessed during exposure to fearful images to investigate differences in responding versus response inhibition in the presence of fearful stimuli. Secondly, the inhibition task was contrasted between fearful and neutral stimuli to assess functional recruitment differences in response inhibition when exposed to fearful stimuli.

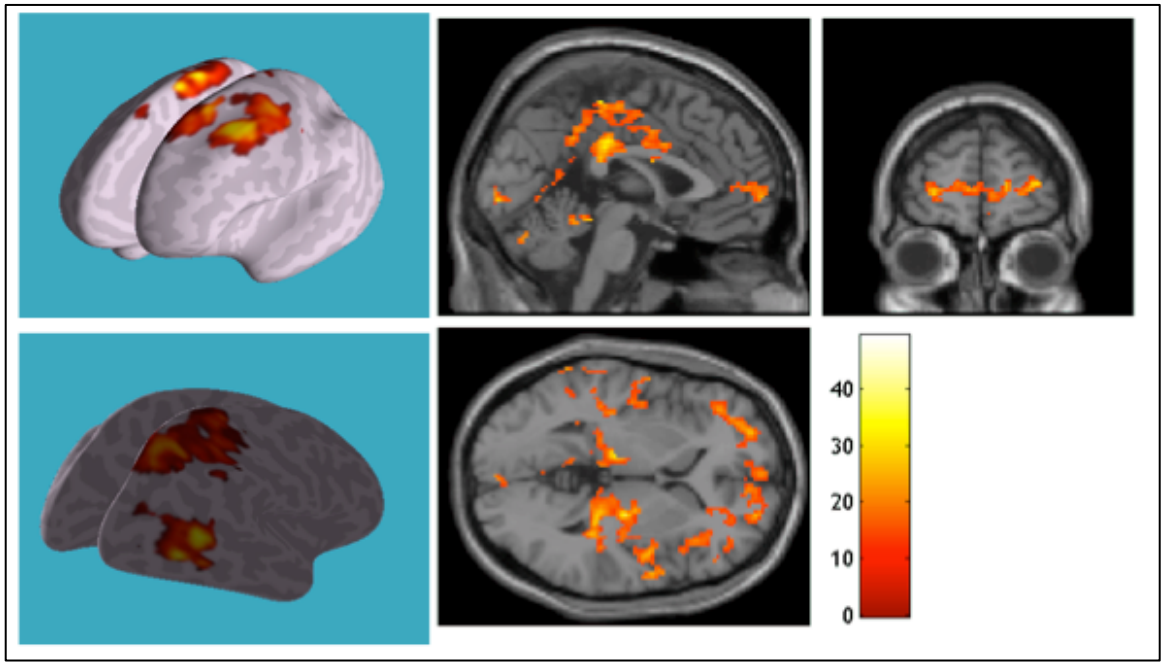


Figure 6 fear nogo vs. fear go: BOLD activation for *fear nogo* > *fear go* (uncorr 0=0.001) (right). Functional connectivity for the same contrast seeded at the amygdala bilaterally (top left) *fear go* > *fear nogo* (FDR q=0.0005). FC seeded at the DLPFC (bottom left) *fear go* > *fear nogo* (FDR q<0.0005)

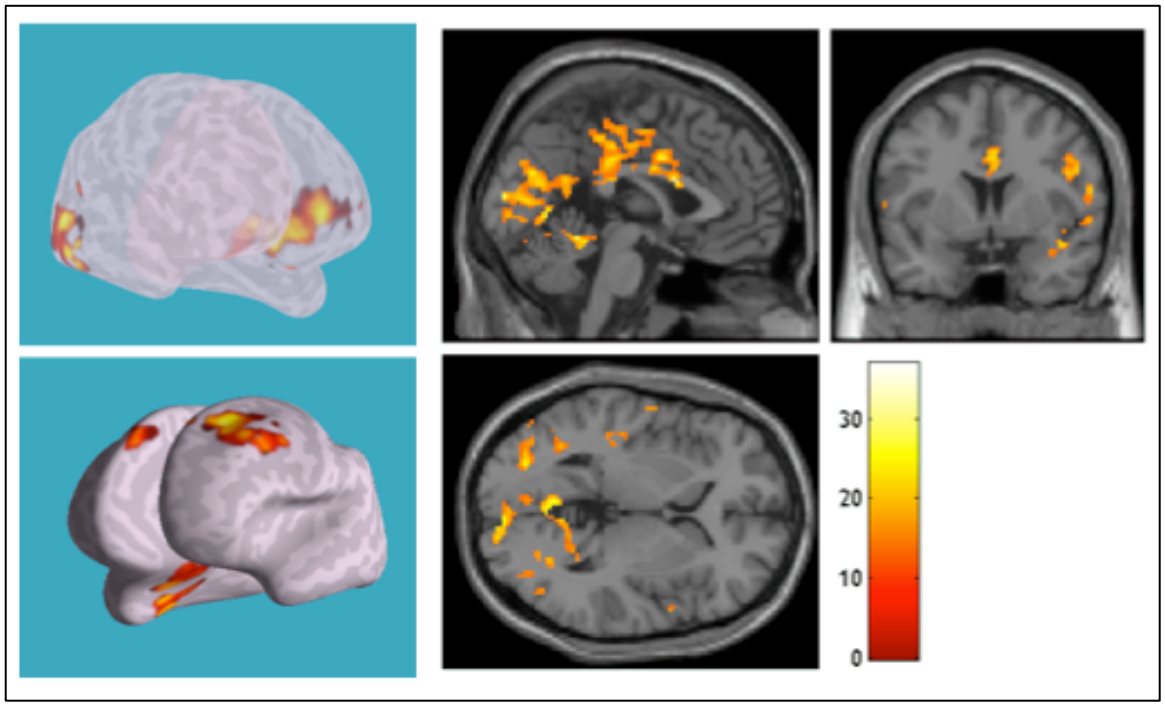


Figure 7 fear nogo vs neutral nogo: BOLD activation for *fear nogo* > *neutral nogo* (uncorr p=0.001) (right). Functional connectivity seeded at the amygdala (top left) with greater connectivity to anterior prefrontal regions for fear nogo (q=0.034) and greater connectivity from the DLPFC to limbic regions during fear nogo task (bottom left) (q=0.003).

When fear stimuli are present during both *go* and *nogo* trials there is significantly greater functional engagement within the limbic network seeded at the amygdala, and the DLPFC rooted emotion regulation circuit during *go* trials (table 2) ($q<0.0005$). The overall BOLD signal, however, is greater for *fear nogo* compared to *fear go* with significant clusters within the medial prefrontal cortex (mPFC, BA 10) and the inferior frontal cortex (BA 47) (uncorrected p=0.001). The limbic circuit was connected regions in the left primary motor (BA 4), somatosensory (BA 5), and dorsal prefrontal (BA 8)

(q=0.0005). The DLPFC was connected to the right fusiform gyrus in the inferior temporal lobe (BA 37) and the angular gyrus in the posterior parietal (BA 39) (q<0.0005), the latter of which forms part of the central executive network.

During response inhibition (*nogo* trials) fear did have a significant impact on the limbic and emotion regulation circuits. When fearful images were displayed with a *nogo* stimulus, the amygdala showed greater connectivity with anterior prefrontal (BA 10) and ventral anterior prefrontal cortices (BA 24) as well as the dACC (BA 32) all on the left side of the brain (q=0.034) (figure 7). The DLPFC was correlated with regions within the temporal lobes (entorhinal and perirhinal) (q=0.003) as well as premotor (BA 6), dACC (BA 32), and ventral ACC (BA24) for *nogo* trials when paired with a fearful image (q=0.007).

Findings are summarized in table 2, below:

Contrast	region	side	BA	voxel count	p-value (FDR corrected)
Fear no-go < Fear go - seed: amygdala	primary motor corted	L	4	192	0.05
	somatosensory association cortex	L	5	124	0.05
	somatosensory association cortex	R	5	29	0.05
	dorsal frontal cortex	L	8	24	0.05
Fear no-go < Fear go - seed: DLPFC	angular gyrus	R	39	29	<0.0005
	somatosensory association cortex	R	5	102	<0.0005
	fusiform gyrus	R	37	101	<0.0005
Fear no-go > Neutral no-go -seed: amygdala	dorsal anterior cingulate	L	32	61	0.03
	anterior prefrontal	L	10	46	0.03
	ventral anterior cingulate	L	24	16	0.03
Fear no-go > Neutral no-go -seed: DLPFC	Premotor	L	6	178	0.007
	dorsal anterior cingulate	L	32	69	0.007
	ventral anterior cingulate	L	24	42	0.007
	posterior entorhinal	R	28	65	0.003
	perirhinal	R	35	57	0.003

Table 2 interaction FC summary: Functional connectivity results summarized for the interaction of emotion and cognitive aspects of the task. Nogo was contrasted with go during fearful image exposure, and fear was contrasted with neutral during a response inhibition task. Both were assessed from amygdala and DLPFC seeds

DISCUSSION

OVERALL NETWORKS DURING CHILDHOOD

Very few studies to date have investigated the functional connectivity of limbic and cognitive control networks in children, and particularly what these functional networks look like during both emotional and cognitive tasks. In the present study we utilized an implicit affective go/nogo task developed in our lab to explore the interplay between top-down executive and emotion regulation networks in middle childhood. The engagement of cognitive faculties to modulate the response to emotional stimuli amounts to emotion regulation, an integral skill for normal behavioural maturation, and one found to be aberrant in affective disorders (Shin & Liberzon, 2010).

Initially, all contrasts across conditions were collapsed to get an overall picture of the developmental state of childhood cognitive networks. Results demonstrated both CEN and SN networks were more diffuse, displaying connectivity with regions outside of the mature adult framework. Interestingly, functional connections that were strongest (having survived corrections for multiple comparisons) were not random diffuse projections but rather connections associated with regional hubs in closely related networks.

The SN and CEN are both integral to emotion regulation through slightly different top-down cognitive faculties but work in tandem to accomplish a main regulatory goal. However while they function closely in the context of stimuli perception and processing, evidence suggests that by adulthood they are functionally distinct networks (Seeley et al.,

2007). With SN playing a role in signaling important events in the environment and the CEN playing a role in cognitively attending to the stimuli. Our findings suggest that developmentally, the less mature structure of the brain in middle childhood is one in which there is less distinct fractionation of networks and a carryover of connections between hubs linking the SN and CEN. Specifically, there exists positive connectivity between the insula and the DLPFC as well as between the dACC and the posterior parietal and DLPFC. These findings suggest that the CEN and SN networks have yet to fully dissociate from one another, a finding which supports previous theories of their development (Fair et al., 2009). Of particular interest is the inclusion of the DLPFC in SN networks seeded at both the dACC and insula. DLPFC (part of the CEN in the adult network) is regularly associated with executive functions such as response inhibition in adults and children (Blasi et al., 2006; Lamm et al., 2012) however, it is also the last cortical region to fully mature, reaching steady grey matter levels in late adolescence (Gogtay et al., 2004). Evidence suggests that it is active in cognitive functions during middle childhood (Lamm et al., 2012) however, given it's protracted development and our findings that it is still bilaterally connected to other networks, it is suggested that in children demands for response inhibition may be achieved by the engagement of a more distributed network involving the DLPFC and other network hubs. This is further exemplified by recent findings that other prefrontal regions, more commonly tied to salience networks (ie OFC), are heavily involved in response inhibition in childhood (Ameis et al., 2014) while both adults and children often recruit different regions (Bunge et al., 2002; Goldstein et al., 2007). To this end, increasing age is associated with an

overall decrease, but not cessation in recruitment of prefrontal regions to perform cognitive tasks, perhaps reflecting a streamlining or improved efficiencies in cortical responses with age (Lamm & Lewis, 2010).

Additionally, seeds from the dACC in the SN and PPC in the CEN are also positively correlated with the posterior parietal cortex (PCC). The PCC is known to be a hub in the default mode resting network (Raichle & Snyder, 2007) and positive connectivity between them is further evidence of broad connectivity that has yet to fully fractionate. Furthermore, SN and CEN seed regions most anatomically adjacent to the PCC, such as the PPC in the CEN, showed the strongest connectivity with the PCC (see figure 4A CEN) and the most distant (i.e. DLPFC, figure 4B CEN) did not show significant connectivity to the region. These findings are consistent with the "local" to "distributed" network hypothesis outlined in Fair et al. 2007 and may explain the observation of some cross-network connections (particularly in caudal regions) between resting state and active networks in children.

An interesting difference between SN seeds at the insula and dACC is their respective remnant connectivity to the PCC. Previous work has highlighted activity in the insula as a functional switch between resting and cognitive networks (Sridharan et al., 2008). Though we did not explicitly examine the interface between resting networks and cognitive networks in this study, our findings of negative connectivity between the insula and PCC suggests an inverse relationship between their respective networks – a sign of a maturing functional organization for rapid cognitive flexibility when a salient stimuli is perceived.

COGNITIVE NETWORKS AND EMOTIONAL INTERACTION

We performed four contrasts: 1) explicitly examining cognitive control by collapsing the fear and neutral dimensions and contrasting go trials with nogo trials, 2) explicitly examining emotion processing, by contrasting fear and neutral blocks 3) exploring cognitive control processes when fear cues are presented implicitly by contrasting fear nogo with fear go, and 4) examining the effects of emotion processing on on response inhibition by contrasting fear nogo with neutral nogo.

Results from the cognitive contrasts suggest greater functional connectivity across both CEN and SN networks for the *go* trials. Given the findings from the CEN overall networks, discussed above, we would expect PPC – DLPFC connectivity to be confined to the left hemisphere as it was in the PPC and DLPFC seeds (figures 4A and 4B CEN). However, this was only the case when the CEN was seeded in the PPC and not when seeded in the DLPFC. As expected, this network showed integration with the salience network as PPC was functionally connected to the dACC in addition to the DLPFC. Conversely, the DLPFC was connected to posterior parietal regions and the posterior cingulate. Mirroring overall network findings, the CEN is still not functionally dissociated from resting networks or from the SN even while performing a cognitive task. Additionally, there does not seem to be an appreciable increase in salience network activity between *nogo* and *go* trials (figure 5, bottom), perhaps indicating that both the response inhibition *nogo* task calls upon similar levels of salience detection as does responding for *go* trials.

Surprisingly, there is significantly greater connectivity in the CEN during performance of the *go* trials. It was expected given previous research (Lamm et al., 2012, 2006) that engagement of executive capacities when seeded in the CEN would show the strongest co-engagement during inhibition of a pre-potent responses (no-go). The go/nogo paradigm is a well validated task to probe executive functions, particularly response inhibition (Ameis et al., 2014; Goldstein et al., 2007; Horn, Dolan, Elliott, Deakin, & Woodruff, 2003; Lamm et al., 2012, 2006) and therefore it is difficult to doubt the sensitivity of the task, particularly given we had a larger sample size than has been historically reported (Phillips et al., 2008). This may reflect a tighter coupling of the CEN when the processing demands are low, and less coupled configuration and the engagement of broader networks when there are heightened processing demands (nogo trials).

The emotion contrast looking at overall BOLD signal differences between fearful images and neutral images found greater activation in the ACC and PCC for fear and no differences within the amygdala, however these findings were uncorrected at p=0.001. When fearful stimuli were presented with a go/nogo task (fear nogo/fear go contrast) there was increased functional connectivity seeded from the amygdala to BA 8 of the dorsal frontal cortex for the fear go compared to fear nogo task. Similarly when the DLPFC was seeded to investigate CEN networks during emotional stimuli, results revealed greater functional connectivity with the right angular gyrus (BA 39) in the parietal lobe for fear-go trials. These findings suggest that during fearful stimuli

presentation, limbic networks as well as cognitive control networks in the CEN are still functioning. Moreover, they reflect the same connectivity present regardless of emotional valence presentation.

Regardless of emotional stimuli presentation, CEN networks function similarly – preferentially activating for go stimuli. However, given our apriori hypothesis regarding the importance of response inhibition we wanted to understand whether the nogo task is affected by fear stimuli. To answer this question, the final contrast was used (fear nogo/neutral nogo). Results revealed greater functional connectivity in a limbic circuit connecting the amygdala with the left dACC and the left anterior prefrontal cortex (BA 10) when withholding responses (*nogo*) with simultaneous presentation of a fearful image. Considering the established importance of the dorsal prefrontal cortex in response inhibition (Blasi et al., 2006), the DLPFC was seeded and increased connectivity was also noted with the left dACC during fear-nogo trials. Collectively, this highlights a functional neural circuit subserving emotion regulation in children. This circuit includes aspects of the dorsal stream, likely for response inhibition, and the ventral stream for salience detection. In-line with previous evidence (Milad et al., 2007; Yamasaki et al., 2002) the presence of the dACC in both the dorsal network (seeded in the DLPFC) and the limbic-anterior prefrontal network is likely that of a inter-network hub, facilitating the necessary communication of this emotion regulation path.

In summary our results suggest that similar to previous work, there exists an amygdala-prefrontal network in children (Gabard-Durnam et al., 2014; D G Gee et al., 2013) that is similar in structure to adult networks (Banks et al., 2007) and is engaged

when there are demands for cognitive response inhibition tasks during the presentation conflicting emotional stimuli (Lamm et al., 2012).

FUTURE DIRECTIONS

As an extension to these findings, comparing these established networks to behavioural data obtained outside of the scanner as well as response times to stimuli will strengthen our understanding of the impact these networks have on measurable external effects.

Paralleling the functional and structural development will be a focus going forward. It is currently unknown how the white matter connectivity develops with respect to the functional development of particular regions, particularly between the uncinate fasciculus connecting the prefrontal and temporal structures examined in this study.

The present study examines the functional correlation between a seed and distal structure, however the variance in BOLD signal in a region of interest as predicted by activation in a seed region is yet to be explored in this sample of children. A direct extension would also be to examine how particular regions actively moderate the variance in BOLD signal time course between two regions. Of notable interest given the findings of this study, would be the moderating effects of the left dACC between the dorsal and ventral cortical circuits and the amygdala.

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