

Emotion Processing in Children and Adolescents with Anxiety Disorders and Typically
Developing Youth

by

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Dedication

To Mom, Dad, Greg, and Alex

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Table of Contents

Dedication	ii
Acknowledgments	iii
List of Tables	vi
List of Figures.....	vii
Abstract.....	viii
Chapter 1 General Introduction.....	1
Anxiety Disorders during Childhood and Adolescence	3
The Role of Amygdala-Prefrontal Cortex Connectivity in Emotion Processing and Regulation	4
Amygdala-Prefrontal Cortex Circuitry as a Mediator for Genetic and Environmental Influences on the Development of Psychopathology	12
Conclusion	19
References.....	21
Chapter 2 Age-Related Changes in the Structure and Function of Prefrontal Cortex-Amygdala Circuitry in Children and Adolescents: A Multi-Modal Imaging Approach	28
Introduction.....	28
Methods	33
Results.....	40
Discussion.....	43
References.....	60
Chapter 3 Changes in Amygdala Activation across a Scanning Session in Children and Adolescents with Anxiety Disorders.....	63
Introduction.....	63
Methods	68
Results.....	75
Discussion.....	78
References.....	92
Chapter 4 Altered Activation of the Rostral Anterior Cingulate Cortex in the Context of Emotional Face Distractors in Children and Adolescents with Anxiety Disorders.....	95
Introduction.....	95
Methods	101
Results.....	105
Discussion.....	108
References.....	121
Chapter 5 Conclusion	124
References.....	133

List of Tables

Table 2.1 Participant characteristics	47
Table 2.2 Bivariate correlations between age, task performance, and symptom scores.....	48
Table 2.3 Relation between age and left amygdala activation.....	49
Table 2.4 Relation between left uncinate fasciculus fractional anisotropy and left amygdala activation to faces	50
Table 2.5 Correlations between left amygdala activation and mean fractional anisotropy in the left uncinate fasciculus and three control white matter tracts.....	51
Table 3.1 Participant characteristics	83
Table 3.2 Behavioral results by condition and group	84
Table 3.3 Whole-brain activation results for group differences in changes in activation and overall activation.....	85
Table 3.4 Whole-brain activation results for group differences in psychophysiological interaction of left amygdala connectivity during face vs. shape matching	87
Table 4.1 Participant characteristics	113
Table 4.2 Behavioral results for EFSAT by group and condition	114
Table 4.3 Whole-brain activation table for main effects of task (face matching and shape matching) averaging across patient and control groups.....	115

List of Figures

Figure 2.1 Cross-sectional change with age in left and right uncinate fasciculus fractional anisotropy (FA) values.....	52
Figure 2.2 Cross-sectional change with age in left amygdala activation to all faces vs. baseline	53
Figure 2.3 Cross-sectional change with age in left amygdala connectivity with the anterior cingulate cortex (ACC).....	54
Figure 2.4 Negative relation between uncinate fasciculus fractional anisotropy and left amygdala activation to sad faces	55
Figure 2.5 Relation between fractional anisotropy (FA) within the left uncinate fasciculus and left amygdala activation to sad faces vs. baseline.....	56
Figure 2.6 Negative relation between uncinate fasciculus fractional anisotropy and left amygdala activation to happy faces.....	57
Figure 2.7 Relation between fractional anisotropy (FA) within the left uncinate fasciculus and left amygdala activation to happy faces vs. baseline	58
Figure 2.8 The association between structural connectivity and amygdala activation to sad faces is moderated by age	59
Figure 3.1 Example trials of the Emotional Face Assessment Task.....	88
Figure 3.2 There is a significant interaction of group x time within the left amygdala.....	89
Figure 3.3 Changes in left amygdala activation viewed separately for each run	90
Figure 3.4 Left amygdala activation shows a similar pattern of changes in activation across diagnostic categories	91
Figure 4.1 Example trials of the Emotional Faces Shifting Attention Task (EFSAT)	116
Figure 4.2 Activation associated with face matching and shape matching during the Emotional Faces Shifting Attention Task.....	117
Figure 4.3 Controls evidence greater rostral anterior cingulate cortex (rACC) activation during shape matching relative to the anxiety disorder group	118
Figure 4.4 Rostral anterior cingulate cortex activation to shape matching vs. face matching by group and emotion	119
Figure 4.5 There is an age x group interaction for rostral anterior cingulate cortex-amygdala connectivity during shape vs. face matching	120

Abstract

Understanding the typical development of neural circuitry involved in emotion processing has the potential to inform our understanding of the atypical development of this circuitry in populations such as pediatric anxiety disorder patients, and vice versa. The aim of this dissertation is to examine cross-sectional changes with age in prefrontal cortex-amygdala circuitry in typically developing children and adolescents and how functioning of this circuitry is altered in pediatric anxiety disorder patients. The first chapter reviews what is currently known about changes occurring in prefrontal cortex-amygdala circuitry across childhood and adolescence and discusses how knowledge of the development of neural circuitry can help link developmental influences such as genes and environment to outcomes of interest such as symptoms and disorders. The following three chapters provide original research examining this circuitry and its role in emotion processing in typical and atypical child and adolescent populations. In the second chapter, I examine the relation between prefrontal cortex-amygdala structural connectivity and function in typically developing youth. The third chapter aims to further elucidate abnormalities in amygdala function in pediatric anxiety disorder patients. In the fourth chapter, I examine prefrontal cortex function in pediatric anxiety disorder patients during a task that manipulates attention to emotional stimuli. In the fifth chapter, I discuss how this original research informs a model of the typical and atypical development of prefrontal cortex-amygdala circuitry across childhood and adolescence and how this model can be applied in future research to generate novel approaches to examining the development and treatment of anxiety disorders.

Chapter 1 ¹

General Introduction

During the periods of childhood and adolescence, significant structural and functional developments occur in the brain that influence how individuals process and respond to emotions. Although most individuals progress through these developmental periods without disturbance, the stages of childhood through young adulthood encompass the peak onset times for many forms of psychopathology related to altered emotion regulation, including anxiety disorders (Kessler et al., 2005; Paus, Keshavan, & Giedd, 2008). Recent neuroimaging research has begun to shed light on the neural correlates of information processing biases associated with anxiety disorders in children and adolescents. This research has the potential to elucidate how disturbances in function develop and why childhood and adolescence may represent periods of particular sensitivity to these disturbances.

The importance of understanding the neural correlates of emotion processing in childhood and adolescence is apparent in several theoretical frameworks of development. These frameworks highlight the bidirectional influences that occur between genes, brain, behavior, and environment, which unfold across development. The transactional model of development highlights the interdependent nature of these interactions; the model proposes that development is the product of reciprocal interacting influences between the child and environment over time (Sameroff, 2010). The complexity of these interactions is delineated in Gottlieb's theory of probabilistic epigenesis (Gottlieb, 2007a, 2007b). Rejecting the traditional notion that genes lead

¹ Chapter 1 corresponds to a portion of the publication Swartz & Monk (in press).

to neural or functional outcomes in a unidirectional fashion (predetermined epigenesis), Gottlieb argued that the environment also alters gene expression and neural function and that gene-environment interactions are fundamental to understanding development. Indeed, the growing field of epigenetics research has shown that the environment can alter the expression of genes, affecting neural development and function (Meaney, 2010). Importantly, the brain is at the intersection of many of these genetic and environmental influences on mental health outcomes, and as a mediator of these reciprocal interacting influences, provides a unique window into the development and treatment of psychopathology (Caspi & Moffitt, 2006; Cicchetti & Dawson, 2002; Hariri & Weinberger, 2003; Hyde, Bogdan, & Hariri, 2011; Monk, 2008).

The developmental psychopathology perspective holds that the study of typical development can inform the study of atypical development, and vice versa (Cicchetti & Thomas, 2008). Throughout this chapter, I will discuss how the study of neural development can inform our understanding of the development of anxiety disorders by a) elucidating trajectories of typical and atypical brain development and b) linking these trajectories to genetic and environmental influences on development. The three empirical papers in this dissertation will address the former goal by examining cross-sectional change in corticolimbic circuitry in typically developing children and adolescents and alterations of this circuitry and its cross-sectional change in youth with anxiety disorders. In the conclusion I will discuss how these papers inform a model of typical and atypical development of corticolimbic circuitry, and how this can be applied in future research to understand genetic and environmental influences on its development. In this review and in the empirical papers that follow, I focus on amygdala-prefrontal cortex circuitry, given its role in emotion processing and regulation in typically developing individuals and abnormal functioning in anxiety disorder patients.

Anxiety Disorders during Childhood and Adolescence

Adolescence is often defined as beginning at the onset of puberty (around 9-12 years old) and ending with the transition to adulthood, which is sometimes defined as the age of 18 years or the early twenties (Crone & Dahl, 2012). Because many of the studies I review in this chapter include participants ranging in age from around 7 years to the early twenties, I will refer to these stages as late childhood and adolescence. The periods of childhood and adolescence entail substantial changes in socio-emotional functioning. Normative anxiety and fears, such as separation anxiety, decline during childhood (Beesdo, Knappe, & Pine, 2009; Gee et al., 2013). Meanwhile, emotion regulation and associated executive functions including selective attention, inhibition, cognitive control, and reappraisal develop and generally show improvements through childhood and adolescence (Crone & Dahl, 2012; Luna, Garver, Urban, Lazar, & Sweeney, 2004; Luna, Padmanabhan, & O'Hearn, 2010; McRae et al., 2012; Silvers et al., 2012). Failure to evidence declines in anxiety during these periods may be associated with the development of anxiety disorders (Pine, 2009).

Indeed, the periods of childhood and adolescence are the developmental stages when the first onset of an anxiety disorder is most likely to occur. The interquartile range for age of onset of any anxiety disorder is 6 to 21 years, and the median age of onset is 11 years (Kessler, et al., 2005). Certain types of anxiety disorders are more likely to onset during specific developmental stages. Separation anxiety disorder most often onsets during childhood (interquartile range: 6 to 10 years) and social phobia in adolescence (8 to 15 years), whereas generalized anxiety disorder has an interquartile range of age of onset spanning from late adolescence through adulthood (20 to 47 years; Kessler, et al., 2005). Moreover, anxiety disorders in adulthood are frequently preceded by pediatric anxiety disorders or clinical levels of anxiety in childhood (Pine, 2009).

Therefore, an understanding of the changes taking place in the brain during childhood and adolescence could be informative regarding the development of these disorders. In the following sections, I summarize current models of changes in prefrontal cortex-amygdala circuitry occurring during childhood and adolescence and how functioning of this circuitry is altered in pediatric anxiety disorder patients.

The Role of Amygdala-Prefrontal Cortex Connectivity in Emotion Processing and Regulation

Emotion processing and regulation in typical populations engage a number of neural regions that act in concert to process emotional stimuli in the environment. The amygdala is a key component of this circuitry and is involved in detecting socially or emotionally relevant cues, such as emotional faces or signals of threat (Adolphs, 2010). The prefrontal cortex is another important component of this circuitry and is involved in higher-order processing of emotional stimuli and regulating an individual's emotional response. The amygdala has reciprocal connections with the prefrontal cortex and, based on anatomical tracing studies performed in animals, frameworks have proposed that the ventral prefrontal cortex contains the majority of direct connections with the amygdala (Ghashghaei, Hilgetag, & Barbas, 2007; Ray & Zald, 2012). These reciprocal connections allow the amygdala to signal ventral prefrontal regions regarding the emotional salience of stimuli and allow ventral prefrontal regions to modify or regulate amygdala activation based on an individual's goals.

The functions of subdivisions of the prefrontal cortex have been characterized in various ways. Some frameworks have suggested that medial regions of the ventral prefrontal cortex are involved in automatic emotion regulation whereas lateral regions may play a larger role in voluntary emotion regulation processes (Milad & Quirk, 2012; Nelson & Guyer, 2011; Phillips,

Ladouceur, & Drevets, 2008). Etkin, Egner, & Kalisch (2011), argued that dorsomedial regions of the prefrontal cortex may be associated with appraisal of emotional stimuli whereas ventromedial prefrontal cortex regions are associated with regulation. This division of function was based on findings of activation of dorsomedial prefrontal cortical regions during the detection of emotional conflict and cognitive reappraisal tasks and negative connectivity between the amygdala and ventromedial prefrontal regions during tasks requiring emotion regulation. Based on similar lines of evidence, Ochsner and Gross (2005) have suggested that dorsal prefrontal cortical regions are involved in tasks associated with explicitly reasoning about emotional stimuli (such as cognitive reappraisal) whereas ventral regions are associated with valuing and selecting context-appropriate responses to emotional stimuli.

Although it is not possible to assess neural signaling at the cellular level in humans, there are several neuroimaging methods available to assess connectivity between the amygdala and prefrontal cortex at the systems level. Two of the most widely used are functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI). Functional connectivity, or connectivity assessed through fMRI, refers to correlated activity between two or more neural regions. The advantage of this approach is that it can assess the function of corticolimbic circuitry under different task conditions (e.g., when a participant is performing an emotion regulation task or when the brain is “at rest” and not performing a task). DTI provides a measure of structural connectivity, as it assesses the organization of white matter tracts connecting neural regions of interest. The uncinate fasciculus is of particular interest in this regard, as it is one of the major white matter tracts connecting the prefrontal cortex and amygdala (Petrides & Pandya, 2002). A commonly used measure of white matter organization is fractional anisotropy (FA), which indicates the degree to which water molecules diffuse along one direction. Because water

molecules diffuse along the direction of myelin, higher FA values are often interpreted as indicating greater myelination or structural connectivity of white matter tracts (Thomason & Thompson, 2011). One advantage of measuring structural connectivity is that it does not require a task, and thus may yield measurements that are more easily compared across age groups. These measures of connectivity have provided insight into the typical development of corticolimbic circuitry across childhood and adolescence and how development of this circuitry may be disturbed in pediatric anxiety disorder patients.

Changes in Amygdala-Prefrontal Cortex Circuitry across Childhood and Adolescence

Developmental frameworks suggest that a complex interplay occurs between changes in amygdala and prefrontal cortex activation and connectivity throughout childhood and adolescence (Casey, Jones, & Hare, 2008; Casey et al., 2011; Ernst & Mueller, 2008; Somerville, Jones, & Casey, 2010; Steinberg, 2005; Sturman & Moghaddam, 2011; Yurgelun-Todd, 2007). Findings of cross-sectional changes in amygdala activation during this period have demonstrated a somewhat complex pattern (Somerville, Fani, & McClure-Tone, 2011). Several studies have demonstrated a non-linear relation between age and amygdala activation in which adolescents exhibit heightened amygdala activation to emotional faces relative to children and adults (Guyer et al., 2008a; Hare et al., 2008). In contrast, other studies have shown a linear decrease in amygdala activation from childhood to adulthood (Gee, et al., 2013). Differences in findings across studies may be due to differences in tasks or sample size and age ranges.

Research across different imaging modalities suggests that the prefrontal cortex and its connections develop along a relatively protracted time course from childhood to adulthood. These developments are observed with structural MRI through decreases in gray matter volume in the prefrontal cortex (potentially representing synaptic pruning) and increases in white matter

volume, which could reflect increased myelination or axonal width of white matter tracts connecting the prefrontal cortex to other brain regions (Giedd & Rapoport, 2010; Gogtay et al., 2004; Paus, et al., 2008). There is also evidence of changes in functional activation of the prefrontal cortex from adolescence to adulthood during emotion regulation and cognitive control tasks, although these changes encompass a complex pattern of increases, decreases, and non-linear change in prefrontal cortex activation with age, depending on a number of factors including the task performed (e.g., Hare, et al., 2008; Lau et al., 2011; Luna et al., 2001; McRae, et al., 2012; Monk et al., 2003; Pitskel, Bolling, Kaiser, Crowley, & Pelphrey, 2011; Rubia et al., 2000; Tamm, Menon, & Reiss, 2002; Wendelken, Baym, Gazzaley, & Bunge, 2011; Yurgelun-Todd & Killgore, 2006).

Based on evidence of differences in prefrontal cortex and amygdala function in adolescents relative to adults, so-called dual-systems models have proposed that prefrontal cortex function is immature during adolescence whereas activity in limbic regions including the amygdala is increased, resulting in a period of decreased prefrontal regulation coupled with increased amygdala activation during adolescence, leading to increased vulnerability to psychopathology and risk-taking (e.g., Casey, et al., 2008; Somerville, et al., 2010; Steinberg, 2005). Others, noting findings of both increased and decreased prefrontal cortex function in adolescents relative to adults, have suggested that this dual-systems model may be overly simplistic (Crone & Dahl, 2012; Pfeifer & Allen, 2012). Crone and Dahl (2012) concluded from the body of work conducted to date that the prefrontal cortex may not be immature in a strict sense during adolescence, but that activation may be more flexible and dependent on contextual factors such as emotional salience, motivation, and social context. Pfeifer and Allen (2012) suggest that incorporating structural and functional connectivity to examine the role of neural

networks as well as relating brain structure and function to behavior will be important moving forward in developing a more complete understanding of corticolimbic development across adolescence and how this relates to the development of psychopathology.

Structural connectivity of the uncinate fasciculus measured with DTI generally shows a predictable pattern of increases in FA with age across childhood and adolescence and then leveling off in young adulthood (Lebel & Beaulieu, 2011; Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008; Schmithorst & Yuan, 2010; Thomason & Thompson, 2011). In adults, FA values within this white matter region relate to amygdala activation to threatening faces (Kim & Whalen, 2009) and prefrontal cortex-amygdala functional connectivity during an emotion processing task (Tromp et al., 2012), suggesting that increased structural connectivity of white matter tracts connecting the prefrontal cortex and amygdala may facilitate communication between these regions. Moreover, in adolescents and adults, higher FA values within the UF relate to lower trait anxiety and harm avoidance (Kim & Whalen, 2009; Taddei, Tettamanti, Zanoni, Cappa, & Battaglia, 2012), suggesting that increases in structural connectivity of corticolimbic circuitry may be tied to the development of emotion regulation across childhood and adolescence.

Relatively little work has examined cross-sectional changes in functional connectivity during emotion processing tasks, but emerging evidence suggests it follows a similar pattern as for structural connectivity. For example, a recent cross-sectional study spanning from ages 4 to 22 demonstrated a shift in the direction of prefrontal cortex-amygdala connectivity, with younger participants demonstrating positive medial prefrontal cortex-amygdala connectivity and older participants exhibiting increasingly negative connectivity with age during the viewing of fearful faces (Gee, et al., 2013). Moreover, amygdala activation and normative separation anxiety also

decreased during this period, and prefrontal cortex-amygdala connectivity mediated the age-related changes observed in separation anxiety. The authors suggested that the shift from positive to negative functional connectivity represents increased regulation of the amygdala by the prefrontal cortex with age.

In sum, there is emerging evidence from fMRI and DTI that amygdala-prefrontal cortex functional and structural connectivity increases from childhood through adulthood, which may facilitate the development of emotion regulation and normative declines in anxiety. Consistent with this, several studies have shown that amygdala activation decreases from childhood or adolescence to adulthood. This model of typical neural development suggests that risk for developing anxiety disorders may be associated with deviations from this typical developmental trajectory during this developmental window.

Altered Prefrontal Cortex-Amygdala Function in Pediatric Anxiety Disorders

The changes occurring in prefrontal cortex-amygdala circuitry during childhood and adolescence suggest that this period may be particularly sensitive to disruptions or alterations in the developmental trajectory of this circuit. In this section, I will discuss how functioning of this circuitry may be altered in pediatric anxiety disorder patients.

Studies using angry or fearful faces as threatening stimuli have demonstrated abnormalities in amygdala and prefrontal cortex function in pediatric anxiety disorder patients, but the nature of these abnormalities are dependent on the task that participants perform during fMRI. Several studies produce evidence of amygdala hyper-activation in pediatric anxiety disorder patients. Amygdala hyper-activation has been demonstrated using a probe detection task with briefly presented (17 ms) then masked angry faces (Monk et al., 2008), when rating how afraid participants felt while viewing fearful faces (McClure et al., 2007), when rating how

participants expected to be evaluated by disliked peers (Guyer et al., 2008b), and during implicit emotion processing tasks in which participants either passively view or identify the gender of threatening faces (Blair et al., 2011; Thomas et al., 2001). This research will be discussed more extensively in Chapter 3, but these studies suggest as a whole that amygdala hyper-activation to threat may play an important role in the development of anxiety disorders.

A related finding emerging from these studies is that pediatric anxiety disorders are associated with abnormal ventral prefrontal cortex activation and amygdala-prefrontal cortex functional connectivity. For example, when threatening faces were briefly presented and masked, patients exhibited weaker negative connectivity between the amygdala and ventrolateral prefrontal cortex while viewing the briefly presented angry faces, potentially indicating reduced prefrontal regulation of a rapid threat detection response by the amygdala (Monk, et al., 2008). When participants were asked to assess how afraid they felt or how they would be evaluated by peers, adolescent anxiety disorder patients exhibited heightened prefrontal cortex activation (Beesdo et al., 2009; McClure, et al., 2007) and increased connectivity between the amygdala and ventrolateral prefrontal cortex (Guyer, Lau, et al., 2008), potentially representing greater communication of threat from the amygdala to the prefrontal cortex.

These results demonstrate a pattern of altered amygdala-prefrontal cortex function in pediatric anxiety disorder patients during the processing of threatening stimuli. Overall, these studies suggest that the neural correlates of anxiety disorders involve a combination of heightened rapid threat detection (mediated by the amygdala), abnormal communication of threat-related signals from the amygdala to the ventral prefrontal cortex, and altered regulation of the amygdala by the ventral prefrontal cortex, all of which may indicate altered corticolimbic development during childhood and adolescence. Studies have found altered activation and

connectivity of ventrolateral prefrontal cortical regions (Beesdo et al. 2009; Guyer et al., 2008; Monk et al. 2006, Monk et al., 2008) and medial prefrontal cortical regions (McClure et al., 2007; Blair et al., 2011), suggesting alterations in both automatic appraisal processes and more explicit forms of emotion regulation.

Notably, many of the abnormalities identified in amygdala-prefrontal cortex activation and connectivity in pediatric anxiety disorder patients have also been identified in adult anxiety disorder patients. Indeed, heightened amygdala response to threatening faces and reduced prefrontal cortex-amygdala connectivity are observed in adult patients with social phobia or generalized anxiety disorder (Etkin & Wager, 2007; Goldin, Manber, Hakimi, Canli, & Gross, 2009; Klumpp, Angstadt, Nathan, & Phan, 2010; Lorberbaum et al., 2004; Nitschke et al., 2009; Phan, Fitzgerald, Nathan, & Tancer, 2006; Prater, Hosanagar, Klumpp, Angstadt, & Phan, 2013; Schmidt, Mohr, Miltner, & Straube, 2010; Shah, Klumpp, Angstadt, Nathan, & Phan, 2009; Stein, Goldin, Sareen, Zorrilla, & Brown, 2002; Yoon, Fitzgerald, Angstadt, McCarron, & Phan, 2007). Moreover, one of the few studies to directly compare adolescent and adult social phobia patients found that both age groups demonstrated the same altered pattern of neural responses (heightened amygdala and rostral anterior cingulate cortex activation to threatening faces) relative to controls, but that there were no differences in activation across the age groups (Blair, et al., 2011). Although more subtle changes in developmental trajectories are likely to be revealed through longitudinal fMRI studies, these results suggest that altered patterns of amygdala-prefrontal cortex connectivity established early in development may persist and influence risk for developing mood and anxiety-related psychopathology at later stages of development. Perturbations in prefrontal cortex-amygdala circuit function are likely to be influenced by the interaction of genetic and environmental factors during development. In the

next section I review how corticolimbic function can serve as a mediator for understanding how developmental influences shape risk for the development of psychopathology.

Amygdala-Prefrontal Cortex Circuitry as a Mediator for Genetic and Environmental Influences on the Development of Psychopathology

One way in which knowledge of amygdala-prefrontal cortex circuitry can inform future research is by serving as a mediator for genetic, environmental, and gene-environment interaction influences on the development of emotion regulation and psychopathology. Hariri and Weinberger (2003) and Hyde and colleagues (2011), among others, have suggested that neural function can be used as a mediator to examine the influence of genes and gene-environment interactions on behavior. Because genes code for proteins that influence brain development and function, neural function may be a more proximal link to genetic variation, and may help us understand the mechanisms through which genes and the environment interact to influence psychosocial outcomes.

Genetic Influences on Amygdala-Prefrontal Cortex Function

Imaging genetics research, which uses fMRI or other neuroimaging methods to relate genes to neural function, suggests that amygdala-prefrontal cortex connectivity is subject to genetic influence. For example, a functional polymorphism in the promoter region for the serotonin (5-HT) transporter gene has been linked to variation in corticolimbic function. Having one or more copies of the high-expressing allele (L_A) of the serotonin transporter-linked polymorphic region (5-HTTLPR) is believed to lead to greater transcriptional efficiency of the serotonin transporter relative to the low-expressing alleles (S or L_G ; Hu et al., 2006). This in turn affects the rate of serotonin reuptake, which may modify the development and function of corticolimbic circuitry (Daws & Gould, 2011; Fisher & Hariri, 2013). Although it has been

difficult to link variation of the 5-HTTLPR directly to complex phenotypes such as trait anxiety or an anxiety disorder diagnosis, there has been much more success in identifying a relation between 5-HTTLPR genotype and the intermediate phenotype of neural function. In both pediatric and adult participants, the low-expressing alleles of the 5-HTTLPR have been associated with heightened amygdala activation to threat and altered prefrontal-cortex amygdala connectivity (Battaglia et al., 2012; Furman, Hamilton, Joormann, & Gotlib, 2011; Heinz et al., 2005; Lau et al., 2009; Munafò, Brown, & Hariri, 2008; Pezawas et al., 2005). The low-expressing alleles of the 5-HTTLPR also predict reduced FA of the uncinate fasciculus in adolescents and adults (Pacheco et al., 2009). Therefore, genetic variation may contribute to individual differences in corticolimbic structure and function over development.

Environmental Influences on Amygdala-Prefrontal Cortex Function

Evidence is accumulating for the role of environmental influences in shaping amygdala-prefrontal cortex connectivity. For instance, children exposed to severe early socioemotional deprivation due to institutionalization in orphanages, an environmental condition linked to emotional problems in later development, demonstrate reduced FA of the left uncinate fasciculus relative to typically developing controls (Eluvathingal et al., 2006). These results suggest that reduced structural connectivity of the white matter tract connecting the prefrontal cortex and amygdala may serve as a neural correlate of the influence of early environmental adversity on development, which could in turn be related to the development of maladaptive emotional functioning. Moreover, in a separate study, previously institutionalized children were found to evidence increased amygdala activation to fearful faces (Tottenham et al., 2011), suggesting that reduced structural connectivity associated with this maladaptive rearing environment may be associated with decreased regulation of amygdala activation. Likewise, life stress experienced

early in development (such as maternal depression, marital conflict, and family financial stress) is associated with the development of reduced resting state functional connectivity between the prefrontal cortex and amygdala during adolescence (Burghy et al., 2012). In sum, research is beginning to show that environmental adversity influences the development of structural and functional amygdala-prefrontal cortex connectivity, which may help us understand how early life stress increases vulnerability for the development of psychopathology.

Relatedly, environmental influences may affect the association between brain function and behavioral phenotypes. For example, Hyde, Gorka, Manuck, and Hariri (2011) found that perceived social support moderated the relation between amygdala and prefrontal cortex activation to threatening faces and trait anxiety. Individuals with heightened levels of amygdala and prefrontal cortex activation only reported higher levels of trait anxiety when they had low perceived social support. Individuals with higher levels of perceived social support did not exhibit a significant relation between amygdala or prefrontal cortex activation and trait anxiety. Thus, profiles of neural activity typically associated with anxiety disorder development may only be associated with anxiety-related traits or clinical symptoms within certain environmental contexts, further emphasizing the importance of considering multiple levels of analysis when tracing the reciprocal pathways between genes, the brain, environment, and behavior.

Gene x Environment Interaction Influences on Amygdala-Prefrontal Cortex Function

One of the key questions of interest to developmental psychopathologists is why certain individuals are more vulnerable to developing psychopathology after exposure to stress. A related question is why some individuals with purportedly risk-related genotypes go on to develop psychopathology whereas other individuals with these “risk” alleles actually demonstrate better psychological functioning in positive environments relative to individuals with non-risk alleles

(Belsky et al., 2009; Hankin et al., 2011). Corticolimbic circuitry appears to play a role in mediating the influence of gene-environment interactions, and thus has great potential to help us answer these questions.

Several studies now suggest that amygdala activation to threat is influenced by the interaction of genes and the environment (Bogdan, Williamson, & Hariri, 2012; White et al., 2012). For example, Bogdan et al. found a gene x environment interaction on amygdala function for a functional polymorphism of the mineralocorticoid receptor gene, which is related to the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. If participants had at least one copy of the allele associated with reduced HPA regulation (val), they had generally high levels of amygdala activation to threat, whereas participants having two copies of the allele associated with better regulation (iso) demonstrated lower levels of amygdala reactivity, except in the context of childhood neglect, in which case they demonstrated equivalent or higher levels of amygdala activation relative to val allele carriers. Therefore, the association between certain genetic variants and corticolimbic function is dependent on the environmental context. If amygdala activation to threat serves as a marker for risk for psychopathology, this could help to explain why some individuals (such as the iso allele carriers for the mineralocorticoid receptor gene) fare better under positive psychological contexts (or in this case, an environmental context lacking adversity) and may fare worse under contexts of environmental stress.

Genetic and Environmental Influences on Amygdala-Prefrontal Cortex Circuitry depend on Developmental Timing

Understanding the typical and atypical development of corticolimbic circuitry also has the potential to explain why the effects of biological and environmental influences vary depending on their developmental timing. An example of this is the noted paradox in the effects

of altering serotonin levels on anxiety at different stages of development (Sibille & Lewis, 2006). In animal models, administering selective serotonin reuptake inhibitors (SSRIs), during early development leads to increased anxiety-related behaviors in adulthood, whereas chronic administration of SSRIs in adulthood leads to a reduction in anxiety-related behaviors (Ansorge, Zhou, Lira, Hen, & Gingrich, 2004; Troelsen, Nielsen, & Mirza, 2005). Likewise, in humans having copies of the low-expressing alleles of the 5-HTTLPR (which decrease serotonin reuptake) is associated with increased amygdala activation to threat, whereas administering SSRIs during adulthood is an effective treatment for anxiety and depressive disorders. These observations suggest that altered serotonin levels have different effects depending on when they occur during development.

Recent research is beginning to suggest that serotonin may have different effects at different developmental stages because it is not only involved in neural signaling as a neurotransmitter, but also because it influences neural development. Research in gene knockout mice has shown that serotonin is involved in modulating neurodevelopmental processes including neurogenesis, cell specification, synaptogenesis, and dendritic and axonal growth during the early stages of development (Gaspar, Cases, & Maroteaux, 2003). There is also some evidence to suggest that altered serotonin levels early in development can have effects on brain and behavioral development that emerge during adolescence. For example, Ansorge and colleagues (2008) demonstrated that administering SSRIs to mice during early postnatal development led to an increase in anxiety-related behaviors that only became apparent during the period corresponding to adolescence in mice. Moreover, administering SSRIs later in development (during adolescence) had no long-term effects on anxiety-related behaviors. Together, these results indicate that there may be critical periods during which altered serotonin

levels affect neural development and that some of the long-term effects of manipulating serotonin levels during early postnatal development do not emerge until adolescence, a period when corticolimbic circuitry undergoes further development and reorganization. Similar results were observed in a cross-sectional study of typically developing children and adolescents who performed an implicit emotion processing task during fMRI scanning (Wiggins et al., 2012). In this study, we found that children and adolescents with the low-expressing alleles of the 5-HTTLPR evidenced a cross-sectional pattern of increases in amygdala activation and decreases in prefrontal cortex-amygdala connectivity with age. As a result, the typical pattern of activation seen in adults with the low-expressing alleles of the 5-HTTLPR (increased amygdala activation to emotional faces and decreased amygdala-prefrontal cortex connectivity) was only observed for participants in late adolescence. These studies are beginning to elucidate the complex processes involved in the development of brain function and anxiety-related outcomes. The developmental periods leading up to adolescence may serve as sensitive periods when biological and environmental influences have unique effects on development, and some of these effects may not emerge until the period of adolescence, when corticolimbic circuitry is undergoing reorganization.

In addition to sensitive periods of timing for biological developmental events, evidence indicates that environmental influences occurring during early development affect corticolimbic circuit function during late adolescence. For example, the longitudinal study described above (Burghy, et al., 2012) demonstrated that early life stress (assessed during participants' first 12 months) predicted increased cortisol levels assessed in childhood (at 4.5 years), which in turn predicted decreased amygdala-ventromedial prefrontal cortex connectivity in late adolescence (18 years). Decreased amygdala-ventromedial prefrontal cortex connectivity was also related to

greater anxiety symptoms at 18 years. Importantly, this relation was specific to early life stress and childhood cortisol levels; life stress and cortisol levels measured concurrently at 18 years did not significantly relate to amygdala-prefrontal cortex connectivity.

Relatedly, Giovanoli and colleagues (2013) found that stress experienced at different developmental stages predicted different psychopathology-related outcomes in a mouse model: mice experiencing prenatal stress (an infection in utero) and stress at the onset of puberty (foot shock, restraint stress, etc.) evidenced reduced prepulse inhibition of the startle reflex, which was not evident in mice experiencing stress at only one of these time points; mice experiencing peripubertal stress evidenced increased anxiety-related behaviors in the elevated plus maze test whether or not they experienced prenatal stress; and either prenatal stress or peripubertal stress alone or in combination led to disruption of the latent inhibition effect for a conditioned response. Moreover, many of these behavioral effects in stress-exposed mice did not emerge until after adolescence, and stress applied later in adolescence generally did not show these effects.

In sum, evidence is building to suggest that biological and environmental influences occurring early in development may shape the organization of corticolimbic circuitry during adolescence. As I have suggested above, the patterns of connectivity established over these developmental periods may lay a foundation for pathological functioning of this circuitry that continues through adulthood; therefore, further examination of how the timing of developmental influences affects corticolimbic circuitry holds the potential for developing preventions and interventions and identifying developmental periods when these interventions have the greatest chance of effecting long-term changes in the course of psychopathology.

Conclusion

In conclusion, amygdala-prefrontal cortex connectivity plays an important role in emotion processing and regulation. The amygdala relays signals to the prefrontal cortex regarding the salience of emotional stimuli and therefore influences cognitive processes such as attention to and the valuation of social stimuli. The prefrontal cortex also has reciprocal connections with the amygdala, allowing for modification or inhibition of amygdala activity when a task requires that the amygdala response be dampened. Accumulating evidence suggests that changing and fine-tuning of these connections occur across childhood and adolescence, and that altered development of amygdala-prefrontal cortex function and connectivity may play a role in the development and maintenance of anxiety disorders.

The aim of this dissertation is to add to our understanding of the typical and atypical development of this circuitry by examining cross-sectional change with age in corticolimbic structure and function across the periods of childhood and adolescence and to further characterize the nature of abnormalities in corticolimbic function in pediatric anxiety disorder patients. In Chapter 2, I use a multi-modal imaging approach, combining DTI and fMRI, in order to examine cross-sectional change in corticolimbic structural connectivity and function across the periods of childhood and adolescence in typically developing individuals. In Chapter 3, I examine differences in the pattern of changes in amygdala activation over the course of scanning in pediatric anxiety disorder patients relative to typically developing controls. The aim of this chapter is to characterize the contexts in which amygdala hyper-activation is observed, in order to inform future research that uses amygdala function as a neural mediator of pediatric anxiety disorder development. In Chapter 4, I examine prefrontal cortex function in pediatric anxiety disorder patients, focusing on the role of rostral anterior cingulate cortex function and

connectivity, in order to address a gap in our knowledge regarding abnormalities in prefrontal cortex function when participants perform a task in the context of emotional distractors. In the conclusion, I will discuss how this research contributes to a general model for the typical and atypical development of corticolimbic circuitry and future directions for using this model to link genetic and environmental influences to trajectories of brain development, and in turn to symptoms and disorders.

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Chapter 2 ²

Age-Related Changes in the Structure and Function of Prefrontal Cortex-Amygdala

Circuitry in Children and Adolescents: A Multi-Modal Imaging Approach

Introduction

Successful social functioning requires the development of processes related to perceiving, interpreting, and appropriately responding to the emotional signals expressed on others' faces. Indeed, abnormal emotion processing is associated with a range of psychiatric disorders (Phillips, Drevets, Rauch, & Lane, 2003; Pine, 2007). Because the neural circuitry associated with emotion processing undergoes substantial change during childhood and adolescence (Nelson, Leibenluft, McClure, & Pine, 2005), youth may be a time when sensitivity of this circuitry to genetic and environmental influences is increased. Understanding the structure and function of neural networks involved in emotion processing in childhood and adolescence will be an important step in understanding the development of emotion processing and how abnormalities arise (Chapter 1; Cicchetti & Dawson, 2002; Hyde, Bogdan, & Hariri, 2011; Swartz & Monk, in press).

Theoretical frameworks have identified several key neural networks that play a role in emotional face processing (Burnett, Sebastian, Cohen Kadosh, & Blakemore, 2011; Haxby, Hoffman, & Gobbini, 2002; Nelson, et al., 2005; Scherf, Behrmann, & Dahl, 2012). The “core” face processing network, composed of the fusiform gyrus, inferior occipital cortex, and posterior superior temporal sulcus (STS), is involved in the perceptual processing of faces (e.g.,

² Chapter 2 corresponds to the publication Swartz et al. (revise and resubmit).

recognizing a stimulus as a face). In addition, emotional face processing consistently activates regions in limbic and prefrontal areas associated with evaluating and regulating responses to emotional stimuli (sometimes referred to as “extended” face processing regions), including the amygdala, orbitofrontal cortex, ventrolateral prefrontal cortex, and anterior cingulate cortex (Fusar-Poli et al., 2009; Tahmasebi et al., 2012).

The extended face processing circuitry comprising the amygdala and prefrontal cortex is of particular interest for the development of socio-emotional function, given its role in interpreting and regulating responses to emotional faces. Ventral regions of the prefrontal cortex receive signals from the amygdala and send signals to regulate the amygdala through direct white matter pathways, including the uncinate fasciculus, one of the major white matter tracts connecting the frontal lobe with the temporal lobe and limbic system (Petrides & Pandya, 2002). Diffusion tensor imaging (DTI) identifies the microstructural properties of white matter tracts (Thomason & Thompson, 2011), which I refer to as structural connectivity. One of the most frequently examined measures of structural connectivity is fractional anisotropy (FA) or the degree to which water molecules diffuse along one direction, which may relate to myelination, fiber organization, or axonal packing (Beaulieu, 2002). Higher FA is interpreted as indicating greater structural connectivity between regions.

Studies conducted in adults that have combined DTI and functional MRI suggest that structural connectivity of the uncinate fasciculus is related to activation as well as connectivity within prefrontal cortex-amygdala circuitry. In particular, FA within this white matter region has been shown to relate to amygdala activation to fearful faces (Kim & Whalen, 2009), and functional connectivity between the anterior cingulate cortex and amygdala during emotion processing (Tromp et al., 2012; Wang et al., 2009). These results suggest that, for adults, greater

structural connectivity within the uncinate fasciculus may facilitate prefrontal communication with the amygdala through enhanced connectivity of this circuit. However, because brain structure and function undergo substantial changes during the periods of childhood and adolescence, it is still not known how structural connectivity relates to prefrontal cortex-amygdala function in early life.

Longitudinal and cross-sectional studies of the uncinate fasciculus generally demonstrate a pattern of increased FA with advancing age across childhood and adolescence (Lebel & Beaulieu, 2011; Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008). Longitudinal studies have also demonstrated variability in individual trajectories, with some individuals demonstrating increases, decreases, or maintained levels of FA in this tract over time (Lebel & Beaulieu, 2011). Given prior observed variance in white matter integrity in youth, it is possible that developmental variation in white matter could be associated with differences in brain function or psychosocial outcomes.

Several fMRI studies have demonstrated age-related changes in neural activation associated with emotional face processing across childhood, adolescence, and adulthood. For instance, in a large cross-sectional study with participants ranging in age from 4 to 22 years old, Gee and colleagues (2013) demonstrated a linear decrease with age in amygdala activation to fearful faces. Other studies have demonstrated greater amygdala activation to emotional faces in adolescents relative to adults (Guyer et al., 2008; Hare et al., 2008; Monk et al., 2003; Passarotti, Sweeney, & Pavuluri, 2009). Research focusing strictly on child and adolescent samples has also shown changes in emotion processing associated with development. These changes include increased amygdala response to sad faces with age (Pfeifer et al., 2011), decreased amygdala activation to neutral faces, and decreased ventrolateral prefrontal cortex activity to fearful faces

with pubertal development (Forbes, Phillips, Silk, Ryan, & Dahl, 2011). Overall, these results suggest a complex, non-linear pattern of development dependent on the nature of the emotion processing task and emotional stimuli used, with the most consistent trend indicating that amygdala activation to emotional faces decreases from adolescence to adulthood.

There is also emerging evidence for changes in prefrontal cortex-amygdala functional connectivity from childhood to adulthood. Using a psychophysiological interaction analysis, Gee et al. (2013) demonstrated a shift in the direction of functional connectivity from childhood to adulthood with the youngest age group (4 to 9 years old) exhibiting positive amygdala-prefrontal functional connectivity while viewing fearful faces whereas older participants evidenced negative functional connectivity that grew increasingly stronger with age. This shift from positive to negative connectivity was suggested to reflect increased prefrontal regulation of amygdala activation with age. Another study implementing a correlational functional connectivity analysis found that across children, adolescents, and adults, the amygdala was negatively connected with the ventral prefrontal cortex during an emotional face go/no go task and the strength of connectivity related to greater amygdala habituation (Hare, et al., 2008). However, although the amygdala and ventral prefrontal cortex showed differences in activity across the three age groups, changes in connectivity with age were not directly tested. A different study by Guyer and colleagues (2008) directly compared functional connectivity across adolescents and adults, and reported no difference in prefrontal cortex-amygdala connectivity between groups during emotion processing, indicating that these effects may be dependent on the task performed or the functional connectivity approach used. All together research that examines amygdala function and connectivity in early life shows structural connectivity between the prefrontal cortex and amygdala increases with age, amygdala activation decreases from

adolescence to adulthood, and there is emerging evidence for increased functional connectivity with age.

Though generally examined separately, it is important to consider brain structure and function simultaneously when examining development, as it is possible that changes in brain structure constrain changes in function, or vice versa (Cicchetti & Dawson, 2002). Moreover, it is important to include behavioral measures in order to examine how changes in brain structure and function relate to emotion regulation and psychological outcomes of interest (Pfeifer & Allen, 2012). The only study yet to examine these relations in an adolescent sample used event-related potentials (ERPs; Taddei, Tettamanti, Zanoni, Cappa, & Battaglia, 2012). Taddei et al. (2012) found that N400 ERP amplitudes (a response evoked by viewing emotional faces) to angry faces measured at ages 8-9 negatively predicted FA in the left uncinate fasciculus at ages 14-15. Moreover, scores on a measure of harm avoidance collected during childhood negatively predicted right uncinate fasciculus FA values in adolescence. These results demonstrate that neural activity in response to processing faces is related to structural connectivity of the uncinate fasciculus; however, because of the use of ERPs in this study, the relation between uncinate fasciculus structural connectivity and amygdala activation or functional connectivity during adolescence remains untested.

The objective of the present study was to examine the relation between structural connectivity of the uncinate fasciculus, functional activation and connectivity of prefrontal cortex-amygdala circuitry, and internalizing symptoms during the periods of late childhood and adolescence. My first hypothesis was that greater structural connectivity of the uncinate fasciculus assessed with DTI would predict reduced amygdala activation to emotional faces. Second, I hypothesized that increased structural connectivity of the uncinate fasciculus would

predict greater functional connectivity between the amygdala and prefrontal cortex. Third, I hypothesized that greater structural and functional connectivity, as well as decreased amygdala activation, would predict lower internalizing symptoms. Fourth, I examined whether the brain structure-function relationship was moderated by age. Because this circuitry is undergoing development during childhood and adolescence, the strength of the relationship between brain structure and function may differ across this age range.

Methods

Participants

Participants were recruited from the community through fliers. Parents reported that participants had no history of psychiatric diagnoses. Moreover, all participants were below the clinical cutoff score for internalizing symptoms on the Child Behavior Checklist (CBCL) (Achenbach & Rescorla, 2001). Participants 18 years and older provided informed consent; minor participants gave assent and their parents signed informed consent forms. A total of 79 participants between 8 and 19 years of age underwent fMRI scanning. Nineteen participants were removed from analyses due to: movement $>3\text{mm}$ in any direction (4 participants), technical problems during scanning (2 participants), accuracy $<70\%$ on the behavioral tasks (2 participants), poor normalization or signal dropout within the amygdala or prefrontal cortex (10 participants), and showing elevated scores on a measure of autism symptoms (1 participant), leaving a total of 60 participants with valid fMRI data. Of these participants, 49 also completed DTI during the same scanning session. One participant was removed for exhibiting $>3\text{mm}$ movement during DTI scanning and 9 participants were removed due to white pixel artifact in the DTI images. Removal of these participants resulted in a total of 39 participants (72% male) with fMRI and DTI data (Table 2.1). fMRI data from these participants have been reported

previously (Swartz, Wiggins, Carrasco, Lord, & Monk, 2013; Weng et al., 2011; Wiggins et al., 2012).

Procedure

Gender Identification Task Completed during fMRI Scanning. Participants underwent an implicit emotion processing task during fMRI scanning. A trial of this task consists of a fixation cross (500 ms), followed by a face (250 ms), and then a black screen (1500 ms), during which participants identify the gender of the face by pressing the thumb button for male or the index finger for female on a button box. Faces were from the NimStim set (Tottenham et al., 2009). Response times and accuracy were recorded. There were two runs of this task with 60 trials each for a total of 120 trials. There were 30 trials each of the following face expressions: fearful, happy, sad, and neutral.

Emotion Recognition Task Completed after Scanning. Participants completed a similar version of this task post-scanning in which they were asked to identify the emotion (rather than the gender) of the faces presented. The stimuli used and presentation times were the same as for the fMRI task. As stated above, participants that performed gender identification or emotion recognition with less than 70% accuracy were removed from the sample.

fMRI Data Acquisition. MRI images were acquired with a 3 Tesla GE Signa Scanner. A high resolution SPGR image was collected for anatomical reference. Functional data were collected with the following parameters: T2*-weighted BOLD images collected with a reverse spiral sequence, TR=2,000 ms, TE=30 ms, 40 adjacent 3 mm axial slices, flip angle=90 degrees, FOV=22 cm, matrix = 64 x 64.

DTI Data Acquisition. DTI was conducted following fMRI scanning with the following parameters: spin-echo EPI diffusion sequence, TR=9000 ms, TE=82.3 ms, FOV=22 cm, 39

slices, thickness=3mm, skip=1mm, 15 diffusion-weighted acquisitions with $b = 800 \text{ s/mm}^2$, two averages. One non-diffusion weighted image ($b=0 \text{ s/mm}^2$) was also collected in order to transform the diffusion-weighted images to a template in MNI space.

Symptom Measure. Internalizing symptoms were measured using T scores from the Child Behavior Checklist (CBCL) Internalizing scale (Achenbach & Rescorla, 2001). This scale was chosen as it provides a general measure of internalizing problems with a relatively broad range in a typically developing sample, whereas measures used to assess specific clinical symptoms related to disorders may not have produced a sufficient range within a non-clinical sample.

Analyses

fMRI Data Analysis. fMRI data underwent a standard pre-processing procedure. Large spikes in the k-space data were filtered out and data were reconstructed into images using field map correction to decrease distortions. Data underwent slice timing correction and realignment. Functional images were co-registered to the high-resolution anatomical image, which was normalized to the SPM template in MNI space. Smoothing was applied with an 8 mm full width at half maximum Gaussian kernel.

Condition effects were modeled at the individual subject level with the SPM canonical hemodynamic response function and a temporal derivative. Incorrect trials were modeled as a separate condition and excluded from analyses. Group-level analyses were conducted in SPM8.

Functional Connectivity Analysis. Psychophysiological interaction (PPI) analysis was used to examine functional connectivity during emotional face processing. PPI allows for the examination of how psychological conditions modulate the connectivity between two regions (Friston et al., 1997). Regressors for the PPI included amygdala response extracted from the

anatomically-defined left or right amygdala, condition effects, and the interaction between these (amygdala response x condition effects).

DTI Data Analysis. Diffusion-weighted images were analyzed using the FMRIB's Diffusion Toolbox (FDT) in FSL (Smith et al., 2004). First, DTI images underwent eddy current correction and linear registration to the non-diffusion weighted image in order to correct for head motion. Next, brain extraction was conducted using BET. Subsequently, the dtifit procedure in FSL was used to fit diffusion tensor models at each voxel and create an FA image for each participant. FA images were then processed using tract-based spatial statistics (TBSS) in FSL (Smith et al., 2006). FA images were realigned to the FMRIB standard-space image and transformed into MNI standard space. A mean FA skeleton was created and thresholded at .2 and each participant's FA data were projected onto the skeleton.

Regions of interest (ROIs) were created using the Johns Hopkins University White Matter Tractography Atlas (Mori, Wakana, Nagee-Poetscher, & van Zijl, 2005). The left and right uncinate fasciculus regions were binarized and skeletonized in order to extract mean FA values from the left and right uncinate fasciculus for each participant.

Age-Related Change in Uncinate Fasciculus FA, Amygdala Activation to Faces, and Prefrontal Cortex-Amygdala Connectivity. Before testing my primary hypotheses, I examined cross-sectional change for the main measures of interest. The correlation between FA and age was tested using Pearson's correlation, conducted in SPSS Software version 20 with mean FA values extracted from the uncinate fasciculus ROIs. Regression analysis performed in SPM was used to measure age-related changes in amygdala activation and prefrontal cortex-amygdala connectivity.

For this and all subsequent analyses performed in SPM, results were first displayed at $p < .01$ uncorrected and then small-volume correction was applied. Significance was assessed at $p < 0.05$ family-wise error (FWE) corrected using anatomically-defined ROIs created with the Wake Forest University Pickatlas (Maldjian, Laurienti, Kraft, & Burdette, 2003). Significance for amygdala activation was tested with the bilateral amygdala ROI and prefrontal cortex-amygdala connectivity was tested with the bilateral anterior cingulate cortex (defined using the Automated Anatomical Labeling atlas), and ventromedial prefrontal cortex (defined using Brodmann's Areas 10 and 11) ROIs based on previous research (Gee, et al., 2013; Hare, et al., 2008; Tromp, et al., 2012).

For all analyses conducted in SPM, the effects of all faces vs. baseline were tested first. This approach is warranted because age-related changes in amygdala activation have been observed in response to all faces (Hare, et al., 2008), fearful faces (Gee, et al., 2013), sad faces (Pfeifer, et al., 2011), and neutral faces (Forbes, et al., 2011). Thus, hypotheses about specific *a priori* emotions were not selected in advance. When significant effects were observed, emotion-specific effects were examined as post-hoc analyses.

Hypothesis 1: Relation between Uncinate Fasciculus FA and Amygdala Activation. The relation between FA and amygdala activation was examined by conducting multiple regression analyses in SPM8. First, I examined the relation of FA to amygdala activation to all faces by regressing mean FA values extracted from the left or right uncinate fasciculus onto the contrast of all faces > baseline. Significance was tested for the regression of left FA values with the left amygdala ROI and right FA values with the right amygdala ROI. Because this analysis involved two comparisons (left and right amygdala), Bonferroni correction was set to $p < 0.025$. This was

followed up with tests of emotion-specific effects by regressing FA onto the contrast of each emotion (fearful, happy, sad, neutral) vs. baseline.

To examine whether the relationship between amygdala activation and FA values was specific to the uncinate fasciculus, or related to white matter maturation across the brain more broadly, I conducted control analyses in white matter regions where I did not expect to observe similar effects. That is, I selected three major white matter tracts as control regions: superior longitudinal fasciculus (SLF; linking the prefrontal cortex with the posterior parietal cortex), inferior longitudinal fasciculus (ILF; running from the occipital cortex to the temporal cortex), and corticospinal tract (CST; linking the motor cortex with the brain stem and spinal cord; Petrides & Pandya, 2002; Thomason & Thompson, 2011). I examined correlations between amygdala activation and mean FA extracted from (a) the uncinate fasciculus, (b) the SLF, (c) the ILF, and (d) the CST. Control analyses were conducted in SPSS using mean FA values extracted from the white matter ROIs and mean contrast values extracted from the amygdala ROI.

Finally, I conducted additional analyses in SPM using neutral faces as baseline rather than fixation to test the relation between amygdala activation to emotional faces and uncinate fasciculus FA. Based on the results for the first hypothesis, I tested the following contrasts: left amygdala activation to sad > neutral faces and left amygdala activation to happy > neutral faces. The purpose of these analyses was to investigate whether the relationship between FA and amygdala activation was specific to processing associated with the emotional content of faces, rather than face processing more generally.

Hypothesis 2: Relation between Structural Connectivity and Amygdala-Prefrontal Cortex Functional Connectivity. I used a similar approach as outlined for the hypothesis above, except that, here, I tested the relation between FA and functional connectivity. Specifically, I regressed

FA values onto the PPI images in SPM in order to examine regions of the prefrontal cortex where connectivity with the amygdala related to uncinate fasciculus FA.

Additional Analyses. I examined the relation between age and task performance in order to assess potential confounds (Table 2.2). Because gender identification reaction times, emotion recognition accuracy, and emotion recognition reaction times were related to age, any significant results for the first two hypotheses were re-examined with reaction time or accuracy as a covariate. Significant results were also re-examined controlling for gender, as gender differences have been shown in neural development during adolescence (Schmithorst & Yuan, 2010; Tahmasebi, et al., 2012).

Hypothesis 3: Relation between Brain Structure, Function, and Internalizing Symptoms. I used a similar approach as described above for testing the relation between the neuroimaging measures and age in order to test their relation with internalizing symptoms. Specifically, I tested the Pearson's correlation between FA values and CBCL scores in SPSS and I used regression analyses in SPM in order to assess the relation between CBCL scores and amygdala activation and prefrontal cortex-amygdala connectivity.

Hypothesis 4: Moderation by Age. The hypothesis that the relation between brain structure and function would be moderated by age was examined by conducting a moderation analysis using the PROCESS macro in SPSS (Preacher & Hayes, 2004). In order to examine amygdala activation in SPSS, mean contrast values were extracted from the anatomically-defined amygdala ROI. Based on the results for the first hypothesis, I used the contrasts of sad vs. neutral and happy vs. neutral for this analysis. I tested whether age moderated the relation between FA values and amygdala activation. Moderation is examined by testing whether the interaction between the predictor variable (structural connectivity) and the moderator variable (age)

significantly predicts the outcome variable (amygdala activation). If the interaction is significant, then the effect of the predictor variable on the outcome differs depending on the level of the moderator. I followed up significant interactions with the Johnson-Neyman approach, which identifies the level of the moderator at which point an effect changes from significant to non-significant (Hayes & Matthes, 2009).

Results

Cross-Sectional Changes with Age

In line with previous research, I observed significant age-related increases in FA in the left ($r = .46, p = .003$) and right uncinate fasciculus ($r = .42, p = .007$; Figure 2.1). Moreover, I observed a negative relation between age and left amygdala activation to all faces vs. baseline (Table 2.3; Figure 2.2). Post-hoc analyses demonstrated that this effect held for each emotion (Table 2.3). Additionally, PPI of all faces vs. baseline revealed a negative relation between age and left amygdala connectivity with the right anterior cingulate cortex, $t(37) = 3.94, p = .03, xyz = 16, 36, 22$ (Figure 2.3). There was no significant relation with age for right amygdala-prefrontal cortex connectivity.

Hypothesis 1: Relation between Uncinate Fasciculus FA and Amygdala Activation

In support of my first hypothesis, there was a negative relation between left uncinate fasciculus FA values and left amygdala activation to all faces vs. baseline ($p = .01$; Table 2.4). Post-hoc analyses indicated this relationship was driven by left amygdala activation to sad ($p = .004$) and happy faces ($p = .005$; Table 2.4; Figures 2.4-2.7). These remained significant when controlling for gender, reaction time on the task performed during scanning, emotion recognition accuracy, and emotion recognition reaction time. My first hypothesis was not supported for the right amygdala, although the negative relationship between right uncinate fasciculus FA values

and right amygdala activation to all faces approached significance, $t(37)=2.62$, $p=.065$, $xyz=28$, -6 , -20 .

In order to determine the specificity of the results, I examined the relation between amygdala activation and the control ROIs. I performed this control analysis with left amygdala activation and FA extracted from the left hemisphere of the control ROIs. As expected, the only significant predictor of left amygdala activation to sad or happy faces was FA within the left uncinate fasciculus; mean FA of other white matter tracts did not relate to amygdala activation (Table 2.5).

I conducted additional analyses using neutral faces rather than fixation as baseline to determine whether the same pattern of results held when the contrast was restricted to emotional content of faces, as opposed to face processing more generally. Although significance levels were somewhat reduced, I found that the relation between left uncinate fasciculus FA and left amygdala activation held when comparing activations for sad > neutral faces, $t(37)=2.76$, $p=.05$, $xyz=-26$, 2 , -18 , and happy > neutral faces, $t(37)=3.48$, $p=.01$, $xyz=-22$, -8 , -14 . I chose to use these contrasts for testing the third and fourth hypotheses so that I could draw conclusions related to the emotion of the faces specifically.

Hypothesis 2: Relation between Structural Connectivity of the Uncinate Fasciculus and Functional Connectivity

The negative relation between left uncinate fasciculus FA values and left amygdala connectivity with BA 11 approached significance, $t(37)=3.66$, $p=.06$. However, no regions demonstrated a significant relation with left uncinate fasciculus FA for left amygdala connectivity for all faces vs. baseline or for sad or happy faces vs. neutral. Likewise, there was

no relation between right uncinate fasciculus FA and right amygdala connectivity with the prefrontal cortex for all faces vs. baseline.

Hypothesis 3: Relation between Structural Connectivity, Function, and Internalizing Symptoms

I observed the predicted positive relationship between amygdala activation to sad vs. neutral faces and CBCL scores, left amygdala: $t(36)=3.64, p=.015, xyz=-22, -10, -12$ and right amygdala: $t(36)=3.13, p=.049, xyz=22, -6, -14$, indicating that greater amygdala activation to sad faces predicts more internalizing symptoms. The positive relationship between amygdala activation to fearful vs. neutral faces and CBCL scores approached significance in the left amygdala, $t(36)=3.08, p=.054, xyz=-20, -10, -12$. There was no significant relationship between CBCL scores and amygdala activation to happy vs. neutral faces, or structural or functional connectivity.

Hypothesis 4: Moderation by Age

I tested whether age moderated the association between left uncinate fasciculus FA values and left amygdala activation to sad or happy faces vs. neutral faces. The moderation effect for amygdala activation to sad vs. neutral faces was significant, $R^2=.20, F(3, 35)=2.90, p=.049$, indicating that the interaction of age x FA values predicted amygdala activation, $B=.14, SE=.07, t(35)=2.07, p=.046$. As shown in Figure 2.8, the relation between uncinate fasciculus FA values and amygdala activation to sad vs. neutral faces was only significant within younger participants; within older participants this effect was no longer present. Using the Johnson-Neyman approach, the cutoff value of 15.7 years represented the point at which this relation was no longer significant. There was no moderation effect for left uncinate fasciculus FA values and left amygdala activation to happy vs. neutral faces.

Discussion

This study contributes to our understanding of the development of brain structure-function relations by revealing three primary findings. First, this is the first study to my knowledge to demonstrate that increased FA within the uncinate fasciculus predicts reduced amygdala activation to emotional faces in children and adolescents. Second, I demonstrated that greater amygdala activation to sad faces predicts higher internalizing symptoms. Finally, I observed that the relation between uncinate fasciculus FA and amygdala activation to sad faces is moderated by age, with younger participants demonstrating a stronger relation.

Previous research with pediatric samples had demonstrated age-related increases in uncinate fasciculus FA (Lebel & Beaulieu, 2011) and decreases in amygdala activation to emotional faces from childhood to adulthood (Gee, et al., 2013). However, until the present study, no work had demonstrated a direct relation between structural connectivity and amygdala function during these developmental periods. The finding of a negative relationship between uncinate fasciculus FA and amygdala activation suggests that increased structural connectivity of the uncinate fasciculus may improve communication between the prefrontal cortex and amygdala, allowing for more efficient regulation of amygdala activity. Therefore, it may be that increased structural connectivity with age facilitates greater regulation of the amygdala, contributing to the declines in amygdala activation from childhood through adolescence observed in the present study and in previous research. Future research with longitudinal data will be necessary in order to confirm the direction of this effect.

Additionally, the relation between amygdala activation to sad faces and internalizing symptoms suggests that structural connectivity may indirectly affect risk for developing internalizing problems. Specifically, reduced structural connectivity predicts greater amygdala

activation to sad faces, and this in turn predicts more internalizing symptoms. Individuals with decreased structural connectivity may therefore be more likely to exhibit amygdala hyper-activation, which could then place them at risk for the development of internalizing problems.

A significant moderation effect demonstrated that uncinate fasciculus FA values were more predictive of amygdala activation to sad vs. neutral faces in younger relative to older participants. This may be due to greater variability in amygdala activation in late childhood and early adolescence, leading to a stronger relationship between structural connectivity and amygdala activation in younger participants. If confirmed in future research, this moderation effect suggests that the periods of late childhood and early adolescence may be sensitive periods during which structural connectivity of the uncinate fasciculus plays a particularly important role in emotion processing and regulation, given that amygdala activation may be more variable during these developmental stages.

I observed that the relation between uncinate fasciculus FA and amygdala activation was only significant for the left hemisphere and was specific to sad and happy faces. This relation approached significance in the right hemisphere; therefore, I suggest that future research with larger samples will be necessary to determine whether this effect is truly lateralized to the left or whether it will be observable in the right with increased power. Interestingly, although amygdala activation to fearful and neutral faces decreased with age, it did not relate to uncinate fasciculus FA. One potential explanation for this observation is that additional or alternative factors predict amygdala activation to fearful and neutral faces in children and adolescents, such as strength of amygdala-prefrontal cortex functional connectivity or greater emotion recognition with age. Notably, the relation between amygdala activation and internalizing symptoms was specific to sad faces. This concurs with previous research on abnormal processing of sad faces in depression

(Fritzsche et al., 2010) and suggests that amygdala activation to sad faces specifically may be predictive of internalizing problems.

These findings have several implications for future research. First, if confirmed through longitudinal research, these results suggest that development across childhood and adolescence involves increased uncinate fasciculus structural connectivity accompanied by decreased amygdala activation to emotional faces. An important question for future research is whether individuals who deviate from these trajectories, such as showing slower increases in structural connectivity and decreases in amygdala activation relative to same-age peers, are at heightened risk for the development of mood and anxiety disorders if they encounter stressful life events during this period or in adulthood.

Moreover, these findings suggest that individual differences in uncinate fasciculus structural connectivity are predictive of amygdala activation to emotional faces. Structural connectivity of the uncinate fasciculus has been associated with both genetic influences (Pacheco et al., 2009) and environmental risk factors (Eluvathingal et al., 2006). Therefore, uncinate fasciculus structural connectivity and age-related changes in amygdala activation (Wiggins, et al., 2012) may serve as neural mediators for identifying the influence of genetic and environmental factors on psychological development (Hariri & Weinberger, 2003; Hyde, et al., 2011).

Finally, the effect of age on moderating the relation between structural connectivity and amygdala activation suggests that uncinate fasciculus development may play a particularly important role in influencing amygdala activation during late childhood and early adolescence. If future research confirms this moderation effect, then these developmental stages may represent

sensitive periods and could be informative regarding the periods when intervention may be most effective.

The limitations of this study warrant mention. First, because of the multi-modal imaging approach used, a greater number of participants had to be removed from the sample due to unusable fMRI or DTI data than if only one imaging modality had been selected. Nevertheless, the sample size for this study was larger than previous research combining fMRI and DTI data in adults (20 participants in Kim & Whalen, 2009) and is comparable to cross-sectional studies conducted in children and adolescents using only fMRI (45 participants in Gee et al., 2013; 38 participants in Pfeifer et al., 2011). Second, the sample contained a higher proportion of male than female participants. Given that girls tend to exhibit more internalizing symptoms than boys during adolescence, the sample composition could have decreased the ability to observe relations between the neuroimaging measures and internalizing symptoms. Future research using samples with a higher proportion of girls may find effects even stronger than the ones found in the present sample. Third, because of the cross-sectional design, I am unable to draw conclusions regarding within-person trajectories in brain structure-function relations over this period. Future research will be necessary in order to test whether the typical cross-sectional trajectories observed in this study are also observed longitudinally and to better examine the direction of effects between these variables.

In conclusion, structural connectivity of a major fronto-limbic white matter pathway predicts amygdala activation to emotional faces during the periods of childhood and adolescence. By using brain structure and function as neural mediators, future research may help shed light on the trajectories of typical and atypical development of emotion processing and how genetic and environmental factors interact to shape individual differences in these trajectories.

Table 2.1 Participant characteristics

Measure	Mean (SD)	Min-Max
Age (years)	15.3 (2.5)	9.6-19.2
CBCL Internalizing T scores	44.8 (7.9)	33-64
Gender ID Task Accuracy	96.6% (3.9)	80-100%
Gender ID Task RT (ms)	729.6 (133)	536-1042.3
ER Task Accuracy	89% (6.4)	70.8-98.3%
ER Task RT (ms)	1180.1 (252)	810.2-2104.0

Note. CBCL = Child Behavior Checklist; Gender ID Task = gender identification task performed during scanning; RT=reaction time; ER Task = emotion recognition task performed after scanning. One participant was missing data for the CBCL.

Table 2.2 Bivariate correlations between age, task performance, and symptom scores

	Age
Gender ID Accuracy	$r=.04, p=.79$
Gender ID RT	$r= -.49, p=.002$
ER Accuracy	$r=.35, p=.03$
ER RT	$r= -.44, p=.005$
CBCL Internalizing T score	$r= -.29, p=.08$

Note. Bold signifies a significant correlation. Gender ID=gender identification task performed during scanning; RT=reaction time; ER=emotion recognition task performed after scanning; CBCL= Child Behavior Checklist.

Table 2.3 Relation between age and left amygdala activation

Contrast	Negative effect of age on activation, t(37)=	P-value (FWE-corrected)	MNI coordinates (xyz)
All Faces vs. Baseline	3.75	.011	-26, -2, -26
Fearful vs. Baseline	3.91	.004	-20, -8, -10
Sad vs. Baseline	3.14	.023	-20, -2, -14
Happy vs. Baseline	2.81	.045	-20, -8, -10
Neutral vs. Baseline	2.87	.045	-22, -2, -14

Note. FWE-correction is based on structurally-defined bilateral amygdala region of interest for contrast of all faces vs. baseline and left amygdala region of interest for emotion-specific contrasts. FWE=family-wise error; MNI=Montreal Neurological Institute.

Table 2.4 Relation between left uncinate fasciculus fractional anisotropy and left amygdala activation to faces

Contrast	Regression for negative effect of FA on activation, t(37)=	P-value (FWE-corrected)	MNI Coordinates (xyz)
All Faces vs. Baseline	3.49	.01	-22, -4, -14
Fearful vs. Baseline	2.15	.15	-22, -2, -14
Sad vs. Baseline	3.87	.004	-26, 2, -18
Happy vs. Baseline	3.76	.005	-24, -6, -14
Neutral vs. Baseline	1.64	.33	-22, -2, -14

Note. Effects of left uncinate fasciculus fractional anisotropy values were examined with FWE-correction for the structurally defined left amygdala region of interest. FA=fractional anisotropy; FWE=family-wise error; MNI=Montreal Neurological Institute.

Table 2.5 Correlations between left amygdala activation and mean fractional anisotropy in the left uncinate fasciculus and three control white matter tracts

White matter tract	Correlation with left amygdala activation to sad faces vs. baseline	Correlation with left amygdala activation to happy faces vs. baseline	Correlation with left amygdala activation to fearful faces vs. baseline	Correlation with left amygdala activation to neutral faces vs. baseline
Left uncinate fasciculus	$r = -.36, p = .024$	$r = -.41, p = .009$	$r = -.17, p = .30$	$r = -.08, p = .61$
Left superior longitudinal fasciculus	$r = -.21, p = .19$	$r = -.21, p = .19$	$r = -.18, p = .28$	$r = -.13, p = .43$
Left inferior longitudinal fasciculus	$r = -.18, p = .28$	$r = -.11, p = .50$	$r = -.10, p = .54$	$r = -.05, p = .78$
Left corticospinal tract	$r = -.23, p = .17$	$r = -.088, p = .59$	$r = -.05, p = .78$	$r = -.07, p = .71$

Note. Bold indicates a significant correlation.

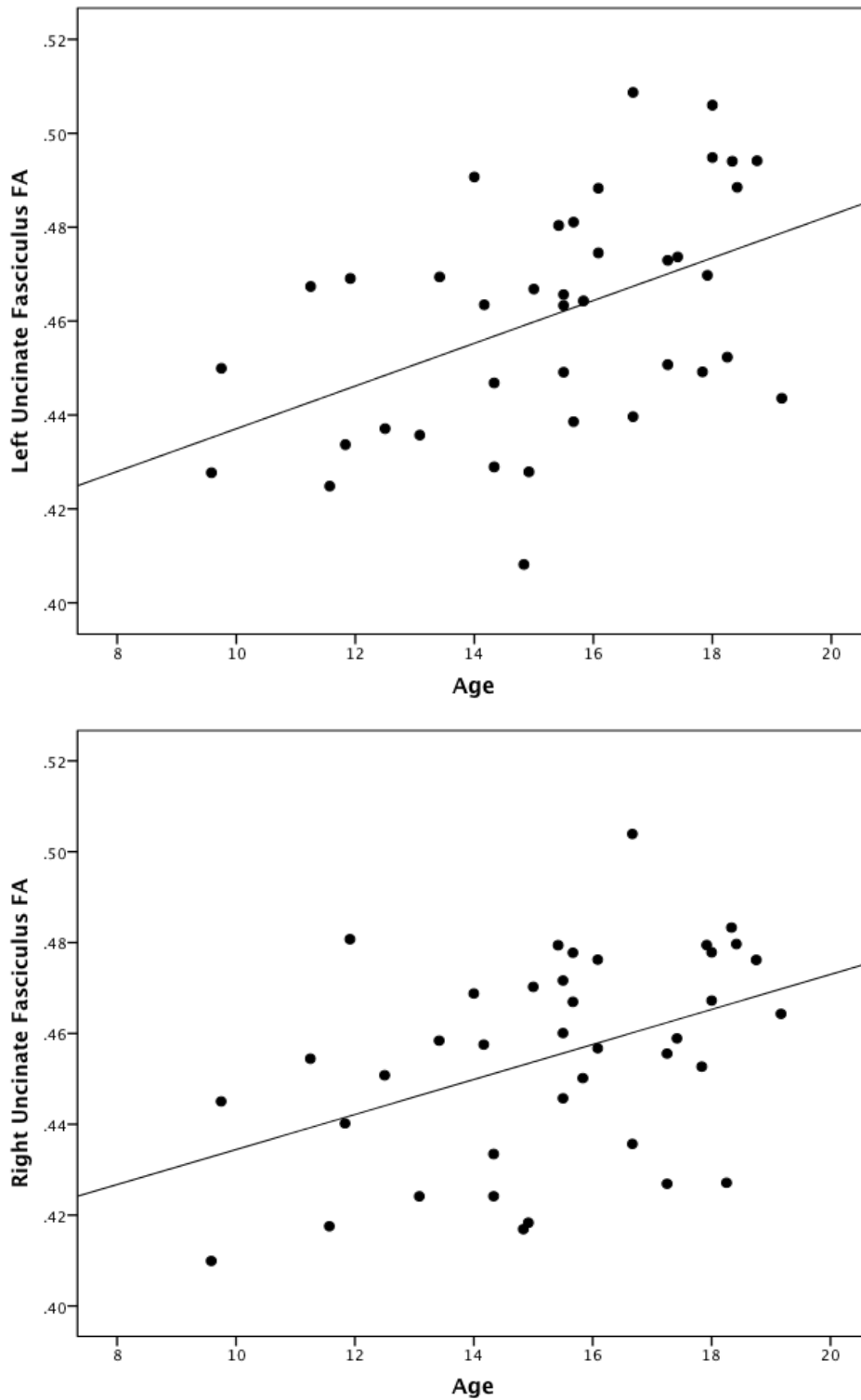


Figure 2.1 Cross-sectional change with age in left and right uncinatus fasciculus fractional anisotropy (FA) values

Age is positively correlated with mean FA values extracted from the left uncinatus fasciculus ($r=.46, p=.003$) and right uncinatus fasciculus ($r=.42, p=.007$).

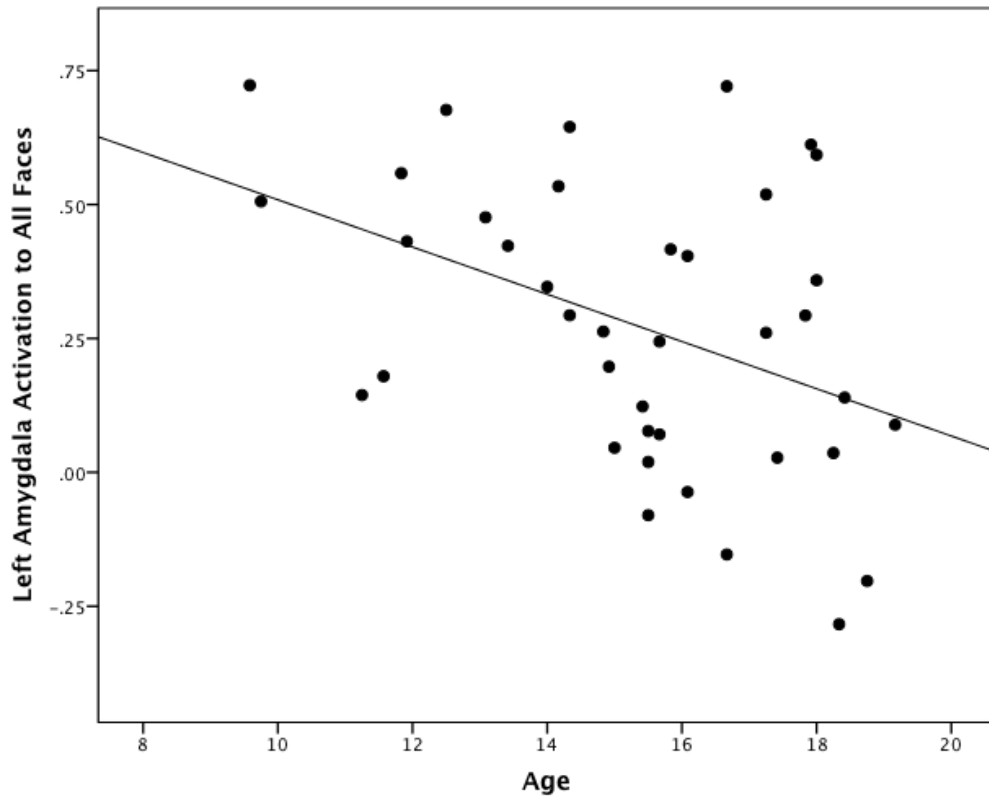
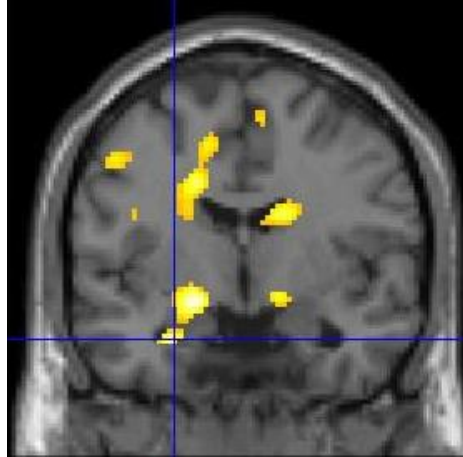


Figure 2.2 Cross-sectional change with age in left amygdala activation to all faces vs. baseline

Age is negatively related to left amygdala activation to all faces, $t(37)=3.75$, FWE-corrected $p=.011$, $xyz=-26, -2, -26$. Figure demonstrates the negative effect of age on activation and is thresholded at $p<.01$ uncorrected in order to demonstrate extent of activation. Scatterplot demonstrates mean amygdala activation extracted from structurally-defined left amygdala region of interest.

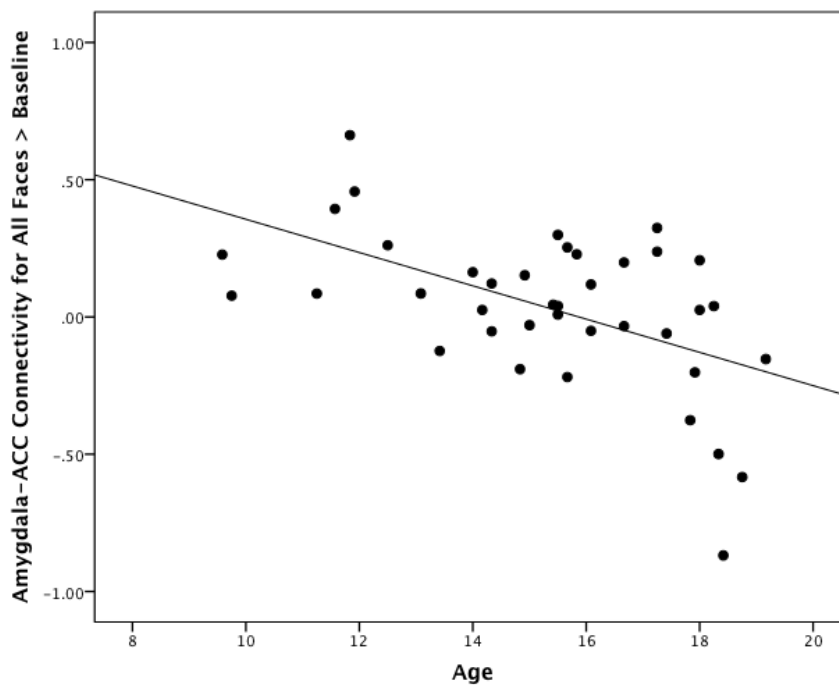
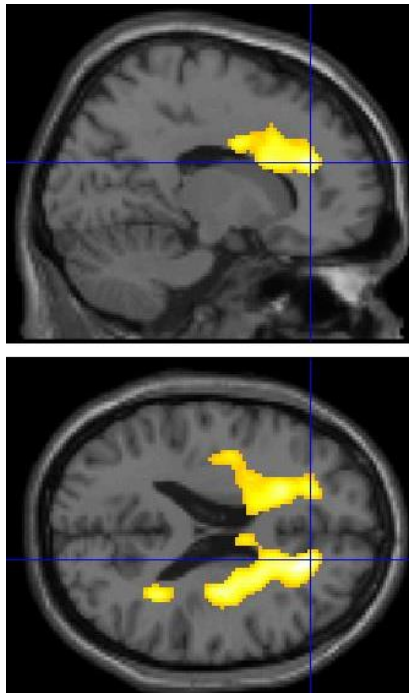


Figure 2.3 Cross-sectional change with age in left amygdala connectivity with the anterior cingulate cortex (ACC)

Age is negatively related to left amygdala-right ACC connectivity, $t(37)=3.94$, FWE-corrected $p=.03$, $xyz=16, 36, 22$. Figure demonstrates negative effect of age on PPI for all faces vs. baseline. Scatterplot demonstrates contrast values for PPI extracted from a 3 mm sphere around peak activation in the ACC.

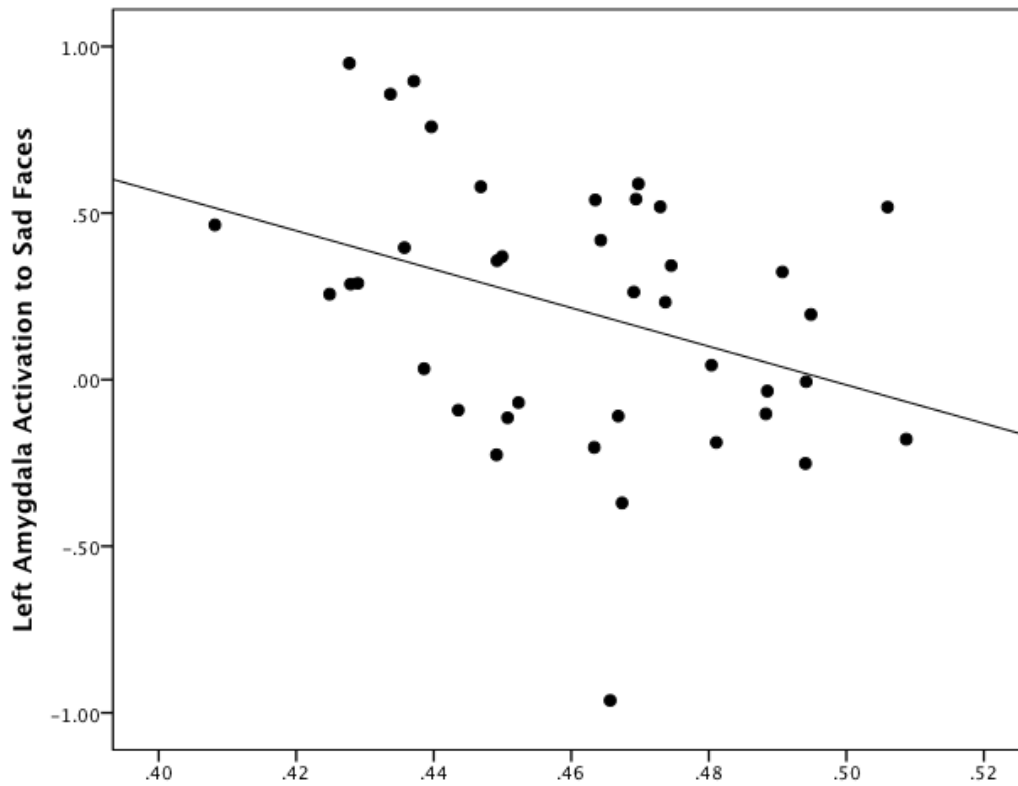
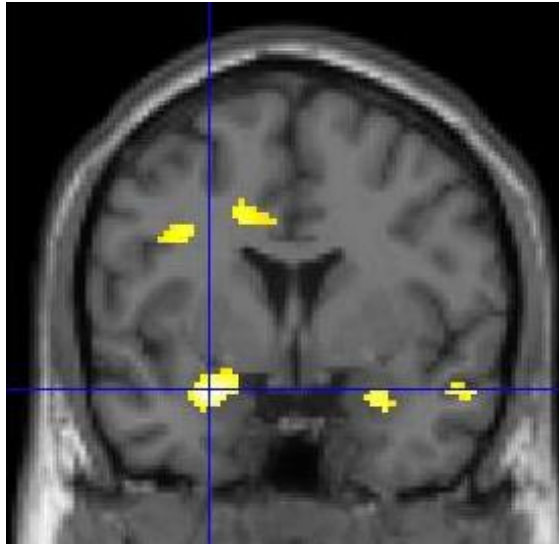


Figure 2.4 Negative relation between uncinete fasciculus fractional anisotropy and left amygdala activation to sad faces

There is a negative relation between fractional anisotropy (FA) within the left uncinate fasciculus and left amygdala activation to sad faces vs. baseline, $t(37)=3.87$, FWE-corrected $p=.004$, $xyz=-26, 2, -18$. Amygdala values for scatterplot are extracted using structural left amygdala ROI.

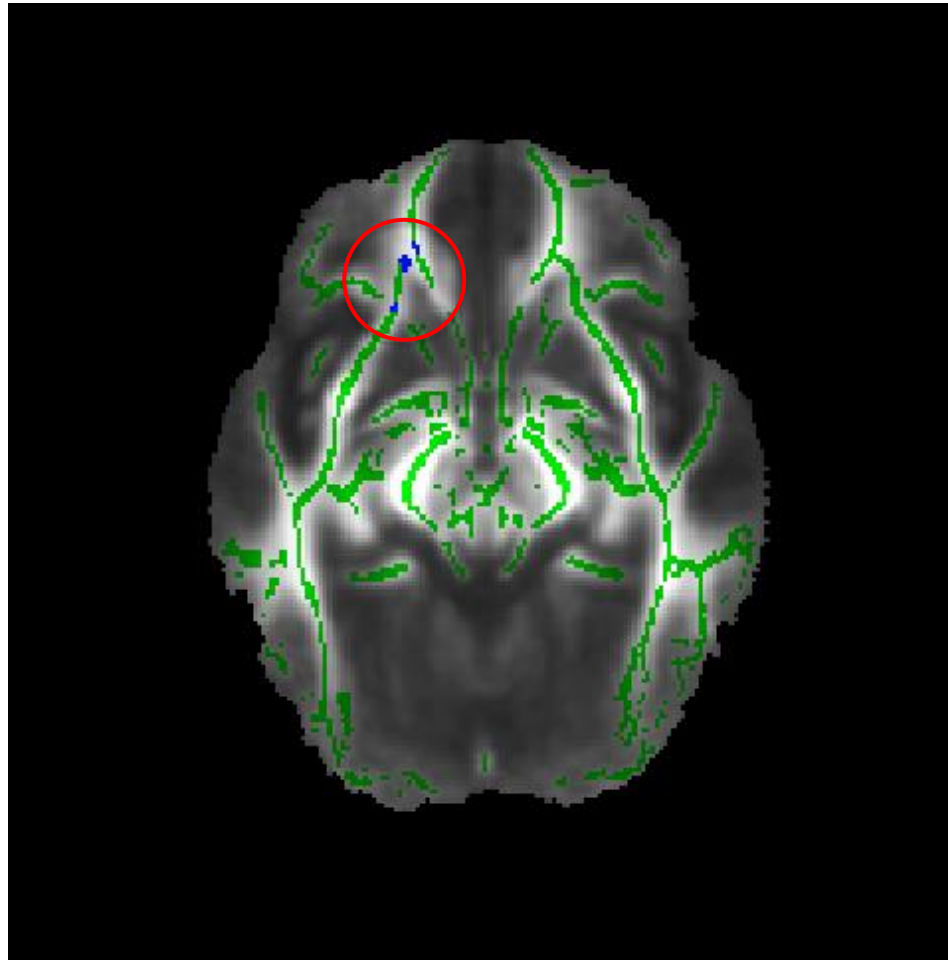


Figure 2.5 Relation between fractional anisotropy (FA) within the left uncinate fasciculus and left amygdala activation to sad faces vs. baseline

Green regions indicate mean FA skeleton and blue regions indicate areas negatively related to left amygdala activation to sad faces vs. baseline. Mean contrast values for amygdala activation were extracted from the anatomically-defined left amygdala region of interest and regressed onto FA. Results are thresholded at $p < .05$, Threshold-Free Cluster Enhancement corrected.

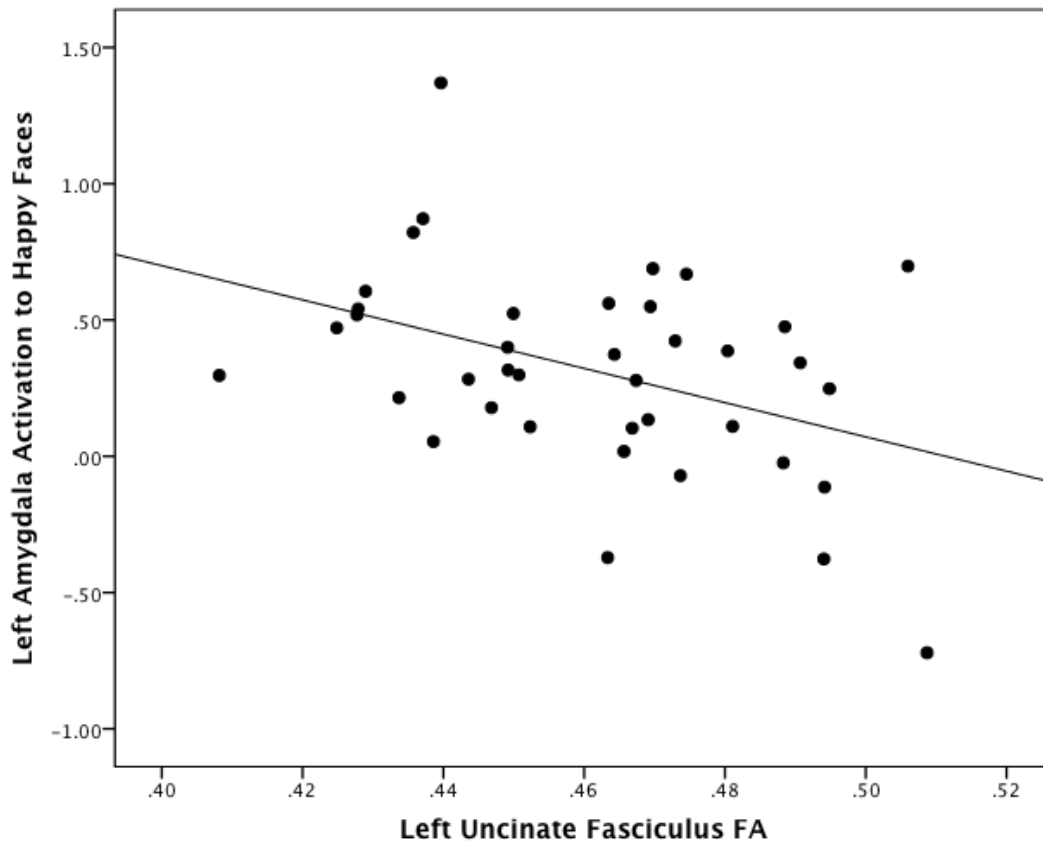
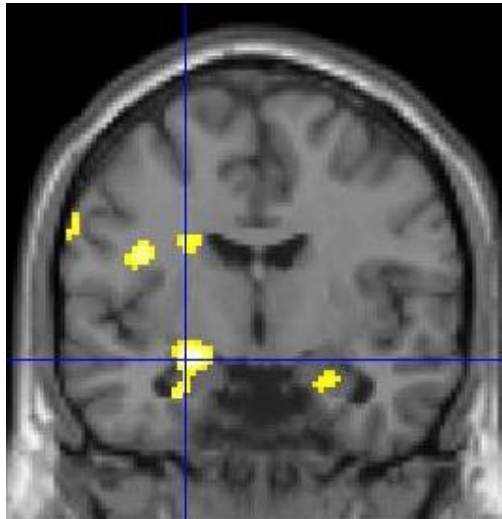


Figure 2.6 Negative relation between uncinate fasciculus fractional anisotropy and left amygdala activation to happy faces

There is a negative relation between fractional anisotropy (FA) within the left uncinate fasciculus and left amygdala activation to happy faces vs. baseline, $t(37)=3.76$, FWE-corrected $p=.005$, $xyz=-24, -6 -14$. Amygdala values for scatterplot are extracted using structural left amygdala ROI.

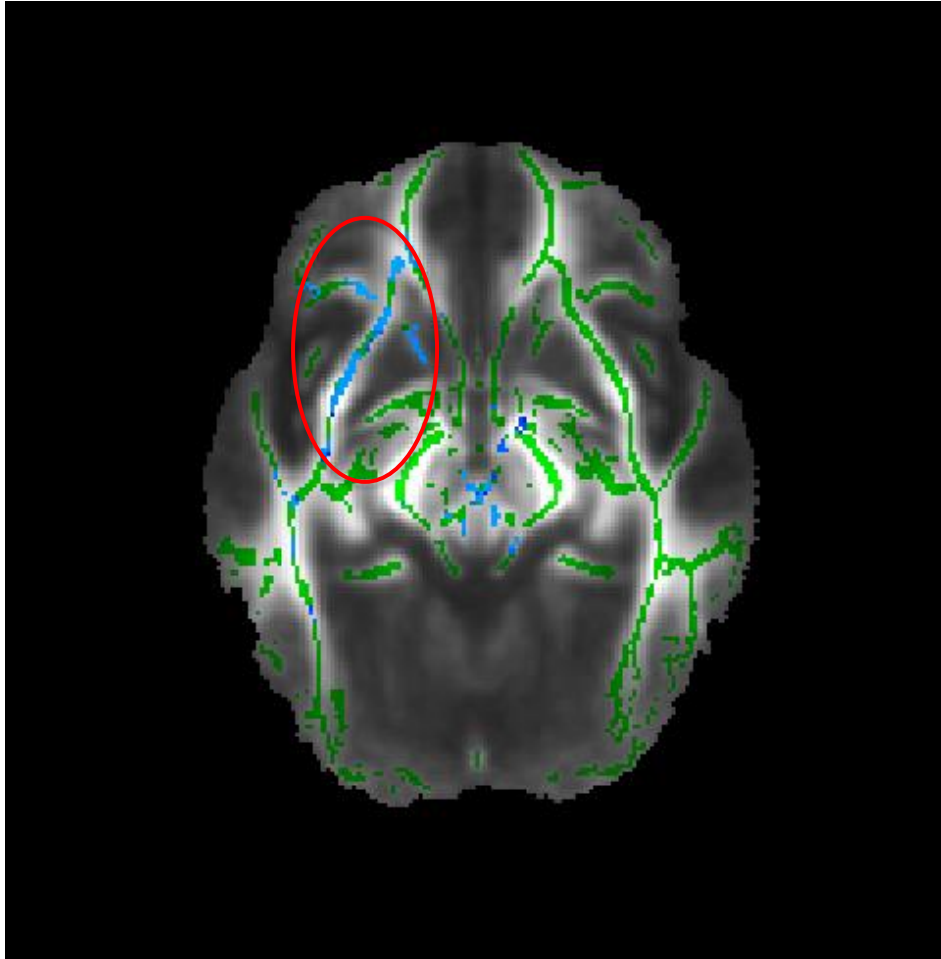


Figure 2.7 Relation between fractional anisotropy (FA) within the left uncinate fasciculus and left amygdala activation to happy faces vs. baseline

Blue regions indicate areas negatively related to left amygdala activation to happy faces at $p < .05$ Threshold-Free Cluster Enhancement corrected.

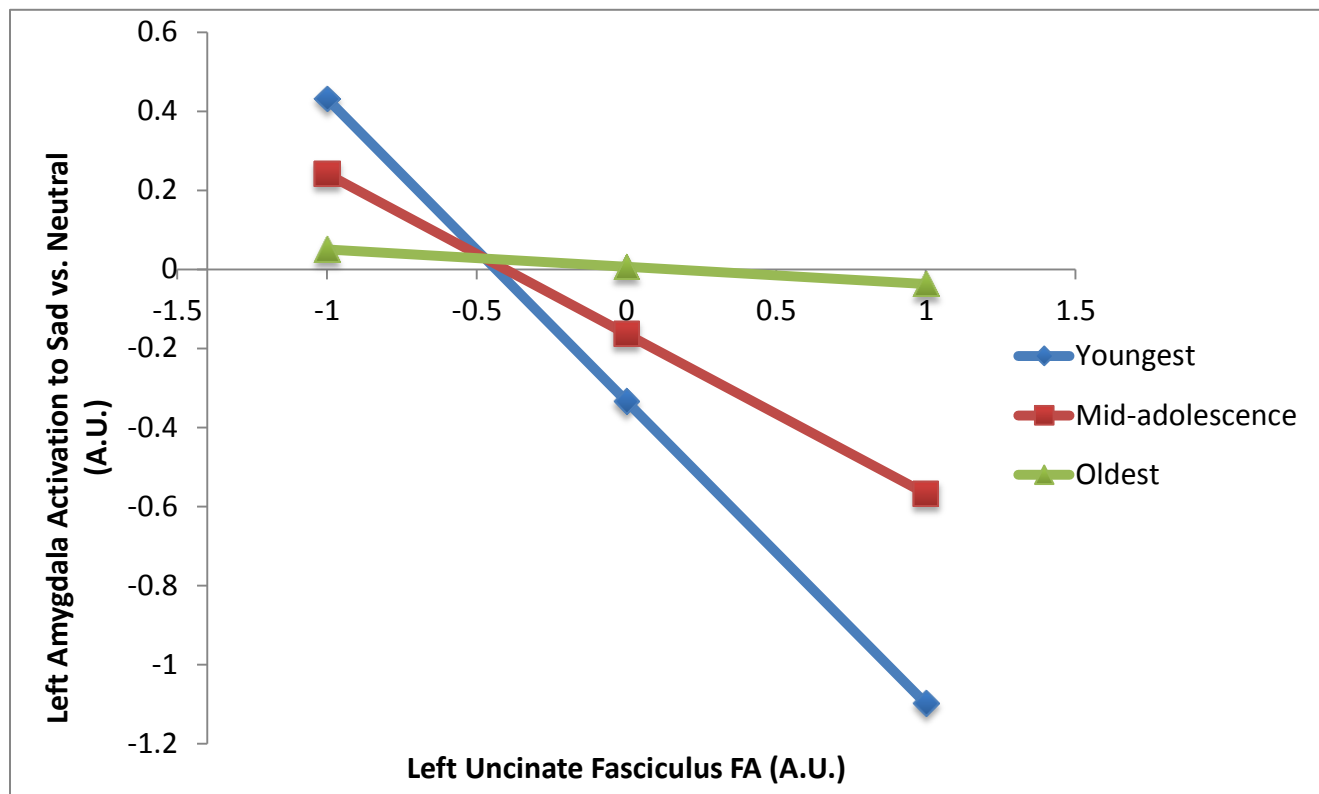


Figure 2.8 The association between structural connectivity and amygdala activation to sad faces is moderated by age

Lines represent estimated outcomes for left amygdala activation to sad vs. neutral faces as a function of left uncinat fasciculus FA and age based on the regression equation for the moderation effect for a) mean age of sample (mid-adolescence), b) 1 standard deviation below (youngest), and c) 1 standard deviation above (oldest).

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Chapter 3³

Changes in Amygdala Activation across a Scanning Session in Children and Adolescents with Anxiety Disorders

Introduction

Characterization of neural abnormalities associated with the processing of emotional stimuli in anxiety disorder patients has the potential to inform our understanding of the development and treatment of anxiety disorders (Hyde, Bogdan, & Hariri, 2011; Paulus & Stein, 2007; Swartz & Monk, in press). By serving as a mediator, neural activation can be used to detect relations between developmental and treatment effects that may alter neural activity (e.g., genes, environments, pharmacological intervention) and outcomes such as symptoms or disorders. In order to be useful for these purposes, it is first necessary to understand the specific contexts in which abnormal neural processing occurs and why it occurs under these conditions. Moreover, identifying abnormalities observable during childhood and adolescence, the developmental stages when anxiety disorders most frequently onset (Kessler et al., 2005), will be critical for advancing our knowledge of the development of these disorders.

Amygdala activation has the potential to serve as a biomarker for the development and treatment of anxiety disorders, given its role in socio-emotional processing (Adolphs, 2010). There is substantial evidence for abnormalities in amygdala activation in anxiety disorder patients. Here, I focus on studies of generalized anxiety disorder (GAD), social phobia (SP), or separation anxiety disorder (SAD) as they share overlap in cognitive and neural abnormalities in

³ Chapter 3 corresponds to the publication Swartz et al. (in preparation-a).

pediatric patients (Pine, 2007). Meta-analytic studies of functional MRI (fMRI) in adult anxiety disorder patients indicate heightened amygdala activation during the processing of threatening or emotion-related stimuli (Etkin & Wager, 2007; Hattingh et al., 2013) supporting its potential use as a neural mediator to study anxiety disorder development.

Several studies have provided evidence of amygdala hyper-activation during emotion processing in children and adolescents with anxiety disorders. Direct emotion processing tasks requiring participants to rate how afraid they felt while viewing fearful faces (Beesdo et al., 2009; McClure et al., 2007), or how they would be evaluated by disliked peers (Guyer et al., 2008a) have elicited heightened amygdala activation in patients relative to controls. Likewise, implicit emotion processing tasks, including identifying the gender of threatening faces (Battaglia et al., 2012; Blair et al., 2011), have also provided evidence for amygdala hyper-activation in pediatric anxiety disorder patients.

Whereas the studies described above used a traditional approach of examining mean group differences in activation across an entire emotion processing task, other research suggests that amygdala activation in anxiety disorder patients may vary depending on the timing of a task. For instance, Sladky and colleagues (2012) found that when adult anxiety disorder patients perform an emotional face matching task, patients exhibit an initial heightened amygdala response to faces relative to controls during the first blocks of the scanning session, but then subsequently demonstrate decreases in amygdala activation over the course of scanning. These results suggest that amygdala hyper-activation in anxiety disorder patients may not be stably high over the entire course of scanning and differences in activation relative to controls may vary at different points of the task (e.g., during initial exposure to novel stimuli versus more prolonged exposure). However, because this task was performed in adults, it is unclear whether

children and adolescents with anxiety disorders would also evidence changes in amygdala activation over time during a direct emotion processing task such as emotional face matching.

Another line of studies conducted in pediatric anxiety disorder patients suggests amygdala activation may vary depending on the length of presentation of stimuli. In the probe detection task, participants view a pair of faces followed by a probe, and are required to indicate the location of the probe with a button press. When threatening faces are presented very briefly (17 ms) and then masked during the probe detection task, pediatric anxiety disorder patients evidence amygdala hyper-activation relative to controls (Monk, et al., 2008). In contrast, when threatening faces are presented for relatively longer presentation times (500 ms) during the probe detection task, anxiety disorder patients do not evidence heightened amygdala activation (Monk, et al., 2006). Instead, they evidence an attentional bias away from threatening faces and increased activation in the ventrolateral prefrontal cortex (Monk, et al., 2006). These results suggest that differences in the dynamics of processing and attending to emotional faces in pediatric anxiety disorder patients may lead to distinct patterns of amygdala activation at different points in time relative to controls. However, this suggestion is based on the observation of differences in activation during tasks that varied in length of presentation of emotional stimuli at the trial level (between 17 ms and 500 ms). To date, no study in pediatric anxiety disorder patients has directly examined change in amygdala activation during a direct emotion processing task over the course of a scanning session, which would allow a more direct comparison of changes in activation during initial exposure to emotional stimuli relative to more prolonged exposure.

An investigation of changes over time in amygdala activation in pediatric anxiety disorder patients is warranted for several reasons. First, the results will have implications for

understanding how factors associated with task design, such as the length of the scanning session, may affect findings of amygdala activation. Additionally, such an investigation will contribute to our knowledge of the dynamics of amygdala dysfunction in pediatric anxiety disorder patients. For example, a finding of changes in amygdala activation over time could indicate that patients evidence different emotion processing styles during first exposure to novel emotional stimuli relative to prolonged exposure. In contrast, a pattern of consistent amygdala hyper-activation over time would suggest a more stable abnormality in emotion processing.

Altered patterns of amygdala activation may be associated with abnormal patterns of connectivity between the amygdala and prefrontal cortex, which has direct anatomical connections to the amygdala and may play a regulatory role in inhibiting amygdala activation (Phillips, Ladouceur, & Drevets, 2008; Ray & Zald, 2012). In pediatric anxiety disorder patients, decreased amygdala-ventral prefrontal cortex connectivity is observed when threatening stimuli are presented briefly (Monk, et al., 2008) but when stimuli are presented for longer presentation times, anxiety disorder patients evidence increased ventral prefrontal cortex activation relative to controls (Monk, et al., 2006). Therefore, abnormal ventral prefrontal cortex-amygdala connectivity may contribute to different abnormalities in amygdala function at different points in time. However, no study to date has examined whether there are changes in amygdala-ventral prefrontal cortex connectivity across the course of scanning and whether these differ in anxiety disorder patients relative to controls.

Age of participants may also affect differences in amygdala activation observed in anxiety disorder patients. As discussed in more detail in Chapters 1 and 2, in typically developing youth, amygdala activation to emotional faces is heightened in children and adolescents relative to adults (Gee et al., 2013; Guyer et al., 2008b; Hare, et al., 2008; Swartz,

Carrasco, Wiggins, Thomason, & Monk, under review). Relatively little work, however, has examined cross-sectional change in amygdala activation in pediatric anxiety disorder patients. Therefore, it is still unknown whether group differences in amygdala activation in anxiety disorder patients relative to controls differ with age across the periods of childhood and adolescence.

The goal of the present study was to further characterize abnormalities in amygdala response in pediatric anxiety disorder patients by examining whether amygdala activation changes over the course of a scanning session. In order to do so, an emotional face matching task was chosen to tap direct emotion processing during fMRI scanning. To my knowledge, this type of emotion matching task has not been used within a pediatric anxiety disorder sample. I hypothesized that I would observe one of two patterns within pediatric anxiety disorder patients: either they would evidence overall amygdala hyper-activation over the course of scanning, or they would evidence an initial heightened amygdala response followed by decreases over time, similar to previous findings in adult anxiety disorder patients performing an emotional face matching task (Sladky, et al., 2012).

Moreover, I hypothesized that changes in amygdala-ventral prefrontal cortex connectivity over the course of scanning would differ between anxiety disorder patients and controls. I also examined whether amygdala response predicted anxiety symptom severity within the patient group and conducted preliminary analyses in order to determine whether differences in amygdala activation existed across the diagnostic categories of pure GAD, pure SP, or comorbid anxiety disorders. Finally, I investigated whether age-related changes in amygdala response varied across the patient and control groups, hypothesizing that control participants would show the previously

observed pattern of decreases in amygdala activation with age, whereas patients would not demonstrate this pattern.

Methods

Participants

Participants with anxiety disorders were recruited through clinics associated with the university and controls were recruited via fliers and postings throughout the community. Primary diagnosis was based on structured clinical interview with the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997) for patients 17 years and younger and with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-IV; First, Gibbon, & Williams, 1997) for patients 18 years and older. Structured clinical interview was also used to confirm a lack of psychiatric diagnosis within the control group. Inclusion criteria for the anxiety disorder group included having a primary diagnosis of GAD, SP, or SAD and exclusion criteria included a lifetime history of bipolar disorder, schizophrenia, mental retardation, or developmental disorders. In line with previous work (Beesdo, et al., 2009; Guyer et al., 2008a; McClure et al., 2007; Monk, et al., 2006), I included participants with GAD, SP, or SAD in order to increase the sample size within the anxiety disorder group and because these disorders are highly comorbid during development (Verduin & Kendall, 2003). Participants with secondary diagnoses (obsessive compulsive disorder, tics, panic disorder, posttraumatic stress disorder, specific phobia) were included if it was determined by the clinician that SP, GAD, or SAD was the primary diagnosis. None of the anxiety disorder participants were currently taking psychotropic medications or undergoing psychotherapy treatment, as most patients were enrolled as part of the pre-treatment phase of a treatment study.

A total of 45 participants with a primary diagnosis of GAD, SP, or SAD and 26 controls completed the emotional face-matching task during fMRI scanning. Three patients dropped out during scanning, 12 participants were removed for having >3mm maximum movement from the reference image or maximum Euclidean distance for translation or rotation during scanning (7 patients and 5 controls), 2 participants were removed due to <60% accuracy on the behavioral task (1 patient and 1 control), and 1 control was removed due to poor normalization, leaving 34 participants in the anxiety disorder group and 19 controls between 7 and 19 years old available for analysis (Table 3.1). There were 9 participants with a primary diagnosis of GAD, 7 with SP, and 18 with comorbid diagnoses involving a combination of GAD, SP, and SAD. Excluded anxiety disorder patients were significantly younger ($M=11.2$ years, $SD=3.5$) than included patients ($M=13.9$, $SD=3.2$), $t(43)=-2.4$, $p=.02$. Excluded patients did not differ from included patients in anxiety symptom severity based on the anxiety measures described in the following section.

Procedure

Experimental Task. Participants performed the Emotional Face Assessment Task (EFAT) during scanning. This task is based on a well-established emotional face-matching paradigm (Hariri, Tessitore, Mattay, Fera, & Weinberger, 2002) and a similar version of this task has been shown to elicit amygdala activation in typically developing adolescents (Forbes, Phillips, Silk, Ryan, & Dahl, 2011). Face-matching trials of the EFAT consisted of three faces in a triangular configuration with a target face on the top row and an emotional and neutral face on the bottom row (Figure 3.1). Participants were instructed to match the emotion of the target face to one of the two faces on the bottom row. The non-matching emotional face on the bottom row was always neutral. Faces were selected from a validated set of emotional face stimuli (Gur et al.,

2002). For the baseline comparison, participants viewed a trio of shapes and were required to match a target shape on the top row with one of two shapes on the bottom row (Figure 3.1). Participants responded with a button box; accuracy and reaction times (RT) were recorded.

The task consisted of 18 faces blocks with 6 blocks each of the following types of emotional faces: fearful, angry, and happy. These blocks were interleaved with 18 shape-matching blocks. The order of emotional face blocks was counterbalanced across participants. Each block was 20 seconds long and consisted of 4 trials lasting 5 seconds each. The task was performed across two runs.

fMRI Data Acquisition. MRI images were acquired on a 3.0 Tesla GE Signa. A high-resolution T1-weighted spoiled-gradient echo (SPGR) image (TR=9ms, TE=1.8ms, flip angle=15 degrees, slice thickness=1.2 mm, 124 slices, FOV=256x256 mm) was acquired for anatomical reference and T2*-weighted BOLD images were acquired using a reverse spiral sequence (TR=2,000 ms; TE=30 ms; slice thickness=3 mm, 43 slices collected parallel to the AC-PC line; 64x64 matrix; 220x220 mm field of view; flip angle=90 degrees) for the functional data.

Measures. Anxiety symptoms were measured with the Multidimensional Anxiety Scale for Children (MASC; March, Parker, Sullivan, Stallings, & Conners, 1997) and the Liebowitz Social Anxiety Scale (LSAS; Heimberg et al., 1999). The former was chosen as a measure of total anxiety symptoms across a range of dimensions (social anxiety, separation anxiety, etc.) whereas the latter provides a more specific measure of social anxiety symptoms, which are particularly relevant to the developmental stages under investigation (Kessler, et al., 2005). Pubertal status was assessed with the Pubertal Development Scale (Petersen, Crockett, Richards, & Boxer, 1988) or an adapted version of this scale from the Youth-Nominated Support Team

study (King et al., 2009) and then converted to Tanner stages. Because age was highly correlated with pubertal development, $r=.87$, $p<.001$, for analyses of cross-sectional changes, I focused on age as the variable of interest as it would be difficult to disentangle the effects of age and puberty within this sample.

Analyses

Behavioral Data Analysis. Mean accuracy and RT were obtained for each condition. Group differences in behavior were examined using repeated-measures ANOVA in SPSS v20.

fMRI Data Analysis. Data underwent a standard preprocessing procedure in SPM8. Large spikes in the k-space data were filtered out and data were reconstructed into images using field map correction to decrease distortions. Functional images were slice-timing corrected and realigned to the first volume of the first run. Coregistration was done in two steps. First, the T1-overlay was coregistered to the realigned functional images. Then the high resolution T1 was coregistered to the (coregistered) T1-overlay. The high resolution T1 was then segmented using voxel-based morphometry (VBM8) and normalized to a template in Montreal Neurological Institute (MNI) space using DARTEL (Ashburner, 2007) and the resulting deformation field was applied to the time-series data. Finally, images were smoothed with a 6 mm full width at half maximum Gaussian kernel.

Condition effects were modeled at the individual subject level. In order to examine changes in activation across scanning, each block was modeled as a separate regressor. The six parameters from the realignment procedure were entered as nuisance covariates in the individual model. The following contrasts were then created: Faces1>Shapes1 (all faces blocks within the first third of the run vs. all shapes blocks within the first third of the run), Faces2>Shapes2 (all faces within the second third of the run vs. all shapes within the second third of the run),

Faces3>Shapes3 (all faces within the last third of the run vs. all shapes within the last third). This was done in order to minimize the effects of signal drift across scanning; if signal drift occurred, it should exert equivalent effects on the blocks of interest (the faces blocks) and the comparison condition (the shapes blocks) within each third of the run, thus the effect of signal drift should be subtracted out through this method. Each run was divided into thirds so that each contrast (e.g., Faces1>Shapes1) contained one face block for each emotion (angry, fearful, and happy). Each third of the task included three faces blocks and three shapes blocks, and combined across two runs, this provided a total of 240 seconds each for the Faces1>Shapes1, Faces2>Shapes2, and Faces3>Shapes3 conditions. Emotion-specific contrasts (e.g., Angry Faces1>Shapes1) were also created in order to examine effects for each type of emotional face block separately.

Psychophysiological Interaction Analysis. Psychophysiological interaction (PPI) was performed in order to examine differences in connectivity between the groups (Friston et al., 1997). PPI was conducted in SPM8 by extracting the time course from the left or right amygdala seed. For the PPI, seeds were based on a functional mask of left or right amygdala activation to the contrast of all faces>all shapes. This approach was chosen in order to ensure that only voxels significantly activated during the viewing of faces were included in the seed. Separate PPI models were created for each third of scanning, thus conditions for the three PPIs were Faces1>Shapes1, Faces2>Shapes2, and Faces3>Shapes3. The PPI model included a regressor for the time course of amygdala activation (the physiological variable), the condition of interest (e.g., Faces1>Shapes1, the psychological variable), and the interaction of these (the psychophysiological interaction). Thus, the PPI indicates regions where connectivity with the amygdala was modulated by task condition (matching faces relative to matching shapes).

Hypothesis 1: Differences in Amygdala Response in Anxiety Disorder Patients Relative to Controls

Because it was possible that changes in amygdala activation over time would occur at several different levels (across runs and within runs), group differences in the pattern of changes over time were examined in several ways. First, in order to examine whether changes from the first run to the second run differed between groups, a group x run interaction was examined (e.g., differences between the groups in amygdala activation for all faces in the first run vs. all faces in the second run). Age was entered as a covariate for this analysis and for all following analyses.

Next, to examine changes within runs, contrasts for face vs. shape matching for each third of the scan were entered into a full factorial model in SPM8. The interaction of time (first third, second third, last third) x emotion (Angry, Fearful, Happy) x group (anxiety disorder, controls) was examined in order to determine whether there were differences in changes within runs between the groups that varied by emotion. If this interaction was not significant, the interaction of time (Faces1>Shapes1, Faces2>Shapes2, Faces3>Shapes3) x group was examined to assess group differences in changes in amygdala activation across all emotion types. This analysis was first conducted collapsing across the two runs, and subsequently was performed separately within each run in order to determine whether effects varied by run. Finally, the main effect of group was examined in order to determine whether there were overall group differences in activation averaged across the entire task (similar to the traditional mean group differences approach). For this analysis and all subsequent analyses performed in SPM8, results were first displayed at $p < .01$ uncorrected and family-wise error (FWE) small-volume correction was applied within the bilateral amygdala region of interest (ROI), structurally defined using the Wake Forest University Pickatlas (WFU Pickatlas; Maldjian, Laurienti, Kraft, & Burdette,

2003). The significance threshold was set at $p < .05$ FWE-corrected for ROI analyses. Whole-brain effects outside of the *a priori* ROI were subsequently examined using a threshold of $p < .001$ uncorrected and a cluster threshold of 10 voxels.

Because prior research points specifically to amygdala hyper-activation to threatening faces in anxiety disorder patients (Beesdo et al., 2009; Blair et al., 2011; McClure et al., 2007; Monk et al., 2008), I also performed a planned post-hoc analysis to examine differences in amygdala activation for the contrasts of angry vs. happy faces and fear vs. happy faces, in order to detect whether there was a threat-specific effect in the present study.

Hypothesis 2: Differences in Overall Connectivity and Changes in Connectivity across Scanning in Anxiety Disorder Patients Relative to Controls

A similar approach as for the first hypothesis was used in order to examine differences in connectivity. Based on the results for the first hypothesis, I collapsed all three emotional face types (angry, fearful, and happy) into a faces condition rather than examine emotional stimuli separately for this analysis. A full factorial model was created using the PPI images for each third of the run. A time (PPI for Faces1>Shapes1, Faces2>Shapes2, and Faces3>Shapes3) x group (anxiety disorder, controls) interaction was examined in order to determine whether changes in connectivity across scanning differed by group. The main effect of group was examined in order to test whether there were overall group differences in connectivity across the entire scanning session. Based on prior research (Monk et al., 2008), I selected the ventrolateral prefrontal cortex (defined using the WFU Pickatlas as Brodmann's Area 47) as the ROI. If a significant PPI was detected, I planned to perform a post-hoc analysis in order to examine whether connectivity predicted amygdala activation.

Secondary Analyses: Amygdala Response and its Relation to Anxiety Symptoms and Diagnosis

Based on the results obtained for the first hypothesis, I calculated the difference between amygdala activation to faces during the first third of scanning and amygdala activation to faces during the last third of scanning in order to create a difference score representing the change in amygdala activation from beginning to end of scanning. I then used partial correlations in SPSS v20 to examine the relation between MASC and LSAS scores and amygdala change, controlling for age, within the anxiety disorder group. Differences across diagnostic categories were examined by performing a group (pure GAD, pure SP, and comorbid diagnoses) x time interaction similar to that used to examine differences in amygdala response with controls.

Hypothesis 3: Cross-Sectional Change with Age in Amygdala Response

The relation between amygdala response and age was examined by conducting a group x age interaction in SPSS using the difference score calculated as described above as the dependent variable. Because there was a significant correlation with age and mean RT on the matching task, $r = -.71$, $p < .001$, mean RT was entered as a covariate in this analysis.

Results

Group Differences in Behavioral Performance

There was no difference in accuracy between groups, but there was a main effect of condition, $F(3,49) = 53.84$, $p < .001$. This was driven by higher accuracy for fearful and happy face matching than the other conditions. Similarly, there was no group difference in RT, but there was a main effect of condition, $F(3,49) = 82.49$, $p < .001$, driven by faster reaction times for shape matching relative to the face matching conditions (Table 3.2).

Hypothesis 1: Differences in Amygdala Response in Anxiety Disorder Patients Relative to Controls

The group x run interaction was not significant, indicating there were no group

differences in changes in amygdala activation across runs. Collapsing across the two runs, the group x emotion x time interaction was not significant within the amygdala, indicating that differences across groups did not differ by emotion. However, in support of the hypothesis of abnormal changes in amygdala activation with time in anxiety disorder patients, the group x time interaction was significant in the left amygdala, $F(2,152)=8.09$, FWE-corrected $p=.023$, size=95 voxels, (-28, 0, -20). As shown in Figure 3.2, this interaction was driven by differences in patterns of activation across the groups. The control group maintained a steady level of activation across scanning, whereas the anxiety disorder group had a heightened initial amygdala response followed by decreases in amygdala activation across the following blocks. Examining the runs separately, the group x time interaction was not significant within the first run alone ($p=.25$) or in the second run alone ($p=.17$), although as shown in Figure 3.3, patients evidenced the same pattern of response within each run characterized by a heightened response relative to controls within the first third, followed by decreases over time.

The main effect of group was not significant within the amygdala, indicating that the groups did not differ in overall amygdala activation averaged across the course of scanning; however, a t-test for the contrast of anxiety disorder patients > controls for all faces > all shapes approached significance, $t(152)=2.92$, $p=.07$, corrected for the bilateral amygdala ROI. Whole-brain results for these tests are presented in Table 3.3. As seen in the table, the only main effect of group on activation (averaging across time) was a difference in right fusiform activation, with controls demonstrating greater activation relative to the anxiety disorder group for all faces. I did not find support for threat-specific effects in the amygdala in the planned post-hoc analyses, as there was no difference in amygdala activation between groups for the contrasts of angry vs. happy or fearful vs. happy face matching.

Hypothesis 2: Differences in Overall Connectivity and Changes in Connectivity across Scanning in Anxiety Disorder Patients Relative to Controls

I focused connectivity analyses on the left amygdala seed based on the results for the first hypothesis. The second hypothesis was not supported, in that there was no group x time interaction for left amygdala connectivity with the ventral prefrontal cortex based on the PPI of face vs. shape matching. There was also no main effect of group within the ventral prefrontal cortex, indicating that the two groups did not differ in left amygdala-ventral prefrontal cortex connectivity across the course of scanning. Whole-brain results for the connectivity analysis are presented in Table 3.4. Notably, controls evidenced greater connectivity between the left amygdala and several regions of the dorsal prefrontal cortex relative to the anxiety disorder group.

Secondary Analyses: Amygdala Response and its Relation to Anxiety Symptoms and Diagnosis

There was no relation between total MASC scores and amygdala response, but the relation between LSAS scores and change in amygdala activation approached significance adjusting for age within the anxiety disorder group, $r=.34$, $p=.06$. The positive correlation indicates that greater social anxiety symptom severity is associated with a greater decrease from the first to third portion of the task. In terms of diagnosis, there was no group x time interaction or main effect of group for different diagnoses within the amygdala, indicating that the pattern of amygdala response was similar across diagnostic categories (Figure 3.4).

Hypothesis 3: Cross-Sectional Change with Age in Amygdala Response

The group x age interaction and main effect of age were not significant for amygdala response.

Discussion

The aim of the present study was to investigate differences in the pattern of amygdala response over time in pediatric anxiety disorder patients relative to controls during an emotional face-matching task. I found that children and adolescents with anxiety disorders exhibit an altered pattern of amygdala response relative to controls across the course of scanning, characterized by initial heightened amygdala response to emotional faces, followed by a decline in amygdala activation across the session. As a result, I found no overall differences in amygdala activation between the groups when averaging across the entire scanning session. I did not find support for the hypotheses that changes in amygdala-ventral prefrontal cortex connectivity across the scanning session would differ between groups or that there would be group differences in age-related cross-sectional patterns.

The finding of abnormal changes in amygdala activation over scanning in pediatric anxiety disorder patients shows a very similar pattern to that observed by Sladky and colleagues (2012). Thus, this is an important extension of these results to a child and adolescent population. Also similar to the results of Sladky et al., I found that controls maintained a relatively steady level of amygdala activation across scanning. The finding of changes in amygdala activation over scanning in pediatric anxiety disorder patients has important implications for future research, as it suggests that certain features of the fMRI task will influence whether amygdala hyper-activation is observed in anxiety disorder patients.

There are several potential factors that may affect whether amygdala hyper-activation is observed in patients. These factors include the nature of the task performed and the length of the task. Amygdala hyper-activation has most consistently been shown in tasks that require participants to evaluate their own emotions or how they will be judged by peers (Beesdo, et al.,

2009; Guyer et al., 2008a; McClure et al., 2007), suggesting that focusing on internal states or evaluations related to emotional stimuli is most likely to produce overall mean group differences in amygdala activation averaged across an entire scanning session, whereas in the current emotion processing task (matching emotional faces), there was no overall group difference in activation. These results also suggest that amygdala hyper-activation is most likely to be observed during the beginning of a scanning session, thus using tasks with a shorter duration or focusing analyses on amygdala response at the beginning of an emotion processing task are approaches more likely to result in a finding of amygdala hyper-activation in pediatric anxiety disorder patients.

The results of this study identified two important abnormalities in amygdala activation in anxiety disorder patients: first, it showed a heightened initial amygdala response during the first third of the task, and second, it revealed an abnormal pattern of decreases in activation during the second and third portions. Further research is necessary in order to elucidate the mechanisms underlying heightened amygdala activation during the first portion of scanning in anxiety disorder patients. A study conducted in spider phobic adults found that spider phobic participants evidenced a faster onset and time to peak of the BOLD response within the amygdala to spider-related pictures relative to controls (Larson et al., 2006). Examining differences in amygdala response at the trial level may therefore be informative regarding the results obtained here at the block level. Additionally, the whole-brain results of the PPI analysis suggested that controls show greater left amygdala connectivity with several regions of the dorsal prefrontal cortex during scanning. Thus, controls may recruit prefrontal regulatory regions in order to regulate amygdala response to novel emotional stimuli at the beginning of a scanning session, whereas failure to do so may result in the heightened initial amygdala response observed in patients.

These results also raise questions regarding the mechanisms through which changes in amygdala activation occur in anxiety disorder patients and whether this is adaptive or related to worse symptom severity. It is possible that individuals with anxiety disorders recruit prefrontal regions in order to regulate amygdala response to emotional faces later in the scanning session, although I was unable to detect differences in ventral prefrontal cortex-amygdala connectivity between groups. Another possibility is that patients begin to avoid attending to the emotional faces across the scanning session, which is consistent with the findings of Monk et al. (2006). This would also be consistent with the finding in the present study of greater overall fusiform activation in controls, which could indicate that the anxiety disorder group was attending less to faces. Given that trials were 5 seconds each but participants responded on average within the first 2 seconds, they could potentially have attended away from faces after responding on each trial without evidencing a decrement in behavioral performance. Future research incorporating eye tracking will be necessary in order to determine whether there are differences in attention to the stimuli during scanning. This could potentially be informative regarding shifts in emotion processing in pediatric anxiety disorder patients when stimuli are first presented at the beginning of a scanning session relative to later, more prolonged exposure towards the end of a session.

Notably, the correlation between LSAS scores and change in amygdala activation across scanning approached significance, suggesting that participants who demonstrated a greater drop in amygdala activation had a higher number of social anxiety symptoms. This could be partially due to the fact that participants with higher initial levels of amygdala activation in the first third of scanning could evidence a greater drop in activation by the end of scanning, although the correlation between amygdala activation within the first third of scanning and LSAS scores was not significant ($p=.17$). These results suggest, then, that the decrease in amygdala activation

across scanning may be maladaptive, in that participants with the greatest decrease have the most severe social anxiety symptoms. Whether the decline in amygdala activation is due to over-regulation or attentional avoidance, failure to fully process emotional stimuli may prevent the ability to learn that they are safe, and may contribute to increased anxiety severity. These results could also be interpreted in the framework of the vigilance-avoidance hypothesis, which proposes that anxiety disorder patients initially evidence vigilance for threatening stimuli, but then a pattern of avoidance of threatening stimuli at longer presentation times when task demands are low (In-Albon, Kossowsky, & Schneider, 2010; Judah, Grant, Lechner, & Mils, 2013; Mogg, Philippot, & Bradley, 2004).

There was no interaction or main effect of age on change in amygdala activation over scanning, suggesting that this effect did not vary cross-sectionally. Given that patients were potentially altering their processing style over the course of the task, this may not have been an ideal task to capture cross-sectional changes in amygdala activation in the anxiety disorder group. Tasks that use briefer stimulus presentation periods in order to capture amygdala hyper-activation in anxiety disorder patients may reveal different cross-sectional associations with age for patients relative to controls.

It is important to note several limitations of the present study. First, as mentioned above, eye-tracking data were not collected, preventing an examination of whether there were differences in eye gaze patterns during performance of the emotional face-matching task. Second, because this was a blocked design, I was unable to separate out effects at the trial level. Future research incorporating a mixed or event-related design could be used to examine whether there are changes in amygdala activation during the first half of a trial (the first 2.5 seconds, during which participants make a behavioral response) and the second half of a trial. Finally, in

terms of examining differences across diagnostic groups, there were a relatively small number of participants within each diagnostic category. Therefore, these results should be considered preliminary and require further investigation with a larger sample.

In conclusion, I found that children and adolescents with anxiety disorders evidence an altered pattern of amygdala response over the course of fMRI scanning characterized by an initial heightened response followed by a reduction in amygdala activation. This suggests that future research using amygdala activation as a neural mediator for anxiety disorder development and treatment could examine the initial amygdala response or use a rapid, event-related design in order to elicit amygdala hyper-activation. Alternatively, the observed drop in amygdala activation from the beginning to end of scanning could potentially serve as a neural mediator for anxiety disorders, as it was found to be related to anxiety symptoms within the patient group. Important questions for future research include whether genetic and environmental influences predict the initial heightened amygdala response found in pediatric anxiety disorder patients and whether successful treatment with pharmacological intervention or psychotherapy is associated with a dampening of this initial hyper-responsiveness or alters the pattern of changes in amygdala activation observed over the scanning session.

Table 3.1 Participant characteristics

	Anxiety Disorder Group, n=34 (M, SD)	Control Group, n=19 (M, SD)	Group difference
Age	13.94 (3.2)	15.07 (4.0)	<i>t</i> (51)=1.13, <i>p</i> =.26
Gender (percent female)	71%	63%	<i>t</i> (51)=-.55, <i>p</i> =.59
Pubertal status	3.3 (1.4)	3.5 (1.6)	<i>t</i> (46)=.61, <i>p</i> =.55
MASC total scores	64.5 (17.6)	31.0 (12.9)	<i>t</i>(51)=-7.26, <i>p</i><.001
LSAS total scores	68.6 (30.6)	12.1 (10.9)	<i>t</i>(47)=-7.34, <i>p</i><.001

Note: Bold indicates a significant group difference. Pubertal status was measured using the Pubertal Development Scale; MASC=Multidimensional Anxiety Scale for Children; LSAS=Liebowitz Social Anxiety Scale. Data on pubertal status were missing for 3 anxiety disorder patients and 2 control participants; LSAS scores were missing for 2 anxiety disorder patients and 2 control participants.

Table 3.2 Behavioral results by condition and group

	Anxiety Disorder Group Accuracy (M, SD)	Control Group Accuracy (M,SD)	Anxiety Disorder Group RT (M ,SD)	Control Group RT (M, SD)
Angry Face	85.4% (9.4)	84.9% (10.3)	1795.1 (376.9)	1679.0 (480.0)
Fearful Face	96.0% (6.7)	97.2% (3.1)	1647.4 (370.7)	1542.8 (469.1)
Happy Face	97.4% (6.2)	97.1% (4.2)	1581.5 (388.0)	1482.7 (476.7)
Shape	91.3% (4.4)	91.5% (3.4)	1098.5 (259.0)	1005.3 (275.3)
All conditions	92.5% (5.2)	92.7% (3.1)	1530.6 (318.5)	1427.4 (399.7)

Note: RT=reaction time in ms.

Table 3.3 Whole-brain activation results for group differences in changes in activation and overall activation

Effect	Direction of effect	Statistic	Number of Voxels	Coordinates	Region
Group x time x emotion		$F(4,458)=5.48$	25	(-24, -22, -16)	Left hippocampus
		$F(4,458)=5.21$	12	(-34, -10, -20)	Left hippocampus
Group x time		$F(2,152)=8.49$	41	(-28, -2, -12)	Left putamen
		$F(2,152)=8.49$	17	(50, -66, 44)	Right angular gyrus
		$F(2,152)=7.70$	11	(-42, -70, 42)	Left angular gyrus
Main effect of group	Controls>AD	$F(1,152)=21.00$	70	(22, -76, -14)	Right fusiform
Group x emotion		No significant clusters			
Main effect of emotion	Threat>Happy	$F(2,458)=19.48$	940	(-38, 20, 0)	Left insula
	Threat>Happy	$F(2,458)=17.77$	639	(34, 24, -4)	Right insula
	Threat>Happy	$F(2,458)=16.02$	615	(2, 28, 42)	Right medial frontal gyrus
	Angry>Happy	$F(2,458)=13.14$	99	(-48, 40, 8)	Left inferior frontal gyrus
	Threat>Happy	$F(2,458)=13.12$	302	(46, 32, 40)	Right middle frontal gyrus
	Threat>Happy	$F(2,458)=13.09$	44	(-44, 50, -14)	Left inferior frontal gyrus
	Happy>Angry	$F(2,458)=12.87$	133	(18, -48, 62)	Right superior parietal region
	Threat>Happy	$F(2,458)=12.15$	164	(-28, -66, -14)	Left fusiform gyrus
	Threat>Happy	$F(2,458)=11.56$	223	(40, 44, 6)	Right middle frontal region
	Threat>Happy	$F(2,458)=11.51$	100	(44, 56, -10)	Right inferior frontal gyrus
	Angry>Happy	$F(2,458)=11.42$	62	(-50, -50, 10)	Left middle temporal gyrus
	Threat>Happy	$F(2,458)=10.34$	39	(-8, -74, -36)	Left cerebellum
	Threat>Happy	$F(2,458)=10.16$	154	(-30, -54, 38)	Left superior parietal lobule
	Angry>Happy	$F(2,458)=9.49$	21	(46, -58, 54)	Right angular gyrus

Threat>Happy	$F(2,458)=8.80$	16	(-36, -64, -32)	Left cerebellum
Threat>Happy	$F(2,458)=8.57$	15	(4, -84, -6)	Right lingual gyrus
Threat>Happy	$F(2,458)=8.46$	29	(10, -10, 12)	Right thalamus
Threat>Happy	$F(2,458)=8.33$	13	(-44, -74, -6)	Lateral occipitotemporal gyrus
Threat>Happy	$F(2,458)=8.00$	20	(-40, -92, 12)	Left occipital pole
Happy>Angry	$F(2,458)=7.86$	11	(-10, -56, 62)	Precuneus

Note: Threshold for whole-brain analyses is set at $p < .001$ uncorrected and cluster threshold of 10. Results were examined first using F-tests and then followed up with t-tests in order to determine direction of effect for main effects. Threat>Happy = Angry and Fearful faces>Happy faces; AD=Anxiety disorder group.

Table 3.4 Whole-brain activation results for group differences in psychophysiological interaction of left amygdala connectivity during face vs. shape matching

Effect	Direction of Effect	Statistic	Number of Voxels	Coordinates	Region
Group x time		$F(2,152)=9.65$	18	(-4, -62, 10)	Posterior cingulate
		$F(2,152)=8.88$	18	(48, -2, 48)	Right precentral gyrus
Main effect of group	Controls>AD	$F(1,152)=22.45$	43	(32, 26, 50)	Right middle frontal gyrus
	AD>Controls	$F(1,152)=21.67$	67	(30, -24, -12)	Right hippocampus
	Controls>AD	$F(1,152)=13.52$	15	(24, 42, 30)	Right superior frontal gyrus
	Controls>AD	$F(1,152)=12.98$	20	(18, 56, 26)	Right superior frontal gyrus

Note: AD=Anxiety disorder group.

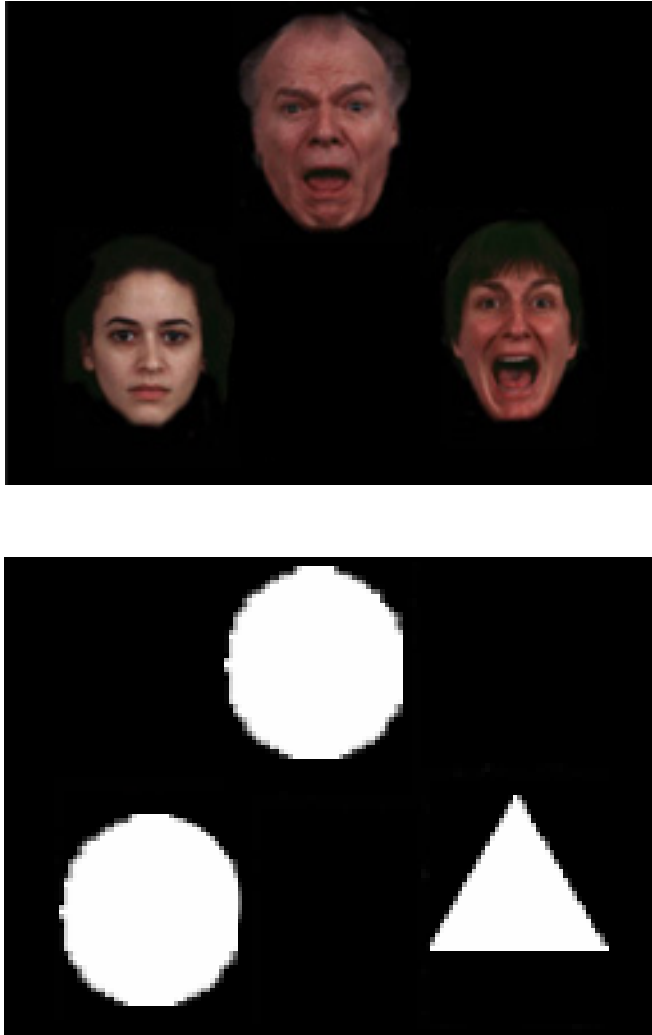


Figure 3.1 Example trials of the Emotional Face Assessment Task

An example trial of face matching with fearful faces (top). Participants used a button to indicate which of two faces on the bottom row matched the expression of the target face on the top row. The baseline comparison task was shape matching (bottom).

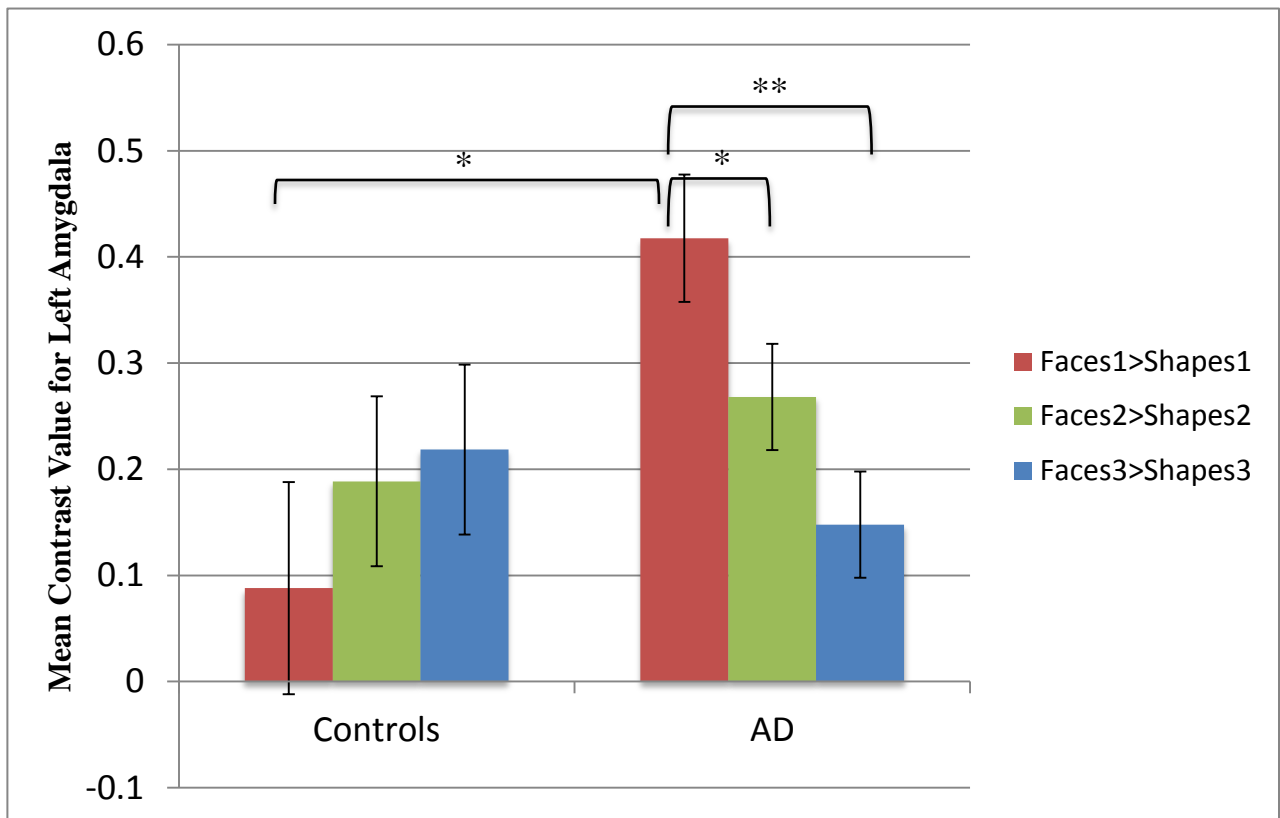
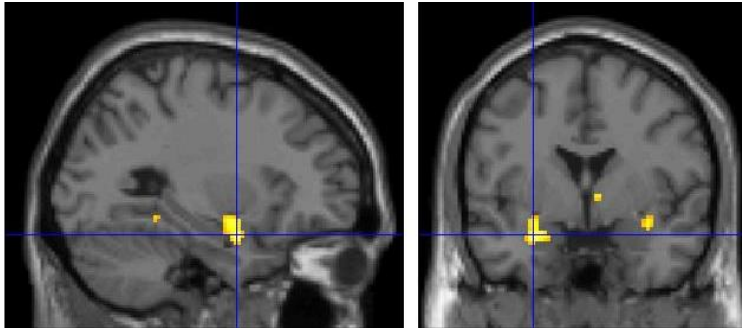


Figure 3.2 There is a significant interaction of group x time within the left amygdala

SPM figure is thresholded at $p < .01$ uncorrected and demonstrates the effect of group x time. Bar graph displays mean contrast values extracted from the anatomically-defined left amygdala for the following contrasts: Faces1>Shapes1, Faces2>Shapes2, and Faces3>Shapes3. AD=Anxiety disorder group. Error bars represent 1 standard error; * = $p < .05$, ** = $p < .001$.

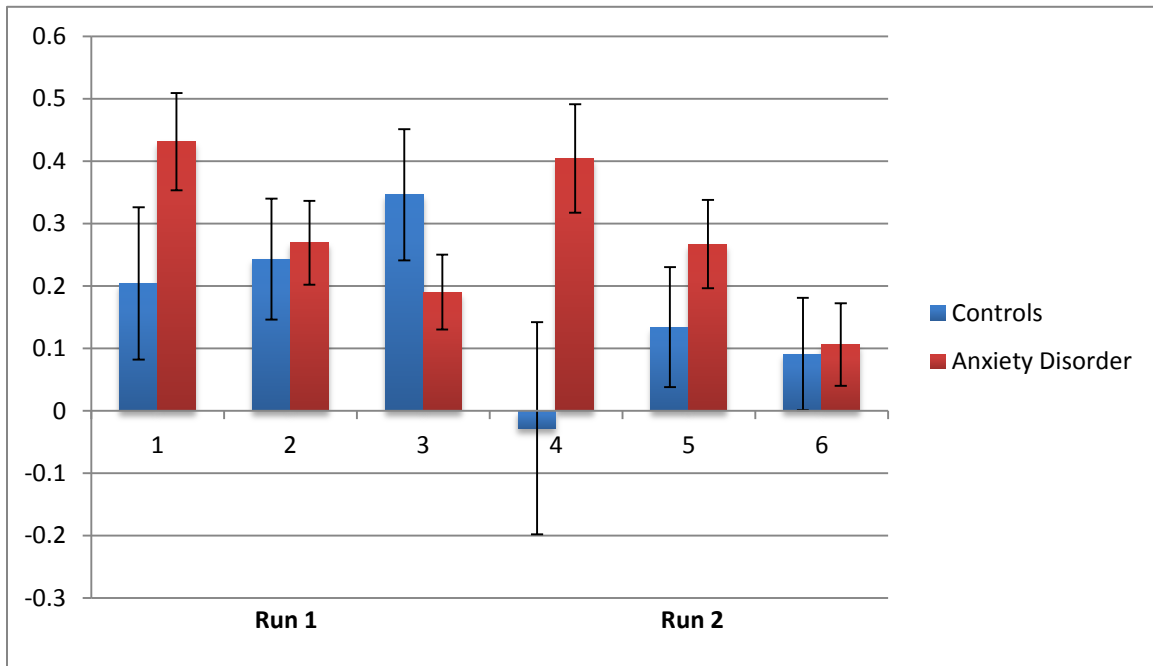
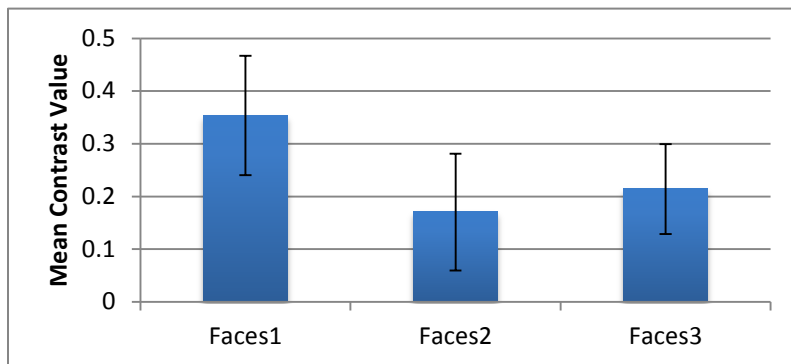


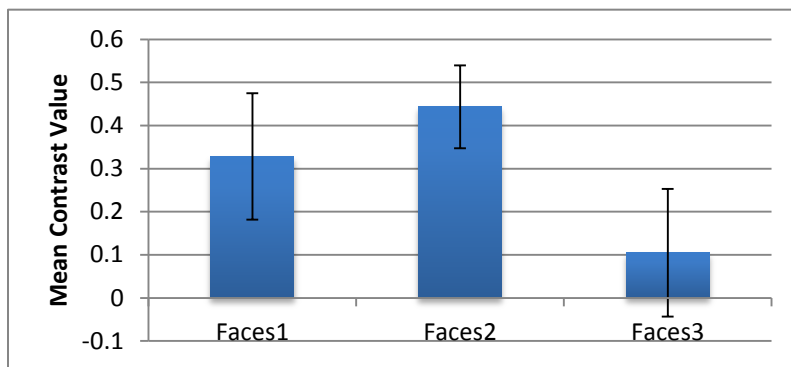
Figure 3.3 Changes in left amygdala activation viewed separately for each run

Bar graph displays mean contrast values extracted from the anatomically-defined left amygdala for the following contrasts: Faces1>Shapes1, Faces2>Shapes2, and Faces3>Shapes3 within the first run (bars 1-3) and within the second run (bars 4-6).

Generalized Anxiety Disorder Group



Social Phobia Group



Comorbid Disorders Group

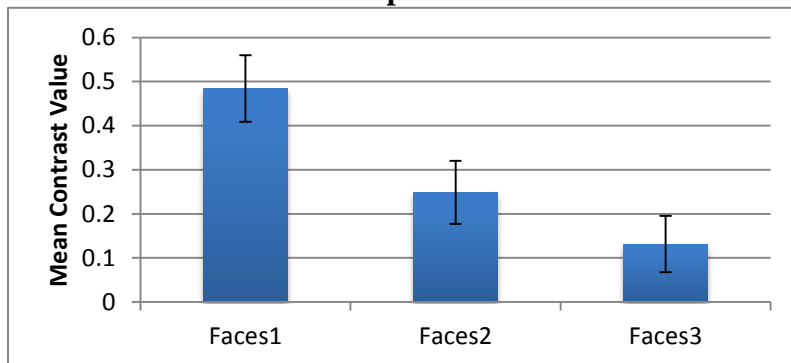


Figure 3.4 Left amygdala activation shows a similar pattern of changes in activation across diagnostic categories

Mean contrast values are extracted from the anatomically defined left amygdala region of interest for Faces1>Shapes1, Faces2>Shapes2, and Faces3>Shapes3.

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Chapter 4 ⁴

Altered Activation of the Rostral Anterior Cingulate Cortex in the Context of Emotional Face Distractors in Children and Adolescents with Anxiety Disorders

Introduction

Research in generalized anxiety disorder (GAD), social phobia (SP), and separation anxiety disorder (SAD) has produced evidence for abnormal amygdala function as a neural correlate of these disorders (Chapter 3). Emotion processing and regulation involves a complex interplay between the amygdala and prefrontal cortex; therefore, in addition to examining the amygdala, it is important to consider how prefrontal cortex function may be associated with the development of anxiety disorders. In this chapter, I examine the function of the rostral anterior cingulate cortex and functional connectivity with the amygdala in the context of a task with emotional face distractors in pediatric anxiety disorder patients.

Research frameworks have proposed that anxiety disorder patients evidence abnormal patterns of attention to threat, characterized by an initial attentional bias to threat followed by either difficulty disengaging attention from threatening stimuli or avoidance of threatening stimuli (Bishop, 2007; Britton, Lissek, Grillon, Norcross, & Pine, 2011; Mogg, Philippot, & Bradley, 2004; Pine, 2007). Whether patients exhibit difficulty disengaging attention from threat or avoidance of threat may be determined by the availability of attentional resources. Based on the hypothesis that avoidance of threat requires attentional control, it has been suggested that when attentional demands for a task are low (placing low demands on participants' attentional

⁴ Chapter 4 corresponds to the publication Swartz et al. (in preparation-b).

control resources), patients will exhibit avoidance of attention to threat with long stimulus presentation periods. In contrast, when a task is attentionally demanding (placing high demands on participants' attentional control resources and theoretically reducing their ability to exercise avoidance), patients will exhibit an attention bias towards threat and difficulty disengaging attention from the threatening stimulus (Judah, Grant, Lechner, & Mils, 2013). Judah et al. illustrated this experimentally using a probe detection task (with a 1500 ms presentation time to assess late attention bias) combined with an n-back task to manipulate working memory load (and thus load on cognitive control resources). When participants high in social anxiety performed this task and working memory load was low, they evidenced a pattern of attentional avoidance of threat. In contrast, when working memory load was high, they evidenced a pattern of attentional bias towards threat. Therefore, when performing a task that places demands on cognitive and attentional control resources, such as the one reported on in this chapter, anxious participants would be expected to evidence difficulty disengaging attention away from threat.

In explaining how attention bias to threat may develop in anxiety disorders, many frameworks draw on biased competition models, which propose that attention to threat is influenced by competition between “bottom-up” sensory processes involved in detecting threat and “top-down” influences on attention, such as a goal to attend towards non-emotional stimuli (Bishop, 2008). Bishop (2007) and others have thus suggested that anxiety disorders may be associated with over-activation of regions associated with detecting threat, such as the amygdala, and altered top-down control by regions associated with goal-directed attention. Top-down control regions within the prefrontal cortex (PFC) include the lateral PFC and the rostral anterior cingulate cortex (rACC), which are recruited to regulate attention to threat or resolve conflict between competing stimuli (Bishop, Duncan, Brett, & Lawrence, 2004; Browning, Holmes,

Murphy, Goodwin, & Harmer, 2010; Etkin, Egner, Peraza, Kandel, & Hirsch, 2006; Klumpp, Angstadt, & Phan, 2012).

Understanding the neural correlates associated with regulating attention to emotional stimuli in children and adolescents with anxiety disorders could have important clinical implications. Attention bias to threat is hypothesized to play a role in the development of anxiety disorders (Pine, 2007), and modifying attention bias to threat by training participants to attend away from threatening stimuli reduces anxiety symptoms (Bar-Haim, Moragi, & Glickman, 2011). Therefore, identifying abnormalities in prefrontal cortex function associated with regulating attention in the context of emotional distractors could help in delineating a potential neural mediator to use in examining the development and treatment of anxiety disorders (Chapter 1).

FMRI research in adult anxiety disorder samples has produced evidence generally consistent with the biased competition model outlined above. Adult anxiety disorder patients demonstrate heightened amygdala activation when performing tasks with threatening stimuli (Etkin & Wager, 2007; Hattingh et al., 2012), consistent with a model of heightened bottom-up salience-driven attention. Moreover, adult GAD patients demonstrate reduced rACC activation and reduced rACC-amygdala connectivity while performing tasks with conflicting or distracting emotional stimuli (Etkin, Prater, Hoefl, Menon, & Schatzberg, 2010; Etkin & Schatzberg, 2011; Klumpp, Post, Angstadt, Fitzgerald, & Phan, 2013). Similarly, Blair et al. (2012) found that participants with SP, GAD, or comorbid SP and GAD evidenced reduced recruitment of the dACC during an emotional conflict task relative to controls. Finally, healthy adults with high levels of trait anxiety show reduced rACC and lateral PFC activation during emotional conflict tasks (Bishop, et al., 2004; Klumpp et al., 2011). In sum, research in adult participants has

generally shown that anxiety is associated with over-activation of regions associated with detecting threat, such as the amygdala, and hypo-activation and connectivity of prefrontal regions associated with top-down goal-directed attention during tasks involving emotional distractors.

Neuroimaging research in child and adolescent anxiety disorder patients has demonstrated abnormalities in the amygdala and prefrontal cortex relative to controls, but the nature of these abnormalities are dependent on the task performed. As discussed in Chapter 3, tasks that are attentionally demanding or briefly present stimuli produce evidence of amygdala hyper-activation in pediatric anxiety disorder patients. Abnormalities in ventral prefrontal cortex and ACC function have also been detected. For example, McClure et al. (2007) used a face processing task in which participants were asked to view emotional faces and either rate how afraid they felt, how hostile the face was, or the width of the nose. When performing this task, pediatric GAD patients demonstrated heightened right ventrolateral prefrontal cortex (vlPFC), left orbitofrontal cortex, and ACC activation compared to controls when rating how afraid they were of fearful faces (Beesdo et al., 2009; McClure et al., 2007), but this pattern did not emerge for the other conditions. McClure et al. also examined functional connectivity during performance of this task and found significant right amygdala-vPFC connectivity across the scanning session, but there was no difference in connectivity between the anxiety disorder and control groups. In a task that also asked participants to rate face stimuli, youth with social phobia or clinical levels of social anxiety demonstrated increased connectivity between the amygdala and vlPFC while evaluating pictures of undesirable peers relative to controls (Guyer et al., 2008).

Abnormalities in vlPFC function in pediatric anxiety disorder patients have also been demonstrated with the probe detection task, which assesses attention bias towards or away from

threat. When pediatric GAD patients performed this task during fMRI scanning and faces were presented for 500 ms, the GAD patients demonstrated an attention bias away from threatening faces and increased right vIPFC activity compared to controls (Monk et al., 2006). When the faces were presented briefly (17 ms) and then masked, pediatric GAD patients demonstrated increased amygdala activation and weaker amygdala connectivity with the right vIPFC relative to controls (Monk et al., 2008).

In summary, consistent with cognitive frameworks and the adult literature, fMRI research with pediatric anxiety disorder patients has shown hyper-activation of the amygdala in response to threatening stimuli. However, conclusions that can be drawn regarding activation of prefrontal regions in the presence of emotional distractors are limited because none of the tasks previously used presented emotional stimuli as distractors during an unrelated task. Participants were either always attending to threatening stimuli while performing the task (Guyer, et al., 2008) or attention bias to threat was measured but not manipulated (Monk, et al., 2006; Monk, et al., 2008). Although some studies required participants to shift the focus of their attention by asking them to either rate how afraid they felt or the nose width of threatening faces (Beesdo, et al., 2009; McClure et al., 2007), participants still attended to threatening stimuli while performing the task. Moreover, although McClure et al. (2007) examined functional connectivity during this task, the use of a correlational connectivity analysis for activation across the entire task (rather than a condition-specific connectivity method such as psychophysiological interaction) precluded the ability to examine prefrontal cortex-amygdala connectivity under different attentional conditions. Therefore, it is still unknown how neural activation and connectivity in pediatric anxiety disorder patients differs from controls when performing a task with emotional face distractors.

The goal of the present paper was to address the aforementioned gap in the literature by examining neural activation and connectivity in pediatric anxiety disorder patients while performing a relatively simple task in the context of the presence of emotional face distractors that were irrelevant to task performance. Participants completed the Emotional Faces Shifting Attention Task (EFSAT), which consists of conditions in which participants must match emotional faces (direct emotion processing) and perform a shape-matching task with irrelevant emotional faces in the same field of view (emotional face distractors). When typically developing youth and healthy adult participants perform this task, matching faces (relative to shapes) results in increased amygdala activation whereas matching shapes (relative to faces) is related to greater rACC activation (Klumpp, et al., 2012; Swartz et al., under review). Additionally, adult patients with SP evidence greater insula activation during face matching and reduced rACC activation during shape matching relative to controls (Klumpp, et al., 2013).

I hypothesized that during the shape-matching condition, the condition with irrelevant but potentially distracting emotional faces, pediatric anxiety disorder patients would demonstrate increased amygdala activation and reduced rACC activation relative to controls. This would be consistent with the biased competition model hypothesizing increased salience-driven (amygdala-associated) attention and reduced top-down goal-oriented (rACC-associated) attention when emotional distractors compete with a goal-directed task in anxiety disorder patients. Second, I hypothesized that anxiety disorder patients would evidence reduced connectivity between the rACC and amygdala during the shape-matching condition. Additionally, I predicted that amygdala and rACC activation and connectivity would relate to anxiety severity within the patient group. Finally, given evidence for extensive changes in this circuitry across childhood and adolescence (Chapters 1 and 2), I also examined whether

amygdala and rACC function was related to age and whether this relation differed between groups.

Methods

Participants

Recruitment procedures and inclusion/exclusion criteria for this study were the same as those described in Chapter 3. Forty-four participants with a primary GAD, SP, or SAD diagnosis and 47 controls performed the EFSAT during fMRI scanning. One control dropped out during scanning, 18 participants were removed for movement (9 patients and 9 controls), 2 controls were removed for accuracy (<60%), and 1 patient was removed for signal dropout in the images, leaving 34 anxiety disorder patients and 35 controls between 7 and 19 years old available for analysis (Table 4.1). The excluded patients did not differ from the included patients in age or anxiety symptoms. Excluded control participants were younger ($M=11.02$, $SD=3.3$) than included control participants ($M=15.2$, $SD=3.9$), $t(44)=-3.2$, $p=.002$. Of the participants in the final sample, 25 patients and 16 controls are overlapping with the sample reported on in Chapter 3; when participants performed both tasks, the EFAT was always performed first and the task reported on in this paper (the EFSAT) was performed second. Data from 25 control participants have been reported on previously (Swartz et al., under review).

Procedure

A trial of the EFSAT consisted of three faces in a triangular configuration, with one on the top row and two on the bottom, and three shapes in an upside-down triangular configuration (Figure 4.1). During the Faces condition, participants were instructed to identify which faces out of the two on the bottom row matched the emotion of the target face on the top row. Likewise,

during the Shapes condition, participants were instructed to match one of the two shapes on the top row with the target shape on the bottom row.

Participants completed two runs of the EFSAT. There were a total of 18 Faces blocks and 18 Shapes blocks with 6 of each of the following conditions: Angry Faces, Fear Faces, and Happy Faces (blocks in which participants were instructed to match faces for faces that were angry, fearful, or happy, respectively) and Angry Shapes, Fear Shapes, and Happy Shapes (blocks in which participants were instructed to match shapes and the unrelated distractor faces were angry, fearful or happy, respectively). The order of conditions was counterbalanced across participants. Each block was 20 seconds long and began with a 4 second instruction screen instructing participants to either match faces or shapes for that block and then 4 trials of the task lasting 4 seconds each. Participants responded with a button box. Accuracy and response times (RT) were recorded.

fMRI Data Acquisition. MRI images were acquired on a 3.0 Tesla GE Signa. A high-resolution T1-weighted spoiled-gradient echo (SPGR) image (TR=9ms, TE=1.8ms, flip angle=15 degrees, slice thickness=1.2 mm, 124 slices, FOV=256x256 mm) was acquired for anatomical reference and T2*-weighted BOLD images were acquired using a reverse spiral sequence (TR=2,000 ms; TE =30 ms; slice thickness=3 mm, 43 slices collected parallel to the AC-PC line; 64x64 matrix; 220x220 mm field of view; flip angle=90 degrees) for the functional data.

Measures. Anxiety symptoms were measured with the Multidimensional Anxiety Scale for Children (MASC; March, Parker, Sullivan, Stallings, & Conners, 1997) and the Leibowitz Social Anxiety Scale (LSAS; Heimberg et al., 1999). Pubertal status was measured with the Pubertal Development Scale (Petersen, Crockett, Richards, & Boxer, 1988) or an adapted version

of this scale and converted to Tanner Stages. Similar to Chapter 3, because of the high correlation between age and puberty within this sample ($r=.84$, $p<.001$), I chose to examine only age for the cross-sectional analyses.

Analyses

Behavioral Data Analysis. Mean accuracy and RT were obtained for each condition. Group differences in behavior were examined using repeated-measures ANOVA in SPSS v20. Behavioral data were missing for one control on the EFSAT.

fMRI Data Analysis. Data were pre-processed using the same pre-processing stream described in Chapter 3. Condition effects were modeled at the individual subject level using the general linear model and the six movement parameters from the realignment procedure were entered as nuisance covariates.

Psychophysiological Interaction (PPI) Analysis. A PPI analysis was conducted using Shapes vs. Faces as the psychological condition. Based on the results obtained for the first analysis, I chose the rostral anterior cingulate cortex as the seed region for the PPI. In order to only include voxels that were activated during the shape-matching task, I created a functional mask based on the contrast of Shapes vs. Faces to use as the seed region for this analysis.

Hypothesis 1: Group Differences in Amygdala and rACC Activation. Before examining group differences, I used a one-sample t-test in order to confirm whether participants evidenced the same pattern of activation as observed previously using this task (Klumpp, et al., 2012; Klumpp, et al., 2013; Swartz, et al., under review). Specifically, I tested whether Faces > Shapes elicited greater bilateral amygdala activation and if Shapes > Faces was associated with greater rACC activation. Results were first displayed at $p<.01$ uncorrected and then family-wise error (FWE) correction was applied within the regions of interest (ROIs). The amygdala ROI was

defined anatomically using the Wake Forest University Pickatlas (WFU Pickatlas; Maldjian, Laurienti, Kraft, & Burdette, 2003). In order to obtain an ROI for the rACC, the anterior cingulate region was intersected with the medial frontal region defined by the Automated Anatomical Labeling atlas in order to define a region encompassing the rostral portion of the ACC. This ROI consisted of 591 voxels. Whole-brain results for these contrasts were also examined at $p < .05$ FWE whole-brain corrected with cluster threshold of 10.

After examining the main effects of task condition, I examined group differences in amygdala and rACC activation. First, an emotion x group interaction was conducted in SPM8 in order to determine whether group differences varied by emotion of the face stimuli. The contrasts used for this interaction were Angry Faces vs. Angry Shapes, Fearful Faces vs. Fearful Shapes, and Happy Faces vs. Happy Shapes. A two-sample t-test was then used to examine overall differences in activation across the groups collapsing across emotions (Faces vs. Shapes). Because of the wide age range in this sample and a significant group difference in reaction times during the task (Table 4.2), I entered age and mean reaction times as nuisance covariates in any second-level analyses conducted in SPM8. Group differences were tested using the amygdala and rACC ROIs described above. Based on the findings of Klumpp et al. (2013), I also examined differences in insula activation during this task as a secondary analysis, using the bilateral insula ROI derived from the WFU Pickatlas. Finally, I conducted a whole-brain analysis at $p < .001$ uncorrected with a cluster threshold of 10 in order to examine other regions outside of the *a priori* ROIs that differed between the groups.

Hypothesis 2: Group Differences in rACC-Amygdala Connectivity. Differences in connectivity were examined by conducting a two-sample t-test in SPM8 for the PPI of Shapes vs.

Faces. Because the rACC was chosen as the seed region for the PPI, I used the bilateral amygdala ROI to test for group differences in connectivity between these regions.

Relation between Anxiety, rACC and Amygdala Activation, and Connectivity. MASC and LSAS total scores were entered as regressors onto the contrast of Faces vs. Shapes in SPM8 within the patient group in order to examine the relation between anxiety symptoms and activation during the task. I used the bilateral amygdala and rACC ROIs to test for significant relations between activation and anxiety symptoms. I used a similar approach with the PPI images, entering symptom measures as regressors onto the PPI contrasts.

Relation between Age, rACC and Amygdala Activation, and Connectivity. I conducted an age x group interaction to examine whether the effects of age on amygdala or rACC activation differed between the groups. I also conducted an age x group interaction for the PPI seeded with the rACC functional mask. These were conducted in SPM8.

Results

Group Differences in Behavior

There was no group difference in accuracy for the EFSAT, although it approached significance ($p=.08$), but there was a task x emotion interaction, $F(2, 65)=10.07, p<.001$. This was due to lower accuracy on face matching, particularly for angry faces. Paired samples t-tests indicated that participants evidenced lower accuracy for angry face matching relative to fearful face matching ($p<.001$) and happy face matching ($p=.002$). There was a group difference in RT during the EFSAT, $F(1,66)=4.65, p=.04$, due to the control group being faster to respond overall. There was also a task x emotion interaction, $F(2, 65)=7.52, p=.001$, with a similar pattern as for accuracy (Table 4.2). Paired samples t-tests indicated that participants evidenced slower reaction times for angry face matching relative to fearful face matching ($p<.001$) and happy face

matching ($p < .001$). They also evidenced slower reaction times for matching shapes with angry faces distractors relative to matching shapes with happy face distractors ($p = .04$).

When performing these analyses with age entered as a covariate, the group differences and emotion interactions were no longer significant. Controlling for age, there was only a main effect of task for accuracy, $F(1, 65) = 4.56, p = .04$, and for RT, $F(1, 65) = 32.2, p < .001$, indicating that across both groups participants were faster and more accurate while matching shapes relative to faces. There were also main effects of age on accuracy, $F(1, 65) = 45.4, p < .001$, and RT, $F(1, 65) = 75.6, p < .001$, due to younger participants evidencing lower accuracy and slower RTs in both groups.

Hypothesis 1: Group Differences in Amygdala and rACC Activation

As predicted, when combining across the groups, Faces > Shapes was associated with activation of the bilateral amygdala, left amygdala: $t(66) = 4.80, p < .001$, size = 109 voxels, xyz = (-18, -6, -14) and right amygdala: $t(66) = 4.56, p = .001$, size = 120 voxels, (18, -6, -16). Additionally, Shapes > Faces was associated with rACC activation, $t(66) = 4.89, p < .001$, size = 207 voxels, (-6, 56, -2; Figure 4.2). Whole-brain results for these analyses are presented in Table 4.3.

For emotion-specific effects, there was no group x emotion interaction or main effect of emotion within the rACC or amygdala. Examining overall group differences collapsing across the three emotion types, there was a group difference in rACC activation for Shapes > Faces. Specifically, controls evidenced greater rACC activation while matching shapes (in the context of emotional face distractors) relative to the anxiety disorder group, $t(65) = 3.63, p = .022$, size = 172 voxels, (8, 56, -2; Figure 4.3). No other regions outside of the rACC were significantly different between the groups for the whole-brain analysis at $p < .001$ uncorrected. Figure 4.4 demonstrates group differences in rACC activation for shape vs. face matching for each emotion condition.

Based on the finding that participants were slower to respond to shapes when distractor faces were angry relative to happy, I performed post-hoc exploratory analyses in order to examine the group difference in rACC activation for each emotion condition separately by using contrast values extracted from the functionally-defined rACC mask to test group differences in SPSS. These post-hoc analyses indicated that the groups evidenced a significant difference in rACC activation for Angry Shape vs. Angry Face matching, $t(67)=2.78$, $p=.007$, but this difference was not significant for the fearful face condition ($p=.40$) or for happy faces ($p=.80$). There were no differences in amygdala or insula activation between the groups.

Hypothesis 2: Group Differences in rACC-amygdala Connectivity

There was no group difference in rACC-amygdala connectivity for the PPI of Shape vs. Face matching.

Relation between Anxiety, rACC and Amygdala Activation, and Connectivity

I did not find a significant relation between MASC or LSAS scores and amygdala or rACC activation within the anxiety disorder group. There was also no relation between anxiety symptoms and rACC-amygdala connectivity assessed with the PPI.

Relation between Age, rACC and Amygdala Activation, and Connectivity

There was no effect of age for rACC or amygdala activation for face vs. shape matching. However, there was an age x group interaction for the PPI of Shapes vs. Faces, significant corrected for the bilateral amygdala ROI: $F(1,64)=12.21$, $p=.034$, size=6 voxels, (22, -10, -10). As shown in Figure 4.5, this interaction was driven by an increase in connectivity with age within the control group, whereas the anxiety disorder group did not show this cross-sectional pattern.

Discussion

The goal of this paper was to examine amygdala and rACC activation in pediatric anxiety disorder patients during a task performed in the context of emotional face distractors. As predicted, results demonstrated that pediatric anxiety disorder patients evidence reduced rACC activation relative to controls. However, the hypothesis of increased amygdala activation in anxiety disorder patients was not supported. In addition, I found an age x group interaction for rACC-amygdala connectivity during shape vs. face matching, with controls evidencing increased connectivity with age whereas the anxiety disorder group did not show this increase.

This is the first study to my knowledge to use a task with emotional face distractors in pediatric anxiety disorder patients. Thus, this fills an important gap in our knowledge regarding abnormalities in prefrontal cortex function during a task requiring attentional control in the context of emotional distractors. Notably, these findings are similar to those obtained in adult anxiety disorder patients, who also evidence decreased rACC activation during the condition in which they must match shapes in the presence of distracting faces (Klumpp, et al., 2013). Given the previously established role of the rACC in goal-directed attention, reduced rACC activation may play a role in attentional biases to threat and difficulty disengaging attention from threat in anxiety disorder patients. The finding of an abnormality in rACC activation that is replicable across studies and a range of ages suggests that this could serve as a potential biomarker for examining anxiety disorder development and treatment.

This study also provides preliminary evidence of differences in emotional modulation of rACC activation between groups. The behavioral results indicated that participants in both groups were slower at matching shapes in the context of angry emotional face distractors relative to happy face distractors. Interestingly, post-hoc exploratory analyses suggested that the

difference between groups in rACC activation for shape vs. face matching was strongest for the angry shapes condition. Therefore, controls may be modulating rACC activation based on the emotional content of the face distractors (increasing rACC activation when face distractors are angry), whereas the anxiety disorder patients fail to evidence this modulation. This lack of modulation of rACC activation when distractors are threatening (or the tendency for threatening distractors to interfere with rACC recruitment) could be associated with attention bias to threatening faces and difficulty regulating attention to threat in anxiety disorder patients. However, due to the post-hoc nature of these emotion-specific analyses, future research will be necessary in order to confirm this effect.

It is important to note that the pattern of results observed suggests an alternative potential interpretation of the group difference in rACC activation. When examining the main effect of task for shape matching vs. face matching, rACC activation was observed alongside activation of the posterior cingulate cortex (Table 4.3, Figure 4.2). Thus, this pattern of activation for shape matching vs. face matching could potentially be the result of deactivation of the default mode network during the more difficult face matching task, which produced lower accuracy and longer response times within both groups. In support of this view, when examining the PPI of shape vs. face matching across both groups combined in a post-hoc exploratory analysis, the rACC demonstrates significant connectivity with the posterior cingulate cortex, $F(1,65)=8.83$, $p=.04$, (2, -46, 28), small-volume corrected for a 5 mm sphere around the peak activation. In this case, the group difference in rACC activation would indicate that controls evidenced default mode network activation during the shape matching task (in other words, default mode network deactivation during face matching), suggesting the shape-matching task was easier for controls relative to the anxiety disorder group. Therefore, these results still support the contention that

performing the shape-matching task in the presence of emotional distractors is more difficult for anxiety disorder patients, although in this interpretation, the difference in rACC activation is due to the patient group not demonstrating default mode network activation during shape matching, as seen in controls. In this case, the emotion-specific effects could be due to the control group evidencing greater deactivation of the default mode network during angry face matching. This possibility is supported by the finding that both groups were slower and less accurate for angry face matching relative to the other emotional face types, indicating that angry face matching was a more difficult condition. Future research with a baseline comparison condition, such as fixation, will be necessary in order to determine whether these results are due to activation during the shape matching condition or deactivation during face matching.

Also similar to the findings of the study conducted in adults (Klumpp et al., 2013), I found no difference in amygdala activation between the anxiety disorder and control groups. It is possible that if anxiety disorder patients had more difficulty regulating their attention in general that they were more likely to attend to emotional faces in both conditions (when matching faces and when matching shapes). In this case, if amygdala activation was generally high across both conditions, this may have made it more difficult to detect a difference in activation between the two conditions in the anxiety disorder group. This hypothesis is supported by the behavioral results, in which anxiety disorder patients tended to be slower to respond for both conditions (face matching and shape matching) relative to the control participants, suggesting that both conditions may have been more difficult for the patient group. Future research incorporating a separate baseline condition with no face stimuli will be necessary in order to test whether amygdala activation is elevated in both conditions. Additionally, although this task was relatively challenging for anxiety disorder patients, as evidenced by slower reaction times for all conditions

relative to controls, making this task even more cognitively demanding (e.g., by shortening the length of trials) may place further demands on patients' attentional control resources and may result in the expected difference in amygdala activation for this task, in line with the prediction that increasing cognitive load will lead to greater difficulty disengaging attention away from threat in anxious individuals (Judah, et al., 2013).

A second novel finding of this study is an age x group interaction in corticolimbic connectivity for pediatric anxiety disorder patients relative to typically developing controls. There are two potential interpretations underlying the observed cross-sectional patterns of connectivity. First, controls may demonstrate increased rACC-amygdala connectivity during shape matching with age, which would be consistent with increased communication between the prefrontal cortex and amygdala during an emotion regulation task with age. An alternative interpretation is that this effect may be driven by a decrease in rACC-amygdala connectivity during face matching in controls with age. Future research with a baseline condition that does not include face stimuli could be used to help distinguish between these two potential explanations.

There are several important limitations to note. As mentioned, the lack of a baseline condition prevents the detection of activation that may occur during both conditions but does not differ between the face-matching and shape-matching conditions. Therefore, there may be overall group differences in activation that were not detected within this task. Likewise, there may have been effects of overall activation for age or symptom correlations that could not be detected. Additionally, the lack of a baseline condition precludes a definitive test of whether the group difference in rACC activation represents deactivation to the face matching task or activation to shape matching. Second, due to the blocked design, I was unable to remove incorrect trials or control for differences in reaction time at the trial level. Future research with

mixed or event-related designs could help to supplement the results found here and address some of these limitations. Additionally, it would be of interest to determine whether rACC activation is associated with reaction time at the trial level (e.g., whether greater rACC activation for a trial is associated with a faster response time).

In conclusion, this study fills an important gap in our knowledge regarding abnormalities in prefrontal cortex function when anxiety disorder patients perform a task in the presence of emotional face distractors. In line with biased competition models of attention in anxiety disorders, patients evidenced reduced rACC activation when required to perform a goal-directed task in the presence of irrelevant emotional face distractors. These results lead to two important questions for future research. First, imaging gene-environment interaction studies will be necessary in order to understand how developmental factors interact to influence this reduction in rACC activation observed in anxiety disorder patients. Second, treatment studies will be needed to examine whether rACC activation is predictive of symptom improvement or is altered following successful treatment.

Table 4.1 Participant characteristics

	Anxiety Disorder Group (M, SD) n=34	Control Group (M, SD) n=35	Group difference
Age	13.84 (3.3)	15.20 (3.9)	$t(67)=1.58, p=.12$
Gender (percent female)	65%	49%	$t(67)=-1.35, p=.18$
Pubertal status	3.38 (1.4)	3.38 (1.4)	$t(61)=.009, p=.99$
MASC total scores	60.5 (18.1)	32.7 (12.3)	$t(67)=-7.49, p<.001$
LSAS total scores	62.7 (32.5)	9.5 (9.1)	$t(46)=-6.59, p<.001$

Note: Bold indicates a significant group difference. MASC=Multidimensional Anxiety Scale for Children; LSAS=Leibowitz Social Anxiety Scale. Note: data on pubertal status were missing for 1 control and 5 anxiety disorder patients. LSAS scores were only collected for a subset of participants and were not collected in 18 controls and 3 anxiety disorder patients.

Table 4.2 Behavioral results for EFSAT by group and condition

	Anxiety Disorder Group Accuracy (M, SD)	Control Group Accuracy (M,SD)	Anxiety Disorder Group RT (M ,SD)	Control Group RT (M, SD)
EFSAT				
Angry Face	73.8% (15.3)	78.7% (14.1)	1692.1 (311.2)	1545.3 (400.9)
Fearful Face	84.4% (14.7)	84.9% (15.0)	1593.6 (341.2)	1420.9 (400.4)
Happy Face	79.7% (18.9)	86.0% (15.1)	1536.9 (273.9)	1394.9 (383.4)
Angry Shape	87.9% (10.9)	89.8% (9.4)	1249.2 (279.2)	1060.5 (299.3)
Fearful Shape	85.2% (15.4)	87.7% (12.8)	1207.7 (283.9)	1054.5 (307.5)
Happy Shape	85.0% (15.5)	91.1% (11.7)	1178.2 (298.8)	1053.9 (315.6)
All conditions	82.7% (9.2)	86.4% (8.0)	1409.6 (268.7)	1257.2 (315.9)

Note: RT=reaction time in ms.

Table 4.3 Whole-brain activation table for main effects of task (face matching and shape matching) averaging across patient and control groups

Effect	Statistic	Number of Voxels	Coordinates	Region
Face	$t(66)=15.38, p<.001$	9156	(-26, -94, -2)	Middle occipital gyrus
Matching	$t(66)=8.85, p<.001$	1194	(42, 14, 26)	Right inferior frontal gyrus
vs. Shape	$t(66)=7.42, p<.001$	635	(-40, 12, 28)	Left inferior frontal gyrus
Matching	$t(66)=6.21, p=.001$	203	(-4, 12, 50)	Left medial frontal gyrus
	$t(66)=5.68, p=.007$	22	(-36, -10, 64)	Left precentral gyrus
Shape	$t(66)=6.06, p=.002$	104	(58, -42, 36)	Right supramarginal gyrus
Matching	$t(66)=5.94, p=.003$	45	(-58, -34, 42)	Left inferior parietal lobule
vs. Face	$t(66)=5.87, p=.006$	54	(2, -28, 44)	Posterior cingulate
Matching	$t(66)=5.41, p=.017$	26	(-48, -60, 38)	Left angular gyrus

Note: P-values are family-wise error whole-brain corrected.

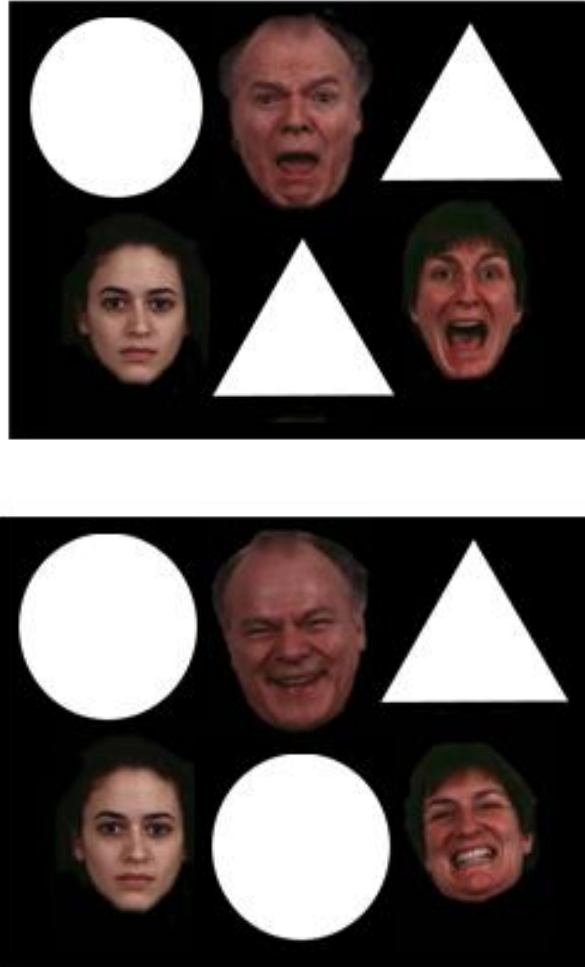


Figure 4.1 Example trials of the Emotional Faces Shifting Attention Task (EFSAT)

Example trials for the fear (top) and happy (bottom) conditions. Trials were presented in block format and participants were instructed at the beginning of each block to either match faces or match shapes for that block. The match faces condition requires attending to the emotional faces whereas the match shapes condition requires performing the shape-matching task in the context of emotional face distractors.

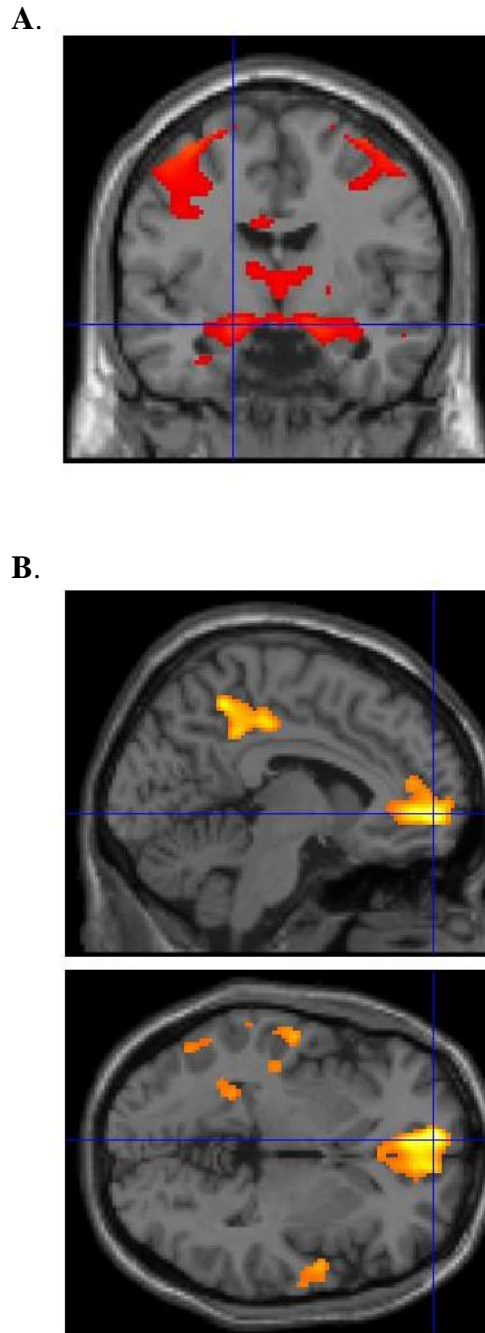


Figure 4.2 Activation associated with face matching and shape matching during the Emotional Faces Shifting Attention Task

Face matching (Faces>Shapes) is associated with greater bilateral amygdala activation (A) whereas shape matching (Shapes>Faces) is associated with greater rostral anterior cingulate cortex activation (B) across all participants. All figures are thresholded at $p<.01$ uncorrected.

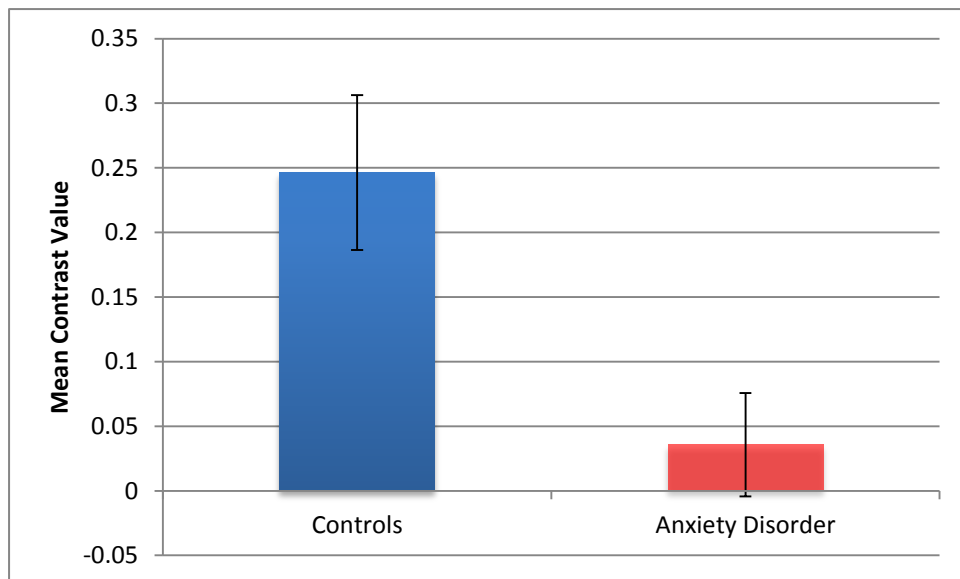
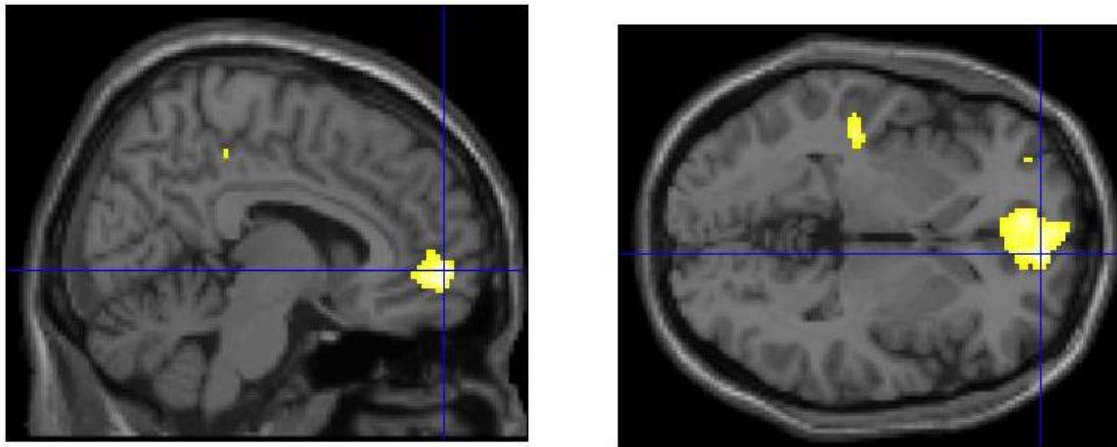


Figure 4.3 Controls evidence greater rostral anterior cingulate cortex (rACC) activation during shape matching relative to the anxiety disorder group

SPM figure demonstrates the contrast of Controls > Anxiety Disorder Group for the contrast of Shapes > Faces. Graph shows mean contrast values extracted from the functional mask of the rACC for the contrast of Shapes > Faces. Error bars represent 1 standard error above and below mean.

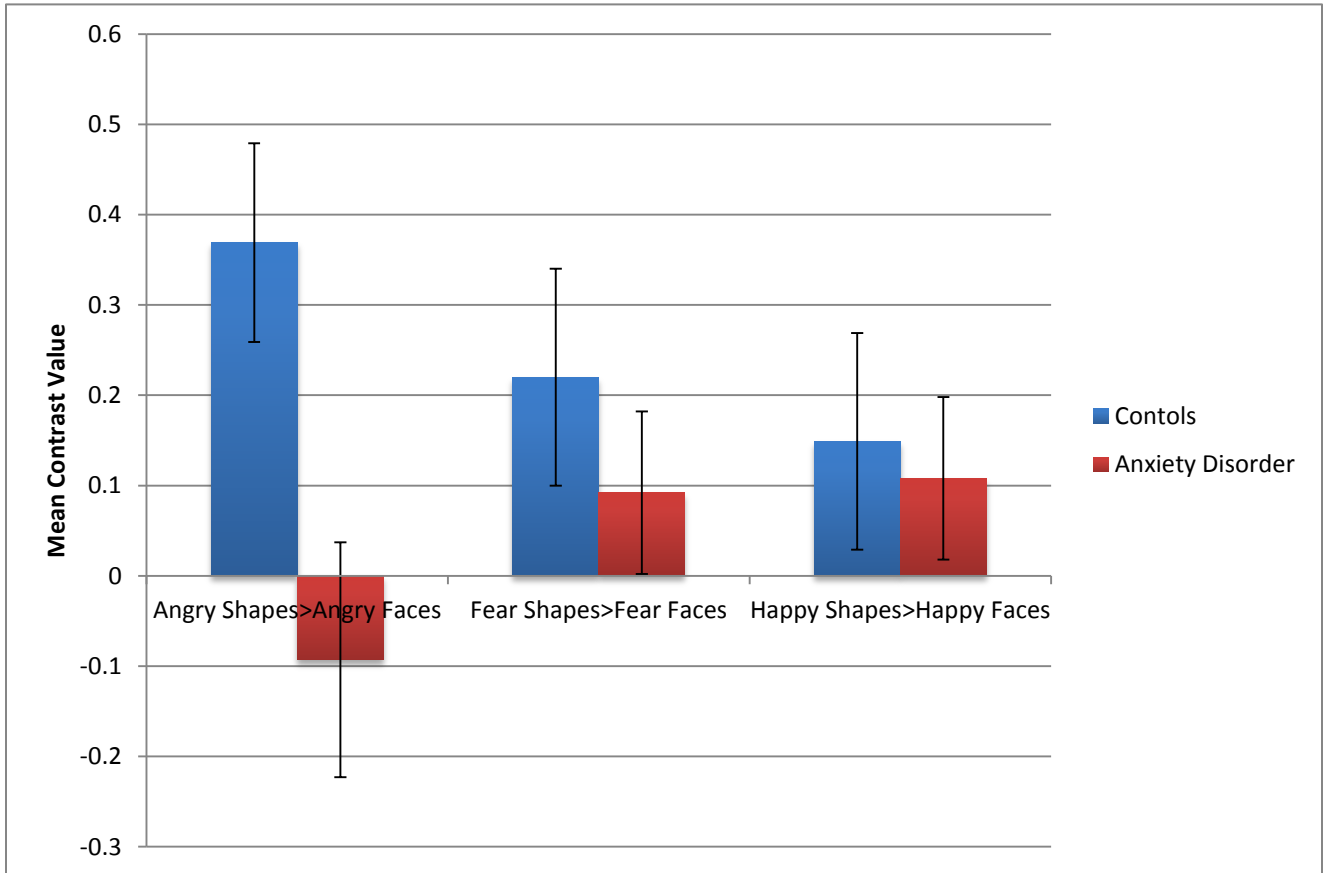


Figure 4.4 Rostral anterior cingulate cortex activation to shape matching vs. face matching by group and emotion

Contrast values are extracted from the functional rACC mask for Shapes > Faces by emotion type (Angry, Fear, and Happy).

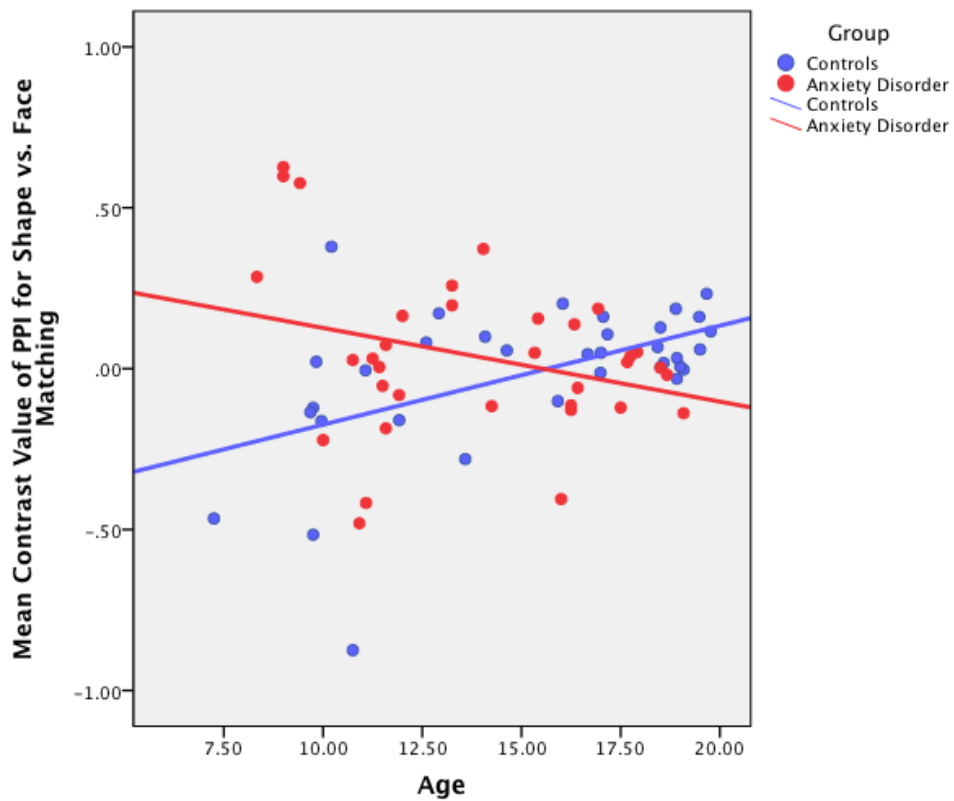
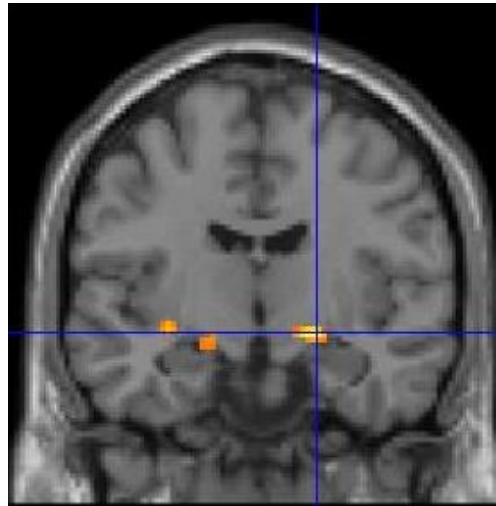


Figure 4.5 There is an age x group interaction for rostral anterior cingulate cortex-amygdala connectivity during shape vs. face matching

Contrast values for the PPI of Shapes vs. Faces are extracted from a 3mm sphere around the peak voxel for the interaction within the amygdala.

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Swartz, J.R., Phank, K.L., Angstadt, M., Klumpp, H., Fitzgerald, K.D., & Monk, C.S. (in preparation-b). Altered activation of the rostral anterior cingulate cortex in the context of emotional face distractors in children and adolescents with anxiety disorders.

Chapter 5

Conclusion

Throughout the dissertation, I have discussed how an understanding of neural development associated with emotion processing and regulation could be used to examine the development and treatment of pediatric anxiety disorders. The aim of the papers in this dissertation was to lay a foundation for this research by examining changes with age in prefrontal cortex-amygdala circuit structure and function in typically developing children and adolescents and how prefrontal cortex-amygdala function differs in pediatric anxiety disorder patients. The results of these papers inform an initial framework with which to formulate new research questions and further elucidate the processes involved in the development of anxiety disorders.

Chapter 2 informs a potential model for the typical changes that occur in corticolimbic structure and function across childhood and adolescence. This was the first paper to demonstrate that structural connectivity of prefrontal cortex-amygdala circuitry and amygdala function are related in late childhood and adolescence by demonstrating that increased uncinate fasciculus FA relates to decreased amygdala activation to sad and happy faces. This suggests that increasing structural connectivity of corticolimbic circuitry with age across childhood and adolescence may facilitate communication between the prefrontal cortex and amygdala, improving regulation of amygdala activity. Moreover, amygdala activation to sad faces related to internalizing symptoms, suggesting that increased activation of the amygdala to sad faces may represent potential risk for the development of internalizing problems such as mood and anxiety disorders.

Chapters 3 and 4 examined abnormal functioning of this circuitry in pediatric anxiety disorder patients. Previous work has suggested that anxiety disorders may be characterized by both difficulty disengaging attention from threat and avoidance of threatening stimuli. Judah and colleagues (2013) proposed that under conditions of low attentional demands, anxiety disorder patients will exhibit a pattern of avoidance of threatening stimuli, given that attentional control resources will be relatively free to divert attention away from threatening stimuli. In contrast, under high working memory load or during attentionally demanding tasks, anxiety disorder patients will exhibit hypervigilance for threat, lacking the attentional resources to disengage attention away from threat. This pattern was generally born out in Chapters 3 and 4. In Chapter 3, patients performed an emotional face matching task that was relatively easy, given that participants only viewed faces or only viewed shapes for each trial, and so it was easy to remember which type of stimuli to match for a given block. During this task, anxiety disorder patients exhibited initial amygdala hyper-activation to faces during the first third of scanning, but then demonstrated an abnormal pattern (relative to controls) of decreases in amygdala activation across the subsequent blocks. This pattern is consistent with the hypervigilant-avoidant hypothesis proposed for anxiety disorder patients, demonstrating an initial amygdala hyper-activation followed by reduced amygdala activation later in scanning.

In Chapter 4, participants performed a more difficult task, the EFSAT, which required them to hold in working memory whether they needed to match faces or shapes for each block (because both faces and shapes were displayed on every trial). During this more attentionally demanding task, anxiety disorder patients evidenced reduced rostral ACC activation during shape matching (in the context of emotional face distractors) relative to controls. This could be associated with difficulty regulating attention in the presence of threatening distractors when

attentional resources are limited during the performance of a difficult task. Additionally, an interaction between age and group suggested that rACC-amygdala connectivity during this task increased for control participants with age, whereas this pattern was not observed within patients.

Moreover, these results add to our previous knowledge of abnormalities in prefrontal cortex function (including abnormal ventrolateral and ventromedial prefrontal cortex activation) in pediatric anxiety disorder patients by providing new evidence of abnormalities in dorsal prefrontal cortex function (Chapter 3) and rostral anterior cingulate cortex function (Chapter 4). These results suggest widespread abnormalities across a range of the functions associated with different subdivisions of the prefrontal cortex that are differentially tapped by the type of task used during fMRI scanning.

These studies point to a potential model for the typical and atypical development of corticolimbic circuitry across childhood and adolescence. Typical development appears to involve increased structural connectivity between the prefrontal cortex and amygdala with age, accompanied by increased functional connectivity and decreased amygdala activation (Chapter 2; Gee et al., 2013; Lebel & Beaulieu, 2011). It is possible that the abnormalities seen in pediatric anxiety disorder patients may be associated with deviations from this typical trajectory, including reduced increases in structural connectivity with age, reduced increases in functional connectivity, failure to evidence decreases in amygdala activation with age, or reduced development of regulatory prefrontal cortex function. For example, failure to demonstrate increases in structural or functional connectivity with age may lead to decreased efficiency of amygdala regulation, which could result in the initial amygdala hyper-activation observed in pediatric anxiety disorder patients in Chapter 3. Indeed, this atypical cross-sectional pattern has been shown with adolescents and young adults at risk for psychosis. Gee et al. (2012)

demonstrated that participants at risk for psychosis (evidencing a prodromal syndrome) demonstrated decreased ventrolateral prefrontal cortex activation and increased amygdala activation cross-sectionally with age, whereas typically developing controls evidenced the opposite pattern (increased prefrontal cortex activation and decreased amygdala activation with age). Therefore, deviations from the typical developmental trajectory for corticolimbic circuitry suggested by cross-sectional research could contribute to risk for the development of a range of psychiatric disorders. With these typical and atypical cross-sectional trajectories in mind, this model could lay the foundation for a number of different research directions to further elucidate the processes involved in the development and treatment of anxiety disorders. Below, I illustrate a few examples of these future directions.

Longitudinal Research on the Typical and Atypical Development of Corticolimbic Circuitry

A limitation of much of the research conducted to date is that it relies on cross-sectional data. Cross-sectional research has the advantage of capturing a wide age range within one study, whereas collecting a longitudinal sample ranging from childhood to adulthood would take as many years as the age range covered. However, in order to begin to make inferences regarding within-individual developmental trajectories and causal processes, it will be necessary to conduct longitudinal research as well, or combine cross-sectional and longitudinal research designs. One important question for longitudinal research will be disentangling the direction of relationships between the variables examined in this dissertation (brain structure, function, symptoms, and disorders). With multiple waves of data within the same individuals, a longitudinal study could investigate whether brain structure or function at one time point can predict symptoms or disorders at a later time point, controlling for initial symptom levels.

Also, with multiple waves of data for individuals, we can begin to look at within-person trajectories and whether trajectories of development over adolescence are predictive of mental health outcomes at later stages of development. Similarly, longitudinal research following adolescents into adulthood will be important in determining which abnormalities in adult anxiety disorder patients may be persistent perturbations in neural function developed during the periods of childhood and adolescence and which perturbations are unique to the stages of adulthood. Notably, to date, even cross-sectional research directly comparing pediatric and adult anxiety disorder patients is relatively limited, with a few exceptions (Blair et al., 2011).

Using Neural Function as a Mediator for Developmental Influences

As discussed throughout the dissertation, one of the important functions of understanding neural development is to be able to link genetic and environmental influences to brain structure and function and in turn to behaviors and symptoms. There is growing evidence that corticolimbic structure and function is influenced by both genetic and environmental factors (Battaglia et al., 2012; Burghy et al., 2012; Eluvathingal et al., 2006; Furman, Hamilton, Joormann, & Gotlib, 2011; Lau, Goldman, Buzas, Fromm, Guyer, Hodgkinson, Monk, Nelson, Shen, et al., 2009; Munafò, Brown, & Hariri, 2008; Pacheco et al., 2009; Pezawas et al., 2005; Tottenham et al., 2011). Some studies also suggest that trajectories of changes in corticolimbic circuitry are influenced by genetic factors as well. For example, serotonin transporter-linked polymorphic region (5-HTTLPR) genotype influences the cross-sectional pattern of changes observed in typically developing children and adolescents: participants with low-expressing alleles of the 5-HTTLPR evidence increased amygdala activation and decreased ventrolateral prefrontal cortex-amygdala connectivity with age, whereas participants with the high-expressing allele tend to show the opposite pattern (Wiggins et al., 2012). Therefore, an important direction

for future cross-sectional and longitudinal research is to examine how genetic and environmental influences interact to shape change in corticolimbic structure and function across childhood and adolescence, and how these trajectories predict psychological outcomes.

A related implication of this model of corticolimbic development is that we should expect the relation between genes, environment, and the brain to differ at different stages of development. For example, 5-HTTLPR genotype may relate to amygdala activation differently in very young children relative to adolescents or adults. This may also help to clarify why findings different from the adult literature have been observed for imaging genetics studies conducted in pediatric patients. For instance, Lau et al. (2009) reported that pediatric anxiety disorder patients with the higher-expressing allele of the 5-HTTLPR evidenced greater amygdala activation to emotional faces relative to lower-expressing allele carriers, a finding opposite to that often observed in adults. Future research will be necessary to delineate how genes and the environment affect corticolimbic structure and function at each stage of development, as well as changes in structure and function over time.

Moreover, it is important to note that the model of development described in this dissertation provides a useful basis for generating hypotheses, but is overly simplistic and will need to be expanded as we gather more data about the nuances and complexities of these processes. For instance, Hyde et al. (2011) found that perceived social support moderated the relation between amygdala activation and anxiety: only adults reporting low levels of social support evidenced the expected relation between amygdala activation and anxiety (greater amygdala activation associated with increased anxiety), whereas there was no relation between amygdala activation and anxiety in individuals with higher perceived social support. Likewise, one could hypothesize that increased structural connectivity of the uncinate fasciculus may not

directly relate to anxiety symptoms in a uniform fashion and may be maladaptive within certain contexts if it allows for more communication of threat signals from the amygdala to the prefrontal cortex. Indeed, both decreased ventrolateral prefrontal cortex-amygdala connectivity (Monk et al., 2008) and increased connectivity (Guyer et al., 2008) have been observed in pediatric anxiety disorder patients, depending on the task they perform. Therefore, another important direction for research is the careful measurement of environmental and contextual variables and close attention to task design in order to gather more data regarding how specific contexts and tasks (as well as the developmental stage of participants) affect the relationship between brain structure, function, and anxiety symptoms and disorders.

Timing of Developmental Influences

As reviewed in Chapter 1, emerging evidence is beginning to suggest that late childhood and adolescence may be important stages for the emergence of abnormalities in brain function and psychopathology (Ansorge, Morelli, & Gingrich, 2008; Burghy, et al., 2012; Giovanoli et al., 2013). A future direction for research will be to examine why abnormalities in functioning emerge during adolescence specifically. One important factor to consider is the role of puberty in neural development. Frameworks have suggested that the changes accompanying puberty (particularly an increase in pubertal hormones) influence the functioning of neural regions involved in emotion and motivation, including the amygdala (Crone & Dahl, 2012; Scherf, Behrmann, & Dahl, 2012). For example, greater pubertal development at age 13 is associated with increased amygdala activation to emotional faces (Moore et al., 2012). Therefore, the timing of pubertal development may interact with other factors (environmental stressors experienced during early development or concurrently, corticolimbic structural connectivity,

genetic influences on amygdala function and prefrontal cortex-amygdala connectivity) to determine an individual's risk for developing disturbances in function during this period.

Predicting Response to Treatment and Developing Novel Treatments

Amygdala and prefrontal cortex activation have been shown to predict anxiety disorder patients' response to treatment (Klumpp, Fitzgerald, & Phan, 2013; McClure et al., 2007; Nitschke et al., 2009). Therefore, understanding abnormalities in neural function associated with pediatric anxiety disorders could help in targeting treatments to individuals or testing the effects of novel treatments. In Chapter 3 and 4, I presented evidence of abnormalities in amygdala and rACC function in pediatric anxiety disorder patients. Future research could be used to examine whether these abnormalities (initial amygdala hyper-activation, abnormal decreases in amygdala activation, reduced rACC activation) are altered by successful treatments. This information could be helpful in deciding whether a specific form of treatment will be effective for an individual.

Moreover, initial results of biofeedback studies using real-time fMRI-based measures have been promising. These studies use real-time fMRI feedback to train participants to regulate neural activity in regions such as the amygdala and anterior cingulate cortex (deCharms et al., 2005; Johnston, Boehm, Healy, Goebel, & Linden, 2010; Linden et al., 2012). Although fMRI-based biofeedback may be a costly approach to treatment, if performing this type of treatment at earlier developmental stages produces long-lasting effects that prevent persistent disturbances in corticolimbic function or the development of more severe disorders in adulthood, this approach may prove to be more cost-efficient in the long run. Further fMRI research will be useful in determining neural abnormalities to target through biofeedback training and whether certain developmental stages may serve as sensitive periods to this type of training.

Conclusion

In conclusion, the developmental psychopathology framework highlights the importance of understanding typical development in order to understand the processes associated with atypical development, and vice versa (Cicchetti & Thomas, 2008). In this dissertation, I have presented three papers that have looked, first, at how corticolimbic circuitry changes cross-sectionally with age in typical development, and second, at abnormalities of functioning of this circuitry in pediatric anxiety disorder patients. Understanding typical and atypical patterns of changes in corticolimbic structure and function has a number of potential applications for future research, including examining longitudinal changes to further elucidate within-person trajectories of development, using cross-sectional and longitudinal patterns of corticolimbic function to link genetic and environmental influences to behavioral outcomes, and using these trajectories to target treatments to individuals and predict treatment outcomes. These future research directions have the potential to refine our understanding of risk and resilience to mental health disorders, develop better preventions and understand the role of timing of developmental influences on later outcomes, and improve the effectiveness of treatments for anxiety disorders.

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