AMYGDALA AND VENTRAL STRIATAL REACTIVITY IN ADOLESCENTS AT HIGH-RISK FOR DEPRESSION

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Previous research has shown that depression clusters within families. Adolescents from these families (i.e., high-risk) have approximately a three-fold increased risk of developing depression, an earlier mean age at onset, and greater lifetime morbidity in comparison with low-risk adolescents. Understanding the developmental pathways and mechanisms of susceptibility to depression, especially at the level of neurobiological circuits, is critical for the development of more effective intervention and prevention strategies, particularly in high-risk adolescents. The current study examined the functional reactivity of affect- and reward-related neural circuitries in high-risk and low-risk adolescents, as well as the functional coupling between regions of PFC and amygdala and ventral striatum. Adolescents (aged 12-15 years)—stratified according to familial history of depression (i.e., high- and low-risk)—completed two fMRI paradigms known to reliably elicit threat-related amygdala and reward-related ventral striatal reactivity, respectively. Using a conservative threshold, employed because of the very large sample size (> 300 adolescents), the present analyses failed to detect significant differences between these groups at the level of the amygdala and ventral striatum. When a more liberal threshold was applied, hypothesized differences were observed for both the amygdala reactivity paradigm and the ventral striatal reactivity paradigm: high-risk adolescents displayed relatively greater

amygdala reactivity and relatively blunted VS reactivity compared to low-risk adolescents. Additionally, these data offer some evidence to suggest that alterations in functional connectivity of the threat-related amygdala reactivity network (but not reward-related VS reactivity) may vary as a function of risk status during adolescence.

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I. INTRODUCTION

Previous research has provided compelling evidence for the clustering of depression and other affective illnesses within families (Weissman, Leckman, Merikangas, Gammon, & Prusoff, 1984; Williamson et al., 1995). First-degree relatives of adults with Major Depressive Disorder (MDD) have a two-fold increase in rate of depression relative to controls without a family history of MDD (Sullivan, Neale, & Kendler, 2000). Moreover, children who have family members with a lifetime history of mood disorders have approximately a three-fold increased risk of developing first-onset MDD in comparison with children who do not (Williamson, Birmaher, Axelson, Ryan, & Dahl, 2004), with an earlier mean age at onset of MDD compared to a control sample (Weissman et al., 1987). Understanding the developmental pathways and mechanisms of susceptibility to depression and identifying potential protective factors may lead to eventual intervention strategies, particularly for high-risk individuals.

Such efforts can be greatly advanced by examining the underlying neurobiological substrates of the emergent clinical and intermediate behavioral phenomenon (e.g., increased anxious temperament or impulsivity). Identifying neurobiological processes that differentiate premorbid risk for MDD provides tangible targets for the development of either behavioral (e.g., CBT) or pharmacological (e.g., SSRIs) intervention strategies before the emergence of disease. Moreover, given the increasing evidence that neurobiological processes demonstrate strong genetically driven variability, their identification in the context of premorbid risk for MDD also

has the potential to reveal discrete biological mechanisms of familial risk (Viding, Williamson, & Hariri, 2006). Identification of genetically driven variability in neurobiological processes also represents a critical step in mapping the moderating influences of environmental factors on emergent risk for psychiatric disease (Moffitt, Caspi, & Rutter, 2006). Additional traction can be gained by focusing specifically in adolescent populations because this developmental transition from childhood to adulthood (ages 15-20) is characterized by a significant rise in the prevalence of internalizing factors including suicide, depression, anxiety, and eating disorders, as well as externalizing factors such as fighting, violence, car accidents, and reckless behavior (Dahl, 2004; Ozer, 2005).

Depression is characterized both by high levels of negative affect and low levels of positive affect (Clark & Watson, 1991). Depressed individuals tend to display a negative affect bias that contributes to low mood, e.g., preferentially remembering negative information (Matt, Vázquez, & Campbell, 1992), focusing excessively on negative information (Leung, Lee, Yip, Li, & Wong, 2009), tending to interpret events as negative (Dearing & Gotlib, 2009), and ruminating about negative life events (Nolen-Hoeksema, 2000). These biases may be related to alterations in the emotion circuit of the brain that have been previously documented in depression, which may lead to negative information becoming more salient to depressed individuals (Gotlib et al., 2004; Ladouceur et al., 2006; Murphy et al., 1999). High levels of negative emotionality may lead to subjective feelings of low mood and contribute to the onset of depression. These brain differences seen in depressed individuals suggest that depression may reflect a deficit in emotion processing, which may contribute to an increased susceptibility for developing depression (Fales et al., 2008; Fu et al., 2008; Grimm et al., 2008; Harvey et al., 2005; Ladouceur et al., 2006; Sheline et al., 2001). Moreover, depression is also characterized

by a profound inability to experience pleasure (anhedonia) (Clark & Watson, 1991); for example, depressed individuals report less fulfillment from rewards than non-depressed individuals (Nestler & Carlezon, 2006). These differences in reward processing have neural correlates that have been demonstrated in laboratory settings in depressed individuals (Epstein et al., 2006; Forbes et al., 2006; Forbes, Hariri et al., 2009; Surguladze et al., 2005). Presented with the same rewarding stimuli, depressed individuals seem to experience less pleasure relative to non-depressed individuals, and this difference is correlated with a blunting of the reward circuit in the brain. These alterations in reward processing may lead to subjective experiences of anhedonia and a paucity of positive affect, which in turn, can contribute to depression.

An additional mechanism by which depression is thought to develop is via deficits in emotion regulation. Deficits in emotion regulation are neurobiologically characterized by alterations in the functional coupling between 1) limbic regions involved in the processing of emotion and 2) regions of the prefrontal cortex with direct inhibitory connections to limbic regions (functional connectivity). Several studies have demonstrated a relation between the failure to regulate negative emotions and the presence of internalizing and depressive symptoms in children and adolescents (Eisenberg et al., 2001; Rydell, Berlin, & Bohlin, 2003; Silk, Steinberg, & Morris, 2003). Previous neuroimaging studies have implicated a modulatory role for the prefrontal cortex on limbic responses (Beauregard, Levesque, & Bourgouin, 2001; Keightley et al., 2003; Siegle, Steinhauer, Thase, Stenger, & Carter, 2002), and abnormal functional connectivity between these anatomically-connected regions has been previously demonstrated in neuroimaging studies of depressed individuals (Anand et al., 2005a, 2005b; Mayberg et al., 1999; Phillips, Drevets, Rauch, & Lane, 2003). Thus, deficits in the functional interactions of limbic regions and prefrontal cortex appear to play a critical role in the

pathophysiology of affective disorders. These alterations in both emotion processing and reward processing have been repeatedly demonstrated in individuals with depression; however, it remains unclear whether these circuit abnormalities represent a consequence of depression or a vulnerability to depression that may influence the individual to focus more on negative affect and have difficulty in experiencing pleasure (see Figure 1 for depiction of model). One method to assess this question is to examine differences in the neural circuitries of these networks prior to the onset of depression using fMRI.
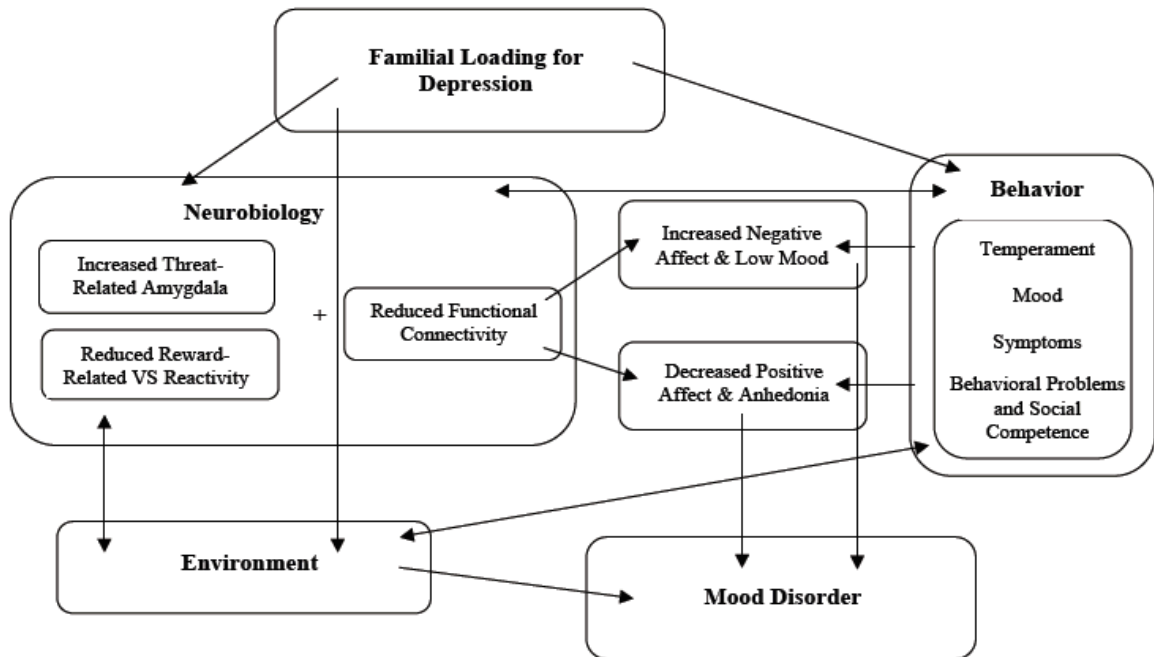
Figure 1: Proposed theoretical model for the mechanism by which familial loading for depression may contribute to the development of depression.

Accordingly, the present study proposed to examine the functioning of two key neurobiological processes—threat-related amygdala reactivity and reward-related ventral striatal (VS) reactivity—regions implicated in both premorbid risk (Monk et al., 2008) and pathophysiology (Drevets, 2003; Elliott, Sahakian, Michael, Paykel, & Dolan, 1998; Epstein et

al., 2006; Fu et al., 2004; Henriques & Davidson, 2000; Seminowicz et al., 2004; Sheline et al., 2001) of mood disorders in adolescents with familial risk for MDD. These two processes are of additional interest because they both exhibit strong genetically driven variability (Forbes, Brown et al., 2009; Munafo, Brown, & Hariri, 2008). Index cases were selected from a high-risk group of 12-15 year old adolescents with at least one first-degree (e.g., parent) and one second-degree (e.g., aunt) relative who have had a lifetime recurrent major depression, a bipolar disorder, or a childhood onset major affective illness. Control cases were selected from a comparable low-risk group with no first-degree relatives with a lifetime history of affective disorders (including parents and siblings) and no more than 20% of second-degree relatives with a lifetime history of affective disorders.

Blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) was used to investigate the functioning of the amygdala, which mediates behavioral and physiological arousal in responses to environmental challenge, and the ventral striatum, which mediates behavioral responses to salient environmental rewards. Amygdala reactivity to threat-related emotional facial expressions was assayed using a well-characterized challenge paradigm that robustly engages the amygdala and interconnected corticolimbic nodes (Hariri et al., 2005). Importantly, this task has been previously shown to effectively engage the amygdala in healthy individuals and patients as well as in pediatric and adult populations (Hariri et al., 2005; Hariri, Tessitore, Mattay, Fera, & Weinberger, 2002; Meyer-Lindenberg et al., 2005; Tessitore et al., 2002; Tessitore et al., 2005; Wang, Dapretto, Hariri, Sigman, & Bookheimer, 2004). VS reactivity was assayed using a more recently developed reward paradigm known to engage the VS in adults (Forbes, Brown et al., 2009; Hariri et al., 2006). Previous pilot data have shown similar patterns of activation in an adolescent population. Using index (i.e., high-risk) and

control cases (i.e., low-risk) and BOLD fMRI challenge paradigms described above, this study aimed to investigate possible premorbid differences between adolescents with high familial loading for depression relative to low familial loading in 1) threat-related amygdala reactivity, 2) reward-related VS reactivity, and 3) the functional coupling between these structures and regions of the prefrontal cortex (PFC) involved in regulating amygdala and VS reactivity.

In addition to contributing to dysfunction at the neurobiological level in high-risk adolescents, high familial loading for depression may contribute to group differences in either observable or psychological behaviors such as temperament, subjective mood, symptoms of depression and anxiety, and behavioral problems and social competence. These behaviors are easily measured via self-report by the child or by the parent about the child. Although the subjects in this study do not meet criteria for depression, these measures allow for the presence of sub-clinical individuals in the current sample. These psychological and behavioral factors via alterations in neurobiology and increases in negative affect (low mood)/decreases in positive affect (increased anhedonia), may also contribute to depression. It may be that scores on these behavioral measures, more than actual risk status, may account for potential differences in the emotion and reward circuits. To this end, the relation between these behaviors and neurobiological reactivity was also explored. For this study, adolescents were evaluated on 1) a measure of temperament, 2) a measure of depressive symptoms (mood), 3) a measure of anxiety symptoms, and 4) a measure of behavioral problems and social competence used to assess internalizing and externalizing factors. Temperament was investigated because prior work has reported that temperamental difficulty is considered a risk factor for later emotional and behavioral problems in both normal and high-risk populations (Tubman & Windle, 1995). Symptoms of depression and anxiety as well as internalizing and externalizing symptoms were

explored to investigate the neurobiology of sub-clinical individuals that do not meet full criteria for mood disorders. The specific measures used in this study are discussed in detail in the methods section.

A.   NEUROIMAGING STUDIES OF THREAT-RELATED AMYGDALA FUNCTION IN CHILDREN/ADOLESCENTS

Numerous studies have shown that depression clusters within families (Weissman, Leckman, Merikangas, Gammon, & Prusoff, 1984; Williamson et al., 1995), with a recent study finding that chronicity of depressive symptoms is also familial (Mondimore et al., 2006). Given that children with high familial loading show an increased risk of developing depression, premorbid and prospective investigation of this population may help identify factors that predict or prevent later depression.

In adults, previous neuroimaging studies have demonstrated significant differences in brain regions associated with emotion processing. Differences in both limbic and cortical brain activation have been observed between depressed and non-depressed individuals (Fales et al., 2008; Fu et al., 2008; Grimm et al., 2008; Harvey et al., 2005; Sheline et al., 2001). Much of this research in adults has utilized populations with either concurrent or remitted depression; therefore, these studies have been unable to demonstrate conclusively that differences observed between depressed and non-depressed individuals are predictors or consequences of depression. Few functional neuroimaging studies have been conducted in depressed children and have

reported conflicting results (Forbes, Hariri et al., 2009; Lau et al., 2009; Roberson-Nay et al., 2006; Thomas et al., 2001).

Because depression is considered a disorder involving alterations in emotion processing and regulation (Baxter et al., 1989; Bench, Friston, Brown, Frackowiak, & Dolan, 1993; Phillips, Drevets, Rauch, & Lane, 2003), both adult and child/adolescent neuroimaging studies (Serene, Ashtari, Szeszko, & Kumra, 2007; Thomas et al., 2001) have focused primarily on functioning of the amygdala given that this brain region plays a significant role in both implicated emotional processes. The amygdala is a small bilateral almond-shaped structure that largely serves as a relay between afferent sensory and visceral information and efferent autonomic responses encompassing increases in behavioral and physiological arousal (LeDoux, 2000). Whereas some studies have reported relatively increased amygdala activity in depressed individuals when viewing affective stimuli (Drevets, 2003; Fales et al., 2008; Fu et al., 2004; Sheline et al., 2001), the majority of these are confounded by current psychotropic medication use and psychiatric comorbidity, especially anxiety, which both can bias amygdala activity (Breiter et al., 1996; Hariri & Fisher, 2007; Perez-Edgar et al., 2007; Rauch et al., 2000). In children, some studies have reported relatively increased amygdala activity in those with anxiety disorders (M. B. Stein, Simmons, Feinstein, & Paulus, 2007; Thomas et al., 2001) but relatively decreased activity in those with MDD (Thomas et al., 2001), and others have reported increases in amygdala activity in children with MDD (Roberson-Nay et al., 2006), and bipolar disorder (Rich et al., 2006). A recent study has shown that directionally consistent differences may exist prior to the onset of depressive symptoms in children and adolescents of depressed parents (Monk et al., 2008). These data point to the existence of differences in amygdala reactivity associated with childhood risk for depression that may continue into adulthood. Due to the paucity of premorbid high-risk

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studies, alterations in amygdala reactivity in adolescents with high familial loading for depression need to be further explored. Future studies need also to examine the dynamic functional interactions of the amygdala and PFC, especially its interconnected medial and ventral extent, which could account for greater variability in emotional behaviors (Drabant et al., 2006; Hariri et al., 2005; Pezawas et al., 2005) and may represent a critical pathophysiological substrate of depression (Siegle, Thompson, Carter, Steinhauer, & Thase, 2007).

B.   NEUROIMAGING STUDIES OF REWARD-RELATED VENTRAL STRIATAL FUNCTION IN CHILDREN/ADOLESCENTS

Because adolescence is a time of increased experimentation and risk-taking (Dahl, 2004), it may also be a time when understanding and processing of reward is changing. Recent investigations have highlighted the possibility that processing of reward cues during this time may be evolving and therefore may contribute to adolescent risk for psychopathology (Bjork et al., 2004). Consequently, investigation of reward-related pathways during this time may help identify neural predispositions to future problems. The reward circuit of the brain is composed of midbrain (substantia nigra/ventral tegmental area), subcortical (ventral striatum/nucleus accumbens, dorsal striatum, amygdala, hippocampus) and cortical (orbitofrontal, medial and dorsolateral prefrontal) regions. The VS plays a critical role in this distributed circuitry as it gates the effects of midbrain dopamine release on cortical, especially PFC regions, and other subcortical regions controlling complex goal-directed behaviors (Kalivas & Volkow, 2005). As such, the VS has

been implicated in reward, addiction, pleasure, and related appetitive or consummatory behaviors.

The reward circuit, especially the VS, has been extensively studied in adults (Fliessbach et al., 2007; Hampton, Adolphs, Tyszka, & O'Doherty, 2007; Hariri et al., 2006; Yacubian et al., 2007); however, the literature in children and adolescents is in need of further development. Despite the paucity of studies, however, there is consensus that adolescents exhibit alterations in the brain regions implicated in reward processing when compared to adults, suggesting the existence of an immature reward system that continues to develop into adulthood. The direction of this effect, however, has varied by study and tended to depend on whether VS reactivity was measured during the anticipation or feedback segment of the scan, with some studies finding reduced VS reactivity in the anticipation of rewarding feedback in adolescents relative to adults (Bjork et al., 2004), and other studies finding greater activation in adolescents in these regions after receiving reward (Ernst et al., 2005; Galvan et al., 2006). In addition to actual developmental differences between adolescents and adults, these differences observed between studies are likely influenced by variations in the design of the fMRI paradigms used to investigate VS function (e.g., performance titration, rewarding stimuli, incentive values) as well as the characteristics of each sample population (e.g., familial risk for addiction versus ADHD). These noted differences have contributed to the difficulty in assessing reward function in children.

Altered VS functioning has also been investigated in depressed adults, with decreased activity generally reported in response to rewarding/positive stimuli (Elliott, Sahakian, Michael, Paykel, & Dolan, 1998; Epstein et al., 2006), potentially creating a predisposition to anhedonia. In addition, reduced VS grey matter volume and reactivity have been correlated with increases in

anhedonia severity (Harvey, Pruessner, Czechowska, & Lepage, 2007; Keedwell, Andrew, Williams, Brammer, & Phillips, 2005). Similarly, studies of depressed children have shown a decrease in reward-related brain reactivity (anterior cingulate cortex, caudate, and inferior orbitofrontal cortex) during both reward anticipation and reward outcome during conditions of loss or low-magnitude reward, relative to non-depressed children (Forbes et al., 2006). Similarly, a recent study of MDD and control adolescents who completed a guessing task involving monetary reward, demonstrated that MDD adolescents exhibited relatively less striatal activity relative to controls during reward anticipation and outcome, and greater dorsolateral and medial prefrontal activity. Moreover, this decreased striatal activation was correlated with lower subjective positive affect in a real-world environment (Forbes, Hariri et al., 2009). Additionally, behavioral data from a longitudinal study of 11 year-old boys indicated that depressed boys presented abnormal responses to reward-related choices—displaying a reluctance to choosing high-probability/high-magnitude reward choices—that predicted depressive disorders and symptoms a year later (Forbes, Shaw, & Dahl, 2007). These studies may explain the low motivation and reduced enjoyment seen in depressed youth. Only one study to date has been conducted in adolescents at high-risk for depression examining possible preexisting differences in reward-related brain function (Monk et al., 2008). Consistent with the general pattern reported in depressed children, this study, which used happy faces as the rewarding stimuli, reported blunted VS activity in children/adolescents at high-risk for depression.

Much like the available literature on premorbid amygdala functioning in high-risk children and adolescents, there is a paucity of research examining premorbid reward-related VS functioning in these populations. Thus, studies of premorbid populations are needed to understand the impact of differential reward-related VS function, if any, on the emergence of

depression in high-risk children and adolescents. These studies should also consider the functional interactions of the VS and regions of the PFC that play an instrumental role in maintaining reward contingencies, mediating inhibitory control, and regulating behavioral strategies (Bechara, Tranel, & Damasio, 2000; Kalivas & Volkow, 2005; Mayberg et al., 2005).

C. NEUROIMAGING STUDIES OF FUNCTIONAL CONNECTIVITY

Emotional problems in adolescence—a period of increased experimentation and coping with social challenges—may be related to difficulties with the regulation of emotions and behavior (Dahl, 2004). These difficulties may arise because of an increased burden on both the functional neural circuits that are critical for mediating arousal, attention, and affect (i.e., amygdala and VS), as well as those necessary for monitoring and regulating the drive of these regions, in order to shape behavior adaptively and avoid negative consequences (i.e., PFC). Proficiency in self-control and the ability to regulate one's emotions crystallizes slowly, and structural neuroimaging studies have shown that cortical, especially PFC, development continues into early adulthood (Casey, Giedd, & Thomas, 2000; Gogtay et al., 2004; Gogtay et al., 2007; Rapoport & Gogtay, 2008; Spear, 2000). Thus, deficits in the functional dynamics between the amygdala, VS, and regions of PFC in adolescents may contribute to risk for the development of affective disorders. Previous studies of functional connectivity in depressed adults have revealed relatively diminished functional coupling between the amygdala and regions of PFC during processing of emotional, especially threat-related, information (Anand et al., 2005a, 2005b; Mayberg et al., 1999), and a recent study has found similarly diminished connectivity in bipolar

children compared to normally developing children (Rich et al., 2008). Few functional connectivity studies have been conducted using reward paradigms, but preliminary data suggest that there is distinct coupling between PFC and subcortical regions, and the strength of this connectivity appears to vary with magnitude of reward-risk (Cohen, Heller, & Ranganath, 2005). Although the functional connectivity involved in processing reward-related information is not fully understood and has not been examined in depression, it is likely that similar patterns of dysregulation are present. Even more so than the extant research on amygdala and VS function, there is little if any research, especially in children and adolescents, examining the predictive value of premorbid functional coupling between regions of the PFC and either the amygdala or VS in the development of depression. As already suggested above, such analyses are critically needed in future studies.

II.   STATEMENT OF PURPOSE

Major depressive disorder remains the most prevalent lifetime disorder in the United States at 16.6 % of the population (Kessler et al., 2005), with an estimated economic burden of 83.1 billion dollars in 2000 (Greenberg et al., 2003). More importantly, depression is associated with a plethora of debilitating symptoms that interfere with an individual's daily life and future. There is a need, therefore, to investigate possible risk and resilience factors for depression so that effective interventions can be employed to reduce these growing numbers.   Studying the underlying neural correlates for the emergence of depression has the promise to expand our knowledge of predictive risk markers and fuel the development of biologically-guided, individually-tailored intervention and prevention strategies. Specifically, because of the deficits in both negative (low mood) and positive affect (anhedonia) often experienced by depressed individuals, understanding dysfunctions in emotion- and reward-related neural circuitries are warranted.   Despite an abundance of studies conducted in depressed adult populations, few studies have been undertaken in child and adolescent populations at high-risk for depression associated with increased familial loading (i.e., positive family history).   Identifying developmental pathways and biological mechanisms of vulnerability (and possible resilience) in high-risk, premorbid populations can greatly inform the refinement of early intervention and prevention of depression and related mood disorders.

To address some of these outstanding needs, this research sought to apply three approaches, which combined represent a novel study of risk for depression in large sample of adolescents. First, the study examined threat-related amygdala reactivity and reward-related VS reactivity in individuals prior to the onset of depression and is therefore not confounded by possible neurobiological consequences of concurrent depression and treatment, as is often found in many of the current studies of depressed adults. Second, because the research is focused on adolescence, results may help elucidate why this is a time period of increased vulnerability to depression. Third, results from this study may offer insight into possible neurobiological predictors of future depression. This knowledge may eventually help to inform potential target interventions at a time when the brain is still continuing to develop and therefore can potentially be most effective. It was hypothesized that adolescents at high-risk for developing depression would display alterations in core brain regions contributing to complex emotion- and reward-related processes, namely the amygdala and VS, as well as the functional connectivity between these regions and the prefrontal cortex, that exist prior to the onset of depression.

III. HYPOTHESES

Based on the previous research in depression and adolescent development reviewed above, the following hypotheses will be tested.

1. Altered threat-related amygdala reactivity in adolescents at high-risk for depression

It was hypothesized that relative to the low-risk group, the high-risk group would have increased amygdala reactivity in response to viewing threat-related faces.

2. Altered reward-related VS reactivity in adolescents at high-risk for depression

It was hypothesized that relative to the low-risk group, the high-risk group would have decreased VS reactivity when processing reward-related stimuli.

3. Differences in functional connectivity between regions of the PFC and amygdala or VS

It was hypothesized that functional connectivity between the amygdala and PFC would be diminished in the high-risk in comparison to the low-risk group. It was predicted that a similar pattern would be observed in the functional connectivity of the VS and PFC regions.

IV. METHODS AND EXPERIMENTAL DESIGN

A. PARTICIPANTS

A total of 333 participants were scanned from a larger community sample of 989 adolescent participants, ages 12 to 15 years old that were recruited from the greater metropolitan San Antonio, Texas area using commercially available mailing/phone lists from Scientific Telephone Services. Of these participants, 17 were excluded based on technical or administrative difficulties and 8 were excluded because of structural brain-abnormalities. Five subjects had unusable amygdala reactivity paradigm data (2 due to excessive movement and 3 due to technical error). Fourteen subjects had unusable ventral striatal reactivity paradigm data (9 due to excessive movement and 5 due to technical error). Of the 308 participants with useable data (51% female), 57% were Caucasian, 29% Hispanic/Latino, 10% multi-racial, 3% African-American, 1% Asian, and < 1% Native-American. One hundred fifty-four index (i.e., high-risk) cases were assessed to have no lifetime psychiatric disorders and were determined to have at least one first-degree (e.g., parent) *and* one second-degree (e.g., aunt) relative who have had a lifetime recurrent major depression, a bipolar disorder, or a childhood onset major affective illness, using defining criteria of the DSM-IV. One hundred fifty-four control (i.e., low-risk) cases were assessed to have no lifetime psychiatric disorders and were established to meet the following criteria: 1) no first-degree relative (including parents and siblings) with a lifetime history of affective disorders (e.g. major depression, bipolar disorder); 2) no more than 20% of

second-degree relatives with a lifetime history of affective disorders; 3) no family history (in first- or second-degree relatives) of psychotic or bipolar depression; 4) no more than one second-degree relative with a recurrent depression or childhood-onset affective disorder; 5) no family history of schizophrenia; and 6) no history of physical or sexual abuse. Additionally, all subjects included in the analysis were free from the following: 1) History of brain injury; 2) Psychosis; 3) Pervasive developmental disorders; 4) Learning disabilities; and 5) IQ < 80. The parent protocol from which the index and control cases were selected was approved by the University of Texas Health Science Center at San Antonio institutional review board. Informed consent was obtained from parents prior to their child's enrollment in the study.

B.   PROCEDURES

Adolescents were assessed at the University of Texas San Antonio Medical Center. Interviews, assessments, and fMRI scans were conducted as described below. Participants were reimbursed for their time at the end of each assessment.

C.   MEASURES

1.   Child psychopathology

Children's lifetime and current psychiatric symptomatologies were assessed using the Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version (K-SADS-PL), a semi-structured diagnostic interview that provides assessments of

present episodes and lifetime history of psychiatric illness in children based on DSM-IV criteria (Kaufman et al., 1997). The unstructured introductory interview was first administered to obtain demographic, health, prior psychiatric treatment, and school and social functioning information. Next, the screen interview was administered to assess the current and most severe past episode. The supplements, used to assess affective disorders, psychotic disorders, anxiety disorders, behavioral disorders, and substance abuse and other disorders in further detail, were administered if at least one score of 3 (threshold) or multiple scores of 2 (sub-threshold) were attained. All interviews were administered by experienced bachelor's- or master's-prepared interviewers. Whereas children with current and lifetime diagnoses of major depressive disorder were excluded, children were permitted in the study if criteria were met for an anxiety disorder (e.g., Generalized Anxiety Disorder (GAD), Separation Anxiety Disorder (SAD), Panic Disorder (PD), etc.). This procedure was adopted because childhood anxiety often preexists the onset of later depressive disorders (Parker et al., 1999; M. B. Stein et al., 2001), and anxiety is common in offspring of parents with major depressive disorder. Exclusion of these individuals may preclude the exploration of a population of adolescents who will go on to develop later depression, and further would decrease the generalizability of the findings. Nevertheless, separate analyses assessing main effect of risk status in both paradigms were conducted with these subjects removed. Diagnoses of the 34 target children (33 high-risk, 1 low-risk with ADHD) with anxiety and/or behavioral disorder diagnoses are displayed in Table 1.

Table 1: Current and lifetime diagnoses of the 34 target children with anxiety and behavioral disorders

Diagnosis	Current	Past
Attention Deficit Hyperactivity Disorder	5	0
Adjustment Disorder	0	2
Adjustment Disorder and Obsessive Compulsive Disorder (OCD)	OCD 1[a]	Adjustment Disorder 1[a]
Anxiety NOS	1	0
Eating Disorder NOS and Cutting	1	0
Generalized Anxiety Disorder	5	1
OCD and Specific Phobia	1	0
OCD	0	1
Panic Disorder (PD)	1	0
PD and Acute Stress Disorder	PD 1[a]	Acute Stress Disorder 1[a]
PD and Specific Phobia	1	0
Separation Anxiety Disorder (SAD) and Specific Phobia	0	1
SAD	0	3
Social Phobia and Adjustment Disorder	0	1
Social Phobia and GAD	GAD 1[a]	Social Phobia 1[a]
Social Phobia	0	1
Social Phobia and Oppositional Defiant Disorder (ODD) and SAD	1	0
Social Phobia and Specific Phobia	1	0
Specific Phobia	4	0

[a]Target child had lifetime diagnosis for listed combination of disorder, with one disorder current and one disorder past

2. Family psychopathology

Parent's lifetime and current psychiatric symptomatologies were assessed using a modified version of the Family History Interview (FHI). This interview was administered to the informant (usually one or both parents) to obtain a complete history of lifetime psychiatric diagnoses of the family pedigree and was used to determine familial risk status (high- or low-risk). This interview assessed DSM-IV lifetime diagnoses for all of the family members identified as 1st- or 2nd-degree relatives of the target child (Weissman, Warner, Wickramaratne, Moreau, & Olfson, 1997). Lifetime diagnoses among relatives were made via consensus of the research team, including the interviewers and the principal investigator, using the best-estimate procedure (Leckman, Sholomskas, Thompson, Belanger, & Weissman, 1982). For the current data, 22 FHIs were conducted with both parents present, 243 with only the mother, 40 with only the father, and 3 with a non-parental guardian/family member. Of the 154 high-risk pedigrees, 84 had mothers diagnosed with a mood disorder, 23 had fathers diagnosed with a mood disorder, 34 had both parents diagnosed with a mood disorder, and 13 had neither father nor the mother with a mood disorder diagnosis (sibling(s) were only first-degree relative with mood disorder diagnosis).

3. Acquisition of Blood Oxygenation-Level Dependent (BOLD) fMRI scans

The fMRI scans were performed at the University of Texas Health Science Center at San Antonio using a Siemens 3T Trio scanner (Siemens Medical Solutions, Erlangen, Germany). BOLD functional images were acquired using a gradient-echo echoplanar imaging (EPI) sequence to obtain 34 axial slices (3 mm thick). The middle slice was aligned to the AC-PC line

to maximize coverage of the limbic regions (TE = 25 msec, TR = 2000 msec, acquisition matrix = 64 × 64, field of view = 20 cm). Prior to collection of fMRI data, a reference EPI scan was acquired and visually inspected for artifacts (e.g., ghosting) and good signal across the entire volume of acquisition, including the amygdala and ventral striatum. Moreover, an autoshimming procedure was administered before the acquisition of BOLD data in each participant to minimize field inhomogeneities. Data from all of the subjects included in the analyses were cleared of such problems.

4.   Amygdala reactivity paradigm

In this paradigm, four blocks of a perceptual face processing task were interleaved with five blocks of a sensorimotor control task (Figure 2A). During the face task, subjects viewed a trio of affective (angry or fearful) faces and were asked to select which of the bottom two faces was identical to the one presented at the top of the trio. The faces were derived from a standard set of pictures of facial affect (Ekman & Friesen, 1976). Each face block consisted of six trios, three of each affect and sex, randomly assigned. Each image was presented for 4 s, with a variable interstimulus interval (ISI = 2-6 seconds) for a total scan time of 390 seconds. The presentation of stimuli for 4 s has previously been shown to allow for the haemodynamic response (dynamic regulation of the blood flow) of the target brain regions to occur (Brown, Manuck, Flory, & Hariri, 2006; Brown et al., 2005; Manuck, Brown, Forbes, & Hariri, 2007). During the control task, the subjects viewed a trio of shapes and were asked to select which of the bottom two shapes was identical to the top of the trio. Each control block consisted of six different images, which were presented for 4 s, with a fixed ISI of 2 s.

A.
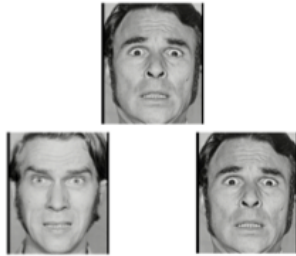
Match Faces   Match Shapes

B.

Reward or No Reward

higher

| Guess Number | ? | 7 | ↑ |

3 s   *lower* 500 ms   500 ms

| | ? | 7 | ↓ |

total scan time = 342 s

Control

| Press Button | X | * | ● |

3 s   500 ms   500 ms
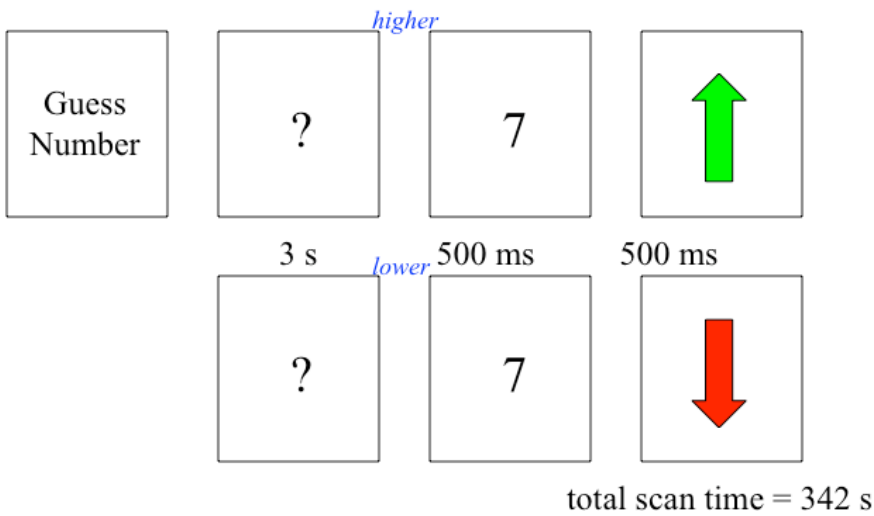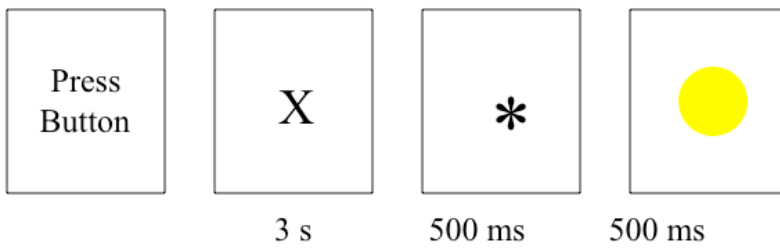
Figure 2: A. Amygdala Reactivity Paradigm, B. Ventral Striatal Reactivity Paradigm.

5. Striatal reactivity paradigm

In this paradigm, subjects were instructed to play a card-guessing game resulting in positive or negative feedback for each trial (Figure 2B.). Subjects were told that their performance on the card game would determine a monetary reward to be received at the end of the game. During each trial, subjects had 3 s to guess, via a button press, whether the value of an upcoming visually presented card would be greater than or less than five. After a choice was made, the numerical value of the card was presented (higher or lower) for 500 ms and followed by appropriate feedback (green "up" arrow for positive feedback on a correct trial; red "down" arrow for negative feedback on an incorrect trial) for an additional 500 ms. A crosshair focus point was then presented for 3 s for a total trial length of 7 s. Each task block was comprised of five trials, with three blocks each of predominantly positive feedback (75% correct) and three of predominantly negative feedback (25% correct). An incongruent trial type within each task block was employed (e.g., one of four trials during positive feedback blocks is incorrect, resulting in negative feedback) to prevent subjects from anticipating the feedback for each trial and to maintain subject's engagement and motivation to perform well. The six task blocks were interleaved with three control blocks. During control blocks, subjects were asked simply to make alternating button presses during the presentation of an "x" (three seconds), which was followed by an asterisk (500 ms) and a yellow circle (500 ms). Each block was preceded by a 2 s instruction of "Guess Number" (for task) or "Press button" (for control), resulting in a total block length of 38 seconds and a total scan time of 342 seconds. Subjects were unaware of the fixed outcome probabilities associated with each block and were led to believe that their performance would determine their net monetary gain, although all subjects received $10 upon completion of the task.

D.   ADDITIONAL DEMOGRAPHIC AND BEHAVIORAL

MEASURES (Summarized in Table 2)

1.   Revised Dimensions of Temperament Survey (DOTS-R)

This measure is a self-report based on the Thomas and Chess (Thomas & Chess, 1976) dimensions of temperament. The factors include activity level (general and sleep), approach-withdrawal, flexibility-rigidity, mood quality, rhythmicity (sleep, eating, and daily habits), and task orientation (distractibility and persistence). The score interpretations are as follows: Activity Level – General: high scorers are characterized by high levels of energy, vigor, and overt motor activity; Activity Level – Sleep: high scorers are characterized by high levels of motor activity during sleep; Approach – Withdrawal: high scorers tend to approach, or move toward, new persons, objects, situations, or events; Flexibility – Rigidity: high scorers tend to respond flexibly to changes in the environment; Mood Quality: high scorers are characterized by high levels of positive affect; Rhythmicity – Sleep: high scorers are characterized by regular timing of the daily sleep–wake cycle; Rhythmicity – Eating: high scorers are characterized by regularity of eating habits pertinent to appetite and quantity consumed; Rhythmicity – Daily Habits: high scorers are characterized by regularity of timing of diurnal activities such as toileting, peak period of feeling full of energy, and taking a rest or a break in daily activities; Task Orientation: high scorers tend to be able to concentrate and maintain perceptual focus despite extraneous stimuli and/or stay with, or continue steadily in an activity for a relatively long period of time. This measure has established factorial validity across samples from early childhood to late adolescence/early adulthood (Windle, 1992; Windle & Lerner, 1986).

2. Mood and Feelings Questionnaire-Parent (MFQ-P) and -Child (MFQ-C)

This is a 33-item measure consisting of descriptive phrases regarding how the child has been feeling or acting within the past two weeks. It is administered to both the parent and child, producing a summary score for each informant that was used in the present analyses. Higher scores on this measure are indicative of greater number of depressive symptoms endorsed (Sund, Larsson, & Wichstrom, 2001; Wood, Kroll, Moore, & Harrington, 1995).   It has been shown to validly identify major depressive episodes or other mood disorders in a variety of populations (Daviss et al., 2006).

3. Child Behavior Checklist for Ages 11-18 (CBCL)

This is a measure administered to parents to assess their child's behavioral problems and social competence. It is composed of 113 Likert-scale items that ask the parent to report the extent to which the listed behavior is true of their child (not true, somewhat or sometimes true, very true or often true).   These items produce a Total Behavior Score, Internalizing Factor Score (measuring anxiety and depressive symptoms), Externalizing Factor Score (measuring aggression and disruptive or antisocial behavior), as well as several subscale scores not used in the present analysis.   Higher scores on the Internalizing and Externalizing measures are indicative of the presence of greater number of anxiety/depressive symptoms and aggression/disruptive/antisocial behavior symptoms, respectively (Achenbach & Rescorla, 2001).

4.   Screen for Childhood Anxiety Related Disorders-Parent (SCARED-P) and -Child (SCARED-C)

This measure consists of 41 items administered to both the parent and child that screens for several types of DSM anxiety disorders including generalized anxiety disorder, separation anxiety disorder, panic disorder, and social anxiety disorders. Additionally, the measure produces a sum anxiety score, which was used in the present analysis. Higher scores on this measure are indicative of the presence of higher number of anxiety symptoms (Birmaher et al., 1997).

5.   Assessments of pubertal status

Pubertal Development Drawings (PDD): The PDD is a self-report measure based on Tanner's stages of development, utilizing drawings based on Tanner's stages of development and illustrates male genitalia and pubic hair, and female breasts and pubic hair (Morris & Udry, 1980). *Pubertal Development Scale (PDS)*: The PDS is a 6-item self-report questionnaire that measures pubertal status administered to both males and females to determine Tanner stage for both breast size and pubic hair development (females) and genitalia size and pubic hair development (males) (Petersen, Crockett, Richards, & Boxer, 1988).

Table 2: Summary of behavioral measures

Measure	Assessment	Summary Scores Used	Number of Cases Available
Revised Dimensions of Temperament Survey (DOTS-R)	Based on the Thomas and Chess dimensions of temperament. Designed to assess dimensions of temperament associated with social maladjustment	Activity Level (General & Sleep), Approach-Withdrawal, Flexibility-Rigidity, Mood Quality, Rhythmicity (Sleep, Eating, Daily Habits), Task Orientation	236
Moods and Feelings Questionnaire - Parent and Child (MFQ - P, C)	Assesses core depressive symptoms derived from DSM criteria	Total Sum	278 (P) 280 (C)
Child Behavior Checklist for Ages 6-18 (CBCL)	Assesses behavioral problems and social competence	Internalizing and Externalizing Scores	230
Screen for Childhood Anxiety Related Disorders - Parent and Child (SCARED - P, C)	Measure of anxiety symptoms and presence of a DSM anxiety disorder	Total Sum	278 (C) 270 (P)
Assessment of Pubertal Status -- Tanner Stage	Determination of Tanner Stage for males and females	Tanner Stage for Pubic Hair (Male & Female) and Breast (Female) and Genitalia (Male) development	115 (F) 116 (M)
Self-Report Parental Education	Parental education	Categorical Highest Education Level	256

6. Demographics

Sex of child, race, and date of birth were collected during the interview. Highest level of parental education was used as a measure of socioeconomic status (a) less than 9th grade, (b) 9th to 12th grade (no diploma), (c) high school graduate (including GED), (d) some college, no degree, (e) associates degree, (f) bachelors degree, (g) graduate or professional degree.

7. Administration of behavioral measures

Due to the experimental design, it was not always possible to administer the behavioral measures at the time of the scan, which is potentially problematic for assessments such as the MFQ-C and MFQ-P that specifically probe symptom presence "in the past two weeks." Because the date of assessment relative to the scan date are potentially critical in determining valid and meaningful relations between some of these measures and fMRI data, data were analyzed with and without the time between scan and assessment entered as a covariate. The results did not differ between these two analyses. Additionally, because of difficulties in coding the data, not all measures were available for all of the subjects (See Table 2).

V. DATA ANALYSES

All data were examined in a step-wise fashion, first examining the data using simple univariate (histograms, boxplots) and bivariate (scatterplots, tables) summaries. Next, standard approaches to explore the data were employed using generalized linear models. For any exploratory analyses conducted, we adjusted for multiple comparisons.

A. BOLD FMRI ANALYSES

Analysis of the fMRI data was completed using Statistical Parametric Mapping (SPM5) software (http://www.fil.ion.ucl.ac.uk/spm). Images for each subject were realigned to the mean volume in the time series to correct for head motion, spatially normalized into a standard stereotactic space (Montreal Neurological Institute template) using a 12 parameter affine model and smoothed to minimize noise and residual differences in gyral anatomy with a Gaussian filter, set at 6 mm full-width at half-maximum. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean. Determination of appropriate thresholds for neuroimaging data has been a long-standing problem (Genovese, Lazar, & Nichols, 2002; Thirion et al., 2007). Due to the unprecedented large sample size of this neuroimaging study, the data were examined using two thresholds. The more conservative threshold of $p < 0.05$, with a region of interest correction (FDR) for multiple comparisons, was first used to identify significant responses for all

comparisons. A more liberal threshold of $p = 0.05$, using a small volume-correction procedure within a region of interest was also employed to detect potential sub-threshold differences between groups.

B.   AMYGDALA AND STRIATAL REACTIVITY PARADIGMS: MAIN EFFECTS OF TASK AND BETWEEN-GROUP ANALYSES

For each subject and scan, predetermined condition effects at each voxel were calculated using a *t*-statistic, producing a statistical image for each contrast of interest: (faces > shapes) for the amygdala-reactivity paradigm, and [(positive feedback > control) > (negative feedback > control)]—"reward > no reward"—for the VS-reactivity paradigm. These individual contrast images were then used to determine task-specific regional responses using predetermined regions of interest including bilateral amygdala and VS at the group-level for the entire sample (main effects of task) and direct comparisons between groups (main effect of risk status). To test the hypothesized differences between the high- and low-risk groups, regressions were conducted to assess group differences in the two fMRI paradigms.

Regions of interest were constructed using the Talairach Daemon option of the WFU PickAtlas Tool, version 1.04 (Wake Forest University School of Medicine, Winston-Salem, North Carolina). The amygdala region of interest was dilated once on both the right and left hemispheres. Due to structural and functional heterogeneity of amygdala nuclei implicated in the processing of threat-related cues (Kim, Somerville, Johnstone, Alexander, & Whalen, 2003; J. LeDoux, 1996; Whalen, 2007), the ventral and dorsal amygdala, which encompass the amygdala's principal input and output regions, respectively, were independently examined.

These subregions were created for the right and left using MarsBaR (v 0.41) using an anatomically-based method elsewhere described (Manuck et al., under review). The ventral striatum region of interest was defined as a sphere of 15 mm in radius centered on the Talairach coordinates of x = 0, y = 10, z = -2, therefore encompassing the VS in both the right and left hemispheres.

C. FUNCTIONAL CONNECTIVITY ANALYSES

Functional connectivity is a method employed by the neuroimaging community as a measure of correlated activity between a reference and a target region using BOLD fMRI to assess aspects of functional integration. Briefly, reference regions (amygdala and VS) were chosen from functional clusters identified by the main effects of task. Region of interest masks were created using the WFU PickAtlas for anatomical regions within the main effect of task activation patterns. Mean activity within these reference regions of interest was correlated with target regions (in PFC) with which they was thought to be functionally correlated. Risk group differences in connectivity were investigated in a linear regression analysis.

Mean values were extracted from clusters identified using the maximally-activated voxel in each of the predetermined reference and target regions of interest using the Main Effect of Task contrasts (e.g., faces > shapes, reward > no reward). For the amygdala reactivity paradigm, data were extracted from the right and left amygdala, right and left dorsal amygdala, right and left ventral amygdala (reference regions) and BA 11, BA 25, right and left BA 47, and BA 32 (target regions). These regions of interest were selected because of previous findings reporting significant recruitment of these areas when subjects are engaging in this paradigm. For the

ventral striatal reactivity paradigm, data were extracted from the VS (reference region) and medial prefrontal cortex, lateral orbital frontal cortex, and dorsolateral prefrontal cortex (target regions). These target regions have been previously correlated with interindividual variability in delay discounting (DD) using this paradigm. Specifically, this previous study demonstrated a positive correlation between DD and activity in the medial PFC and negative correlations with activity in the lateral OFC and dorsolateral PFC (Hariri et al., 2006).

When the values were extracted, BA 25, BA 32 (amygdala task), lateral OFC, and dorsolateral PFC (reward task) target regions did not overlap with the main effect of tasks analyses ($p < 0.05$, corrected for multiple comparisons) and extraction analyses therefore yielded null results. As a result, these target regions were not included in future analyses. Connectivity was thus assessed in 18 regression analyses in SPSS using the extracted data for the amygdala reactivity paradigm (each of the 6 reference regions correlated with each of the remaining 3 target regions) and 1 regression for the ventral striatal reactivity paradigm (1 reference region correlated with the 1 remaining target region). The significance of the interaction between connectivity and risk status was calculated using a linear regression in SPSS by creating a dummy code for risk status and an interaction term for connectivity by risk.

D.   EXPLORATORY ANALYSES

To explore potential associations between task-related brain activity and measures of behavior (e.g., SCARED, MFQ), data were first extracted from the main effects analysis using pre-defined regions of interest. These regions were defined as stated above using the WFU PickAtlas. These regions were the same regions investigated in the functional connectivity analyses: for the

amygdala reactivity paradigm—right amygdala, left amygdala, right dorsal amygdala, left dorsal amygdala, right ventral amygdala, left ventral amygdala, BA 11, BA 25, BA 47, and BA 32; for the ventral striatal reactivity paradigm—ventral striatum, medial prefrontal cortex, lateral orbital frontal cortex, and dorsolateral prefrontal cortex. These extracted values were then visually examined using scatter plots, and outliers were removed from further analyses if the extracted values were > 3 SD above or below the mean (Gianaros et al., 2008). These values were then entered into correlation analyses with the behavioral measures of interest.

VI.   RESULTS

A.   SAMPLE DEMOGRAPHICS

Demographic information for all subjects by risk status group is detailed in Table 3. As a group, the high-risk adolescents did not differ significantly from low-risk adolescents with respect to age, sex distribution, race, Tanner stage, or parental education (all p values > 0.1).

Table 3: Descriptive statistics by risk status

		N	Mean	SD	df	F	Significance
Race % White	High Risk	154	55		1		
	Low Risk	154	58		306	.210	.647
	Total	308	56		307		
Age at MRI	High Risk	154	13.57	.988	1		
	Low Risk	154	13.59	.955	306	.033	.857
	Total	308	13.58	.970	307		
Sex (% Female)	High Risk	154	50		1		
	Low Risk	154	50		306	.116	.733
	Total	308	50		307		
Tanner Female Pubic Hair	High Risk	54	3.65	1.119	1		
	Low Risk	61	3.74	1.015	113	.203	.653
	Total	115	3.70	1.061	114		
Tanner Female Breast	High Risk	54	3.54	1.041	1		
	Low Risk	61	3.61	.862	113	.153	.696
	Total	115	3.57	.946	114		
Tanner Male Pubic Hair	High Risk	62	3.40	.858	1		
	Low Risk	54	3.20	.939	114	1.429	.234
	Total	116	3.31	.898	115		
Tanner Male Genitalia	High Risk	62	3.13	.859	1		
	Low Risk	54	3.09	.896	114	.050	.824
	Total	116	3.11	.872	115		
Parental Education	High Risk	111	3.88	1.803	1		
	Low Risk	131	3.93	1.642	240	.048	.827
	Total	242	3.91	1.714	241		

B.   MAIN EFFECT OF TASK

1.   Amygdala reactivity paradigm

Consistent with previous findings, BOLD fMRI showed robust amygdala, hippocampal, fusiform, and PFC reactivity associated with the perceptual processing of fearful and threatening faces relative to control blocks of shapes when applying a statistical threshold of $p = 0.05$, corrected for multiple comparisons (Figure 3).

Figure 3: Main effect of task: Amygdala reactivity paradigm. Statistical parametric map of brain activation during the perceptual processing of fearful and threatening faces across all 308 subjects. Activations are shown overlaid onto an averaged structural magnetic resonance image. Color bar represents *t* scores for activations.

2. Ventral striatal reactivity paradigm

Contrasting with previous studies, the present study failed to detect an association between BOLD fMRI in the VS with either positive and negative feedback blocks, relative to control blocks when applying a statistical threshold of $p = 0.05$, corrected for multiple comparisons. However, when applying a more liberal threshold of $p = 0.05$, using a small volume-correction procedure within the VS (a similar method to that used by Monk et al., 2008), a pattern consistent with previous findings is seen: BOLD fMRI results in a right striatal activation cluster associated with positive feedback blocks relative to control. This effect also survives at a threshold of $p = 0.005$, with a small-volume correction procedure. Additionally, at this more liberal threshold, VS activity varies by type of feedback, with greater left activation in response to predominantly positive, relative to negative, feedback blocks (Figure 4).

Figure 4: Main effect of task: Ventral striatal reactivity paradigm. Statistical parametric map of brain activation during the processing of positive feedback relative to negative feedback masked for a VS region of interest across all 308 subjects. Activations are shown overlaid onto an averaged structural magnetic resonance image. Color bar represents *t* scores for activations. Maximally activated voxel: x = -14, y = 6, z = -4, t = 3.48, 410 voxels, *p* < 0.001 (small volume-correction with a threshold of *p* < 0.05). All neuroimaging data are reported using the coordinate system of Talairach and Tournoux.

C.   MAIN EFFECT OF RISK

1.   Altered threat-related amygdala reactivity in adolescents at high-risk for depression

To test the hypothesis that relative to the low-risk group, the high-risk group of adolescents would have increased amygdala reactivity in response to viewing threat-related faces, a regression analysis of the BOLD fMRI data was conducted with risk status entered as the covariate of interest. When correcting for multiple comparisons, risk status was not significantly correlated with amygdala reactivity for the faces > shapes contrast. However, when a more liberal threshold was applied (*p* = 0.05, using a small volume-correction procedure within the amygdala), adolescents at high-risk for depression displayed relatively greater bilateral amygdala reactivity relative to low-risk adolescents (left amygdala: F(1,301) = 4.95, *p* < 0.05; left amygdala; F(1,301) = 7.00, *p* < 0.001) (Figures 5a, 5b). An additional analysis was conducted without the 34 adolescents with anxiety and/or behavioral disorder diagnoses, resulting in very similar findings.
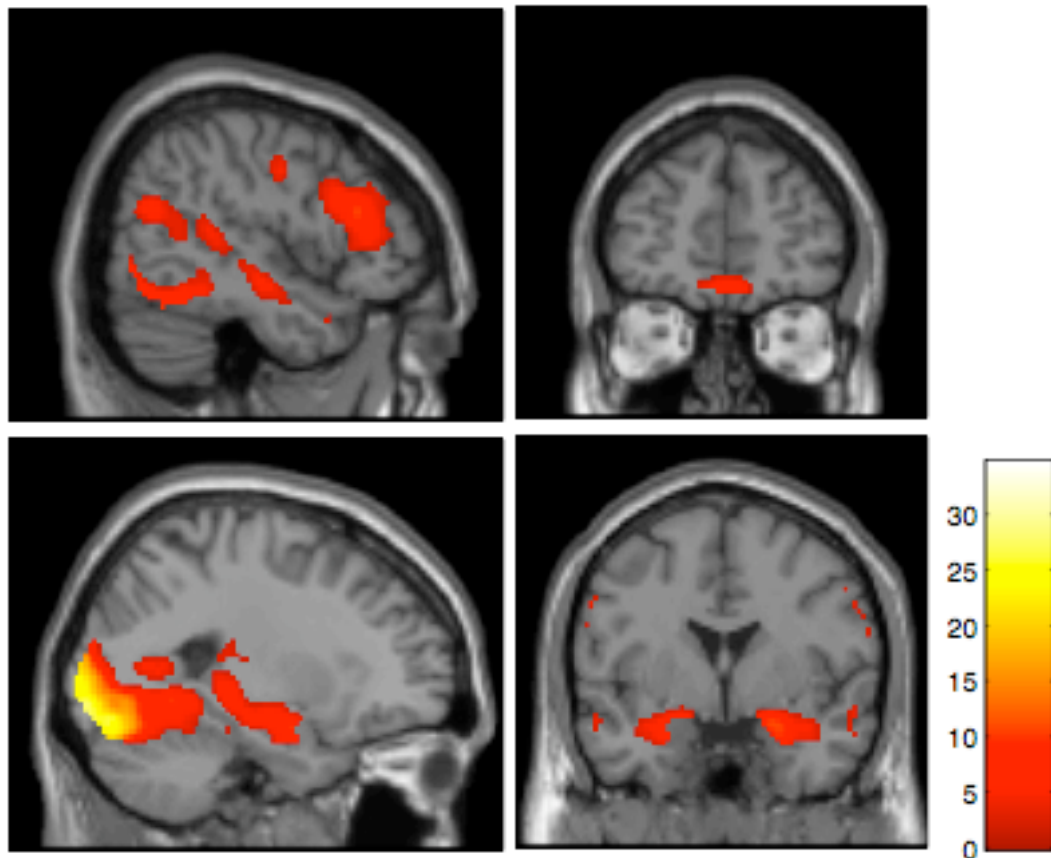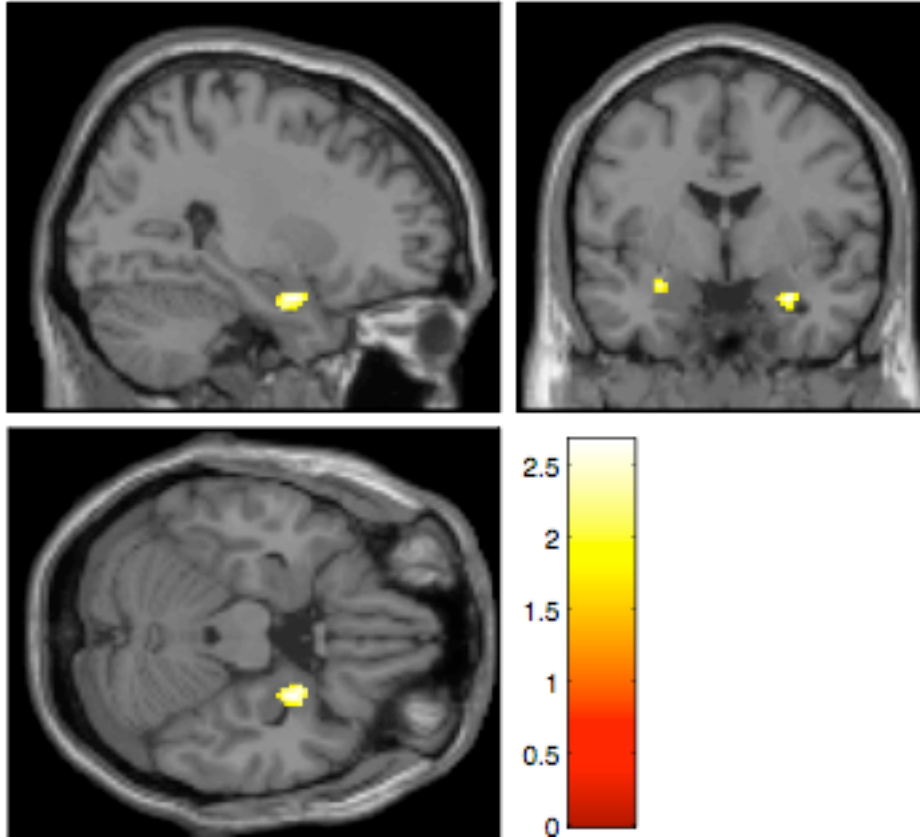
Figure 5a: Main effect of Risk Status: Amygdala reactivity paradigm. Statistical parametric map of brain activation during the perceptual processing of fearful and threatening faces masked with an amygdala region of interest: high-risk adolescents > low-risk adolescents. Activations are shown overlaid onto an averaged structural magnetic resonance image. Color bar represents *t* scores for activations. Maximally activated voxel: left: x = -28, y = -5, z = -15, t = 2.36, 37 voxels, *p* = 0.009; right: x = 28, y = -3, z = -18, t = 2.67, 77 voxels, *p* = 0.004 (small volume-correction with a threshold of *p* < 0.05).
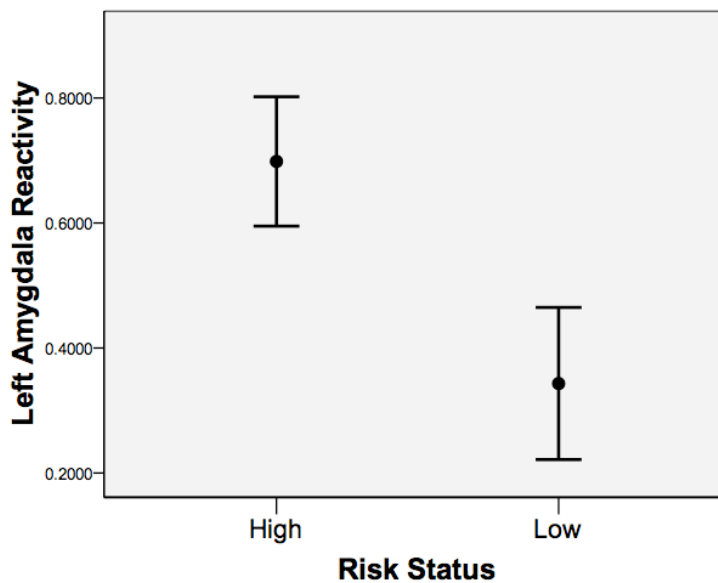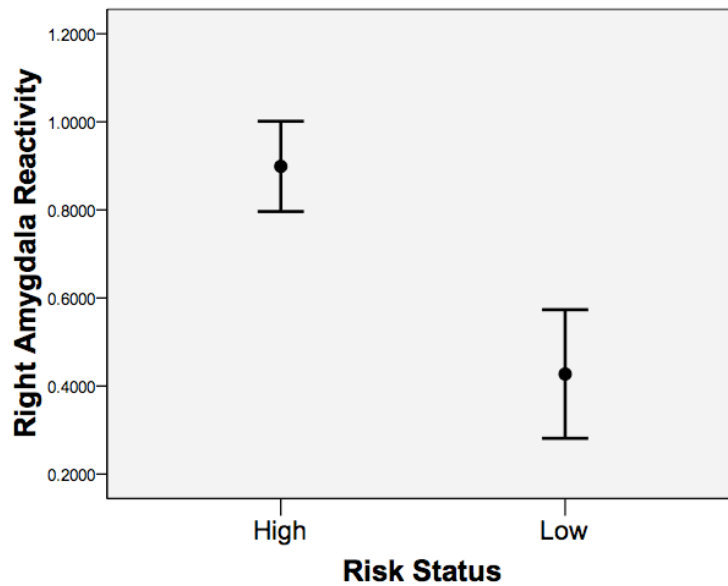
Figure 5b: Main Effect of Risk Status: Amygdala reactivity paradigm. Boxplots displaying extracted mean activation values for the left and right amygdala (arbitrary units) by risk status during the perceptual processing of fearful and threatening faces. Coordinates are maximally activated voxel: left: $F(1,301) = 4.95$, $p < 0.05$; x = -28, y = -5, z = -15, t = 2.36, 37 voxels, $p = 0.009$; right: $F(1,301) = 7.00$, $p < 0.001$; x = 28, y = -3, z = -18, t = 2.67, 77 voxels, $p = 0.004$ (small volume-correction with a threshold of $p < 0.05$).

2. Altered reward-related VS reactivity in adolescents at high-risk for depression

To test the hypothesis that relative to the low-risk group, the high-risk group would have decreased VS reactivity when processing reward-related stimuli, a regression analysis of the BOLD fMRI data was conducted with risk status entered as the covariate of interest. When correcting for multiple comparisons, risk status was not significantly correlated with VS reactivity for the reward > no reward contrast. However, as with the amygdala reactivity paradigm, when a more liberal threshold was applied ($p = 0.05$, using a small volume-correction procedure within the VS), adolescents at high-risk for depression displayed relatively blunted left VS reactivity relative to low-risk adolescents ($F(1,292) = 11.80$, $p < 0.001$) (Figures 6a, 6b). This effect also survives at a threshold of $p = 0.005$, with a small-volume correction procedure. Data from this moderately conservative threshold is displayed in the Figures 6b bloxplot. As with the amygdala reactivity paradigm, an additional analysis was conducted with the 34 adolescents with anxiety and/or behavioral disorder diagnoses removed, which resulted in very similar findings.
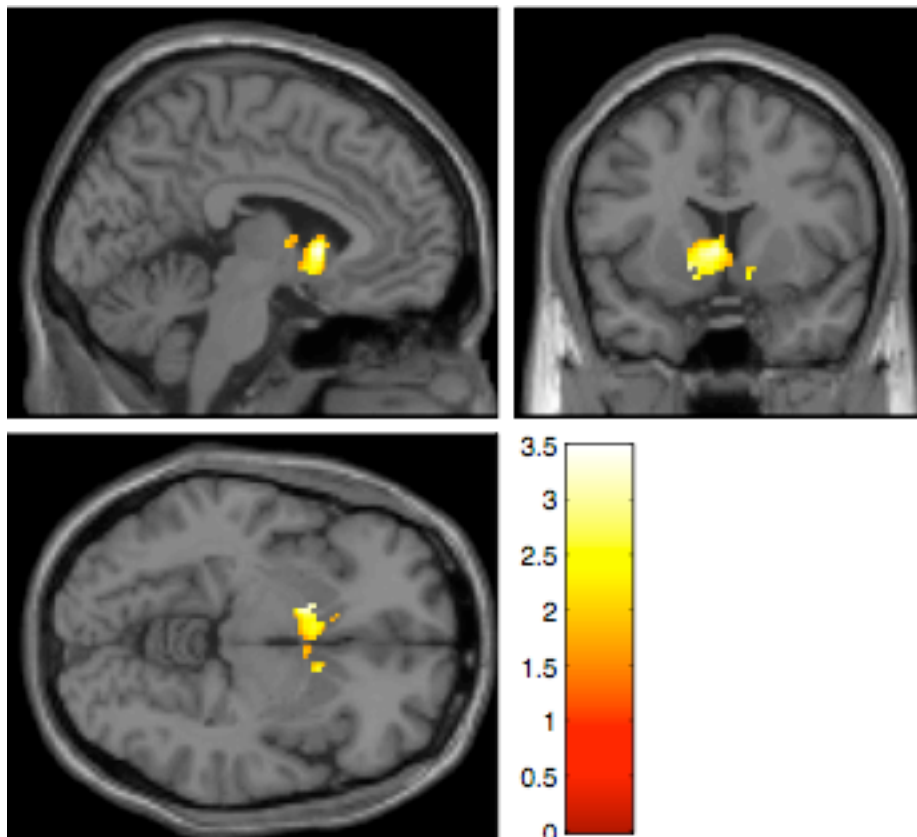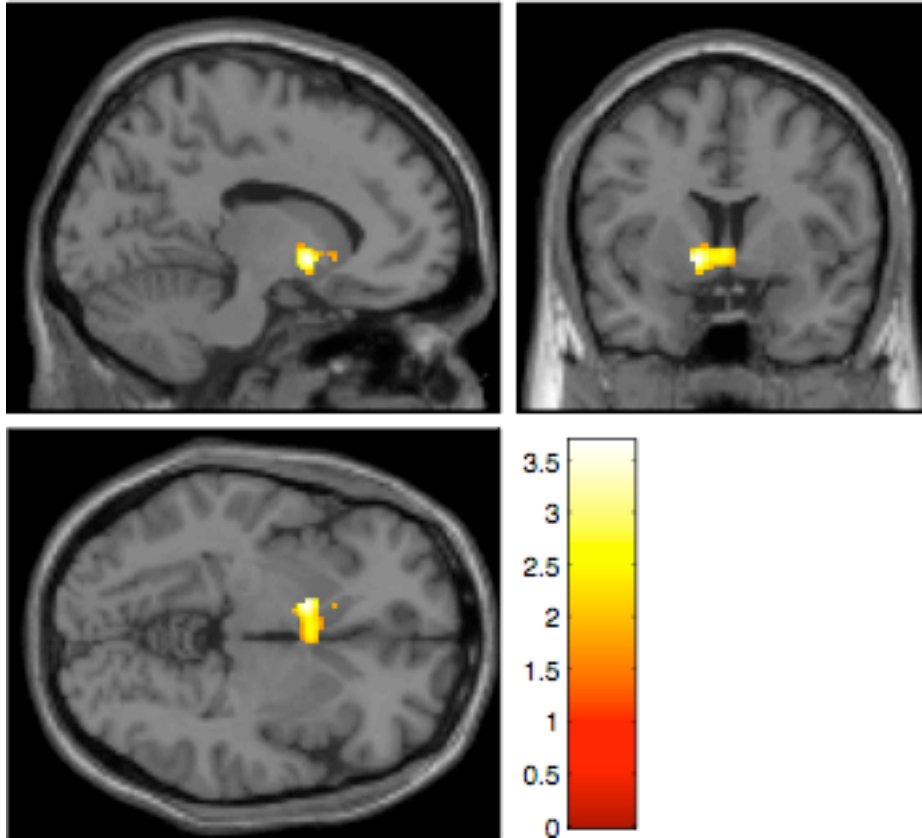
Figure 6a: Main effect of Risk Status: Ventral striatal reactivity paradigm. Statistical parametric map of brain activation during the processing of positive feedback relative to negative feedback masked with a VS region of interest: low-risk adolescents > high-risk adolescents. Activations are shown overlaid onto an averaged structural magnetic resonance image. Color bar represents t scores for activations. Maximally activated voxel: x = -12, y = 6, z = -4, t = 3.67, 119 voxels, $p < 0.001$ (small volume-correction with a threshold of $p < 0.05$).
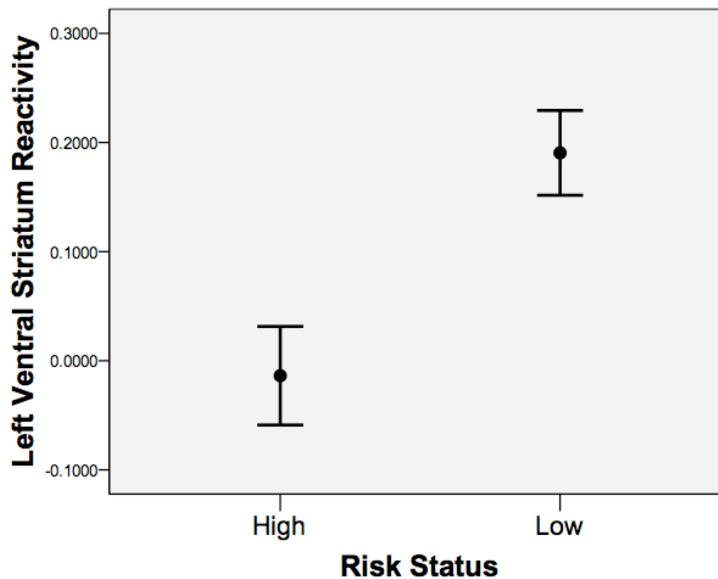
Figure 6b: Main Effect of Risk Status: Ventral striatal reactivity paradigm. Boxplot displaying extracted mean activation values for the left VS (arbitrary units) by risk status during the processing of positive feedback relative to negative feedback. Coordinate is maximally activated voxel: $F(1,292) = 11.80$, $p < 0.001$; x = -12, y = 6, z = -4, t = 3.67, 31 voxels, $p < 0.001$ (small volume-correction with a threshold of $p < 0.005$).

3.   Differences in functional connectivity between regions of the PFC and amygdala or VS

To test the hypothesis that functional connectivity between both the amygdala and PFC as well as between the VS and PFC would be diminished in the high-risk compared to the low-risk group, data were extracted as described previously in the data analysis section. Investigation of these regressions using SPSS yielded two statistically significant differences in functional connectivity as a function of risk group. For the amygdala reactivity paradigm, the connectivity between (a) right BA 47 and right ventral amygdala ($p < 0.05$), and (b) left BA 47 and left total amygdala differed as a function of risk status ($p < 0.05$). Further analyses revealed that the simple slope of the correlation between right BA 47 and right ventral amygdala was significantly different from 0 in the low-risk group ($t = 3.78$, $p < 0.001$) but not in the high-risk group ($t = 0.45$, $p > 0.5$), suggesting a stronger coupling of these two regions in the low-risk group.   The simple slope of the correlation between left BA 47 and left total amygdala was significantly different from 0 in both the low-risk ($t = 2.83$, $p < 0.01$) and the high-risk group ($t = 5.33$, $p < 0.001$).   These analyses did not survive a Bonferroni correction for multiple comparisons; therefore results should be interpreted with caution.   There were no statistically significant differences in the functional connectivity of regions engaged by the ventral striatal reactivity paradigm as a function of risk group status.

D. EXPLORATORY ANALYSES

1. Correlations with demographic data

To determine if the variables of interest were correlated with risk status, sex, age, race, and parental education, the behavioral measures were first entered into a correlation analysis. This was done to 1) examine whether these behavioral measures were related to risk status (which may account for potential differences between groups), and 2) to explore whether these behaviors may be correlated with other demographic factors. Risk status was correlated with MFQ-P, eating rhythmicity, Total Internalizing score, Total Externalizing score (significant at $p < 0.01$) and SCARED-P (significant at $p < 0.05$). In each of these measures, high-risk adolescents had a greater mean score relative to low-risk adolescents, with the exception of eating rhythmicity, which was lower. Race was correlated with parental education, with White parents reporting more advanced highest levels of education on average ($p < 0.01$). Age was correlated with the four Tanner stage scores ($p < 0.01$). Sex was correlated with MFQ-C and SCARED-C, with females scoring higher than males on average ($p < 0.01$). (Sex was not correlated with Tanner stages because the data were entered as separate measures for males and females.) These data are summarized in Tables 4a and b (separated for ease of viewing). Not all data were available in all subjects (see Table 2).

Table 4a: Correlations between behavioral measures and demographic measures

		Risk	Sex	Age	Race	Parental Education
MFQ-C	Correlation	-.078	-.186[**]	.045	.023	-.029
	Significance	.191	.002	.449	.695	.659
	N	280	280	280	280	235
MFQ-P	Correlation	-.160[**]	.041	.005	.079	-.105
	Significance	.007	.495	.927	.192	.108
	N	278	278	278	278	235
SCARED-C	Correlation	-.076	-.225[**]	.006	.034	.038
	Significance	.206	.000	.915	.574	.563
	N	278	278	278	278	234
SCARED-P	Correlation	-.147[*]	-.068	-.065	-.057	.027
	Significance	.016	.263	.286	.348	.679
	N	270	270	270	270	232
Tanner Female Pubic Hair	Correlation	.042	.[a]	.367[**]	-.073	.080
	Significance	.653	.000	.000	.436	.431
	N	115	115	115	115	99
Tanner Female Breast	Correlation	.037	.[a]	.337[**]	-.029	.004
	Significance	.696	.000	.000	.759	.965
	N	115	115	115	115	99
Tanner Male Pubic Hair	Correlation	-.111	.[a]	.537[**]	.126	-.047
	Significance	.234	.000	.000	.177	.651
	N	116	116	116	116	95
Tanner Male Genitalia	Correlation	-.021	.[a]	.520[**]	.046	.083
	Significance	.824	.000	.000	.626	.423
	N	116	116	116	116	95
Total Internalizing	Correlation	-.242[**]	-.082	-.071	-.029	-.031
	Significance	.000	.216	.281	.661	.669
	N	232	232	232	232	198
Total Externalizing	Correlation	-.162[*]	-.012	-.056	.002	-.028
	Significance	.014	.858	.401	.973	.699
	N	230	230	230	230	198

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level (2-tailed)

Table 4b: Correlations between subscales of the Revised Dimensions of Temperament Survey (DOTS-R) and demographic measures

		Risk	Sex	Age	Race	Parental Education
Approach - Withdrawal	Correlation	.035	.070	-.031	-.043	-.032
	Significance	.595	.284	.635	.516	.651
	N	236	236	236	236	201
Activity Level - General	Correlation	.034	.016	-.023	.042	.026
	Significance	.605	.802	.723	.521	.709
	N	236	236	236	236	201
Activity Level - Sleep	Correlation	.000	-.066	.059	.004	.030
	Significance	1.000	.313	.367	.953	.676
	N	236	236	236	236	201
Flexibility-Rigidity	Correlation	.083	.102	.030	.044	-.030
	Significance	.206	.118	.651	.504	.674
	N	236	236	236	236	201
Mood Quality	Correlation	.101	-.156*	.012	.001	.029
	Significance	.123	.017	.853	.994	.681
	N	236	236	236	236	201
Rhythmicity -Sleep	Correlation	.102	.085	-.018	-.014	.069
	Significance	.120	.195	.789	.836	.327
	N	236	236	236	236	201
Rhythmicity - Eating	Correlation	.181**	.033	-.026	-.036	.077
	Significance	.005	.612	.688	.578	.276
	N	236	236	236	236	201
Rhythmicity -Daily Habits	Correlation	.085	-.006	.042	-.021	-.008
	Significance	.192	.922	.521	.750	.913
	N	236	236	236	236	201
Task Orientation	Correlation	.029	.125	.040	-.120	.054
	Significance	.663	.056	.536	.066	.446
	N	236	236	236	236	201

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level (2-tailed)

2. Correlations between behavioral measures

Behavioral measures were then examined for correlations between variables of interest to confirm that measures expected to correlate based on prior literature were also correlated in the present study. These statistics are outlined in Tables 5a and b (separated for ease of viewing).

Table 5a: Descriptive statistics and intercorrelations for behavioral variables

		MFQ-C	MFQ-P	SCARED-C	SCARED-P	Tanner Female Pubic Hair	Tanner Female Breast	Tanner Male Pubic Hair	Tanner Male Genitalia	Total Internalizing	Total Externalizing
MFQ-C	Corr.										
	Sig.										
	N	280									
MFQ-P	Corr.	.150*									
	Sig.	.012									
	N	276	278								
SCARED-C	Corr.	.603**	.113								
	Sig.	.000	.061								
	N	278	274	278							
SCARED-P	Corr.	.272**	.295**	.221**							
	Sig.	.000	.000	.000							
	N	268	269	268	270						
Tanner Female Pubic Hair	Corr.	.210*	.042	.085	.009						
	Sig.	.024	.662	.369	.924						
	N	115	113	115	112	115					
Tanner Female Breast	Corr.	.020	.076	-.048	-.110	.630**					
	Sig.	.829	.425	.612	.248	.000					
	N	115	113	115	112	115	115				
Tanner Male Pubic Hair	Corr.	.084	.015	.087	.010						
	Sig.	.370	.871	.351	.913						
	N	116	115	116	114			116			
Tanner Male Genitalia	Corr.	.072	.000	.111	.007			.766**			
	Sig.	.442	.994	.237	.939			.000			
	N	116	115	116	114			112	116		
Total Internalizing	Corr.	.146*	.563**	.156*	.293**	-.178	-.149	.045	.017		
	Sig.	.027	.000	.018	.000	.079	.143	.663	.873		
	N	230	228	229	226	98	98	95	94	232	
Total Externalizing	Corr.	.125	.428**	-.086	.148*	.003	.015	.106	.029	.485**	
	Sig.	.059	.000	.196	.026	.976	.883	.303	.779	.000	
	N	228	226	227	224	100	100	96	96	218	230

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level (2-tailed)

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Table 5b: Descriptive statistics and intercorrelations between DOTS-R and the remaining behavioral variables

		MFQ-C	MFQ-P	SCARED-C	SCARED-P	Tanner Female Pubic Hair	Tanner Female Breast	Tanner Male Pubic Hair	Tanner Male Genitalia	Total Internalizing	Total Externalizing
Approach - Withdrawal	Corr.	-.123	.022	-.176[**]	-.102	.120	.104	-.093	-.200[*]	-.106	.048
	Sig.	.058	.742	.007	.122	.232	.302	.348	.043	.135	.503
	N	236	234	236	232	101	101	103	103	201	199
Activity Level - General	Corr.	.310[**]	.098	.263[**]	.100	.117	.036	-.031	.002	.068	.156[*]
	Sig.	.000	.135	.000	.129	.244	.717	.754	.980	.336	.028
	N	236	234	236	232	101	101	103	103	201	199
Activity Level - Sleep	Corr.	.219[**]	.086	.228[**]	.108	.024	.086	.004	.009	.126	.081
	Sig.	.001	.188	.000	.102	.815	.390	.966	.932	.074	.253
	N	236	234	236	232	101	101	103	103	201	199
Flexibility-Rigidity	Corr.	-.282[**]	-.078	-.500[**]	-.120	-.005	.027	.013	-.006	-.163[*]	.027
	Sig.	.000	.233	.000	.068	.959	.792	.896	.953	.021	.701
	N	236	234	236	232	101	101	103	103	201	199
Mood Quality	Corr.	-.238[**]	-.154[*]	-.066	-.119	.051	.128	-.116	-.159	-.177[*]	-.079
	Sig.	.000	.019	.313	.070	.616	.201	.243	.108	.012	.270
	N	236	234	236	232	101	101	103	103	201	199
Rhythmicity -Sleep	Corr.	-.224[**]	-.104	-.070	-.173[**]	-.118	-.014	.098	.113	-.111	-.071
	Sig.	.001	.111	.282	.008	.241	.889	.326	.256	.116	.321
	N	236	234	236	232	101	101	103	103	201	199
Rhythmicity -Eating	Corr.	-.296[**]	-.090	-.171[**]	-.132[*]	-.066	-.055	.008	-.007	-.163[*]	-.090
	Sig.	.000	.171	.008	.045	.509	.585	.933	.943	.021	.207
	N	236	234	236	232	101	101	103	103	201	199
Rhythmicity -Daily Habits	Corr.	-.159[*]	-.162[*]	-.152[*]	-.153[*]	-.182	-.070	.019	.088	-.147[*]	-.206[**]
	Sig.	.014	.013	.020	.019	.068	.489	.851	.379	.038	.004
	N	236	234	236	232	101	101	103	103	201	199
Task Orientation	Corr.	-.267[**]	.038	-.066	-.026	-.119	-.140	-.132	-.167	.029	-.077
	Sig.	.000	.558	.316	.697	.236	.162	.185	.092	.680	.277
	N	236	234	236	232	101	101	103	103	201	199

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level (2-tailed)

3.   Correlations between behavioral measures and extracted BOLD fMRI values

Behavioral measures were examined for potential correlations with extracted BOLD fMRI values. Of the 162 correlations computed, only SCARED-C was significantly correlated with left dorsal amygdala values ($p < 0.05$) and Flexibility-Rigidity was correlated with total right ($p < 0.05$) and right ventral amygdala values ($p < 0.01$). However, these did not survive a Bonferroni correction for multiple comparisons. These correlations are outlined in Tables 6a and b (separated for ease of viewing). There were no significant correlations between the behavioral measures and regions in the ventral striatal reactivity paradigm (Tables 7a and b, separated for ease of viewing).

Table 6a: Correlations between behavioral measures and extracted BOLD fMRI values in the amygdala reactivity paradigm

		Left Amygdala	Right Amygdala	Right Dorsal Amygdala	Right Ventral Amygdala	Left Dorsal Amygdala	Left Ventral Amygdala	BA11	Right BA47	Left BA47
MFQ-C	Correlation	.013	.053	.048	.086	-.068	.054	-.007	-.014	.056
	Significance	.830	.382	.430	.155	.267	.374	.903	.813	.361
	N	273	273	273	272	271	271	271	271	272
MFQ-P	Correlation	.039	.023	.042	.085	.031	.046	.043	-.007	.064
	Significance	.521	.702	.494	.165	.613	.456	.485	.903	.296
	N	271	271	271	270	269	269	269	269	270
SCARED-C	Correlation	-.067	.065	.011	.116	-.125*	-.032	-.031	-.013	.020
	Significance	.270	.289	.855	.057	.041	.604	.608	.829	.748
	N	271	271	271	270	269	269	269	269	270
SCARED-P	Correlation	.000	-.025	-.041	.006	-.051	.056	-.083	-.095	-.036
	Significance	.997	.682	.512	.920	.413	.371	.181	.127	.558
	N	263	263	263	262	261	261	261	261	262
Tanner Female Pubic Hair	Correlation	.087	-.023	.087	-.075	.017	.011	.151	.014	.140
	Significance	.362	.812	.360	.428	.858	.908	.109	.884	.142
	N	113	113	112	113	111	113	114	111	112
Tanner Female Breast	Correlation	.031	-.015	.008	-.104	.041	-.026	.051	-.105	-.009
	Significance	.747	.874	.937	.271	.669	.785	.593	.272	.927
	N	113	113	112	113	111	113	114	111	112
Tanner Male Pubic Hair	Correlation	.095	.067	.018	.067	.029	.098	-.076	.072	-.067
	Significance	.320	.484	.848	.484	.763	.308	.434	.454	.484
	N	111	111	112	112	111	110	109	111	111
Tanner Male Genitalia	Correlation	.010	-.014	.018	-.064	-.033	-.001	-.133	.057	-.115
	Significance	.917	.886	.854	.504	.732	.989	.168	.553	.229
	N	111	111	112	112	111	110	109	111	111
Total Internalizing	Correlation	.018	.058	.007	.052	-.074	-.008	-.003	-.002	-.013
	Significance	.788	.383	.921	.434	.272	.910	.960	.972	.849
	N	226	225	225	225	224	224	223	224	225
Total Externalizing	Correlation	.015	.004	-.008	-.024	-.002	-.030	.081	.010	.056
	Significance	.826	.957	.900	.722	.975	.653	.230	.885	.405
	N	224	224	224	224	222	222	222	222	223

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level (2-tailed)

Table 6b: Correlations between subscales of the DOTS-R and extracted BOLD fMRI values in the amygdala reactivity paradigm

		Left Amygdala	Right Amygdala	Right Dorsal Amygdala	Right Ventral Amygdala	Left Dorsal Amygdala	Left Ventral Amygdala	BA11	Right BA47	Left BA47
Approach - Withdrawal	Correlation	-.026	-.040	-.028	-.016	-.027	.012	-.013	-.008	-.022
	Significance	.697	.548	.672	.806	.685	.853	.844	.901	.746
	N	230	230	231	230	229	228	228	228	229
Activity Level - General	Correlation	-.004	.035	.045	.043	-.015	-.059	.001	.021	.029
	Significance	.948	.601	.499	.517	.820	.372	.987	.756	.664
	N	230	230	231	230	229	228	228	228	229
Activity Level - Sleep	Correlation	-.056	-.122	-.108	-.109	-.057	-.119	-.062	.013	-.110
	Significance	.396	.065	.102	.100	.388	.074	.349	.842	.097
	N	230	230	231	230	229	228	228	228	229
Flexibility- Rigidity	Correlation	.014	-.133*	-.064	-.187**	.062	-.011	-.093	-.049	-.110
	Significance	.829	.044	.333	.004	.347	.863	.162	.458	.097
	N	230	230	231	230	229	228	228	228	229
Mood Quality	Correlation	-.074	-.002	-.032	.043	-.050	-.086	-.020	-.080	-.071
	Significance	.261	.971	.628	.513	.452	.198	.768	.227	.288
	N	230	230	231	230	229	228	228	228	229
Rhythmicity - Sleep	Correlation	-.038	-.053	-.092	-.031	-.036	.003	.090	.019	-.003
	Significance	.571	.420	.162	.644	.586	.969	.175	.771	.966
	N	230	230	231	230	229	228	228	228	229
Rhythmicity - Eating	Correlation	-.023	-.024	-.045	-.017	.026	.007	.059	-.024	-.069
	Significance	.732	.712	.499	.802	.694	.912	.378	.715	.299
	N	230	230	231	230	229	228	228	228	229
Rhythmicity - Daily Habits	Correlation	-.078	.088	.081	.078	-.026	-.046	.015	-.052	-.030
	Significance	.239	.184	.221	.241	.699	.491	.825	.435	.648
	N	230	230	231	230	229	228	228	228	229
Task Orientation	Correlation	-.068	-.060	-.074	-.036	-.104	.005	-.080	.108	-.098
	Significance	.302	.366	.264	.583	.117	.938	.227	.104	.138
	N	230	230	231	230	229	228	228	228	229

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level (2-tailed)

Table 7a: Correlations between behavioral measures and extracted BOLD fMRI values in the striatal reactivity paradigm

		VS Reward > No Reward	MPFC
MFQ-C	Correlation	-.010	.035
	Significance	.871	.573
	N	259	262
MFQ-P	Correlation	.024	-.062
	Significance	.700	.313
	N	260	263
SCARED-C	Correlation	-.005	.005
	Significance	.937	.943
	N	252	255
SCARED-P	Correlation	-.063	.126
	Significance	.516	.192
	N	108	109
Tanner Female Pubic Hair	Correlation	-.093	-.014
	Significance	.337	.887
	N	108	109
Tanner Female Breast	Correlation	-.013	.013
	Significance	.891	.891
	N	109	111
Tanner Male Pubic Hair	Correlation	-.067	-.076
	Significance	.488	.426
	N	110	112
Tanner Male Genitalia	Correlation	.040	-.029
	Significance	.557	.667
	N	216	218
Total Internalizing	Correlation	.054	-.069
	Significance	.433	.313
	N	215	217

Table 7b: Correlations between subscales of the DOTS-R and extracted BOLD fMRI values in the striatal reactivity paradigm

		VS Reward > No Reward	MPFC
Approach - Withdrawal	Correlation	-.055	.026
	Significance	.416	.701
	N	221	222
Activity Level - General	Correlation	.040	-.115
	Significance	.550	.087
	N	221	222
Activity Level - Sleep	Correlation	-.033	-.079
	Significance	.621	.243
	N	221	222
Flexibility-Rigidity	Correlation	.056	.078
	Significance	.406	.245
	N	221	222
Mood Quality	Correlation	-.061	.035
	Significance	.363	.605
	N	221	222
Rhythmicity -Sleep	Correlation	.003	-.054
	Significance	.970	.421
	N	221	222
Rhythmicity -Eating	Correlation	.034	-.014
	Significance	.610	.841
	N	221	222
Rhythmicity -Daily Habits	Correlation	-.059	.007
	Significance	.381	.912
	N	221	222
Task Orientation	Correlation	-.029	.001
	Significance	.669	.984
	N	221	222

VII. DISCUSSION

The goals of the present study were to use BOLD fMRI challenge paradigms to investigate potential premorbid differences between adolescents with high familial loading for depression and those with low familial loading by examining the functioning of both threat-related amygdala reactivity and reward-related ventral striatal reactivity, as well as the functional coupling between regions of PFC and areas within these limbic regions. At a conservative threshold, employed because of the very large sample size (> 300 adolescents), the present analyses failed to detect significant differences between these groups at the level of the amygdala and ventral striatum. When a more liberal threshold was applied—a method reliably employed previously in analyses of smaller sample sizes (< 100)—hypothesized differences were observed for both the amygdala reactivity paradigm and the ventral striatal reactivity paradigm: high-risk adolescents displayed relatively greater amygdala reactivity and relatively blunted VS reactivity compared to low-risk adolescents. Additionally, these data offer some evidence to suggest that alterations in functional connectivity of the threat-related amygdala reactivity network (but not reward-related VS reactivity) may vary as a function of risk status during adolescence.

A. MAIN EFFECT OF TASK

1. Amygdala reactivity paradigm

As expected, the present study replicated previous findings (in both adult and pediatric populations) of a specific limbic and prefrontal network that is more activated during the perceptual processing of threat-related faces relative to a sensorimotor control block of shapes (Hariri et al., 2005; Hariri, Tessitore, Mattay, Fera, & Weinberger, 2002; Meyer-Lindenberg et al., 2005; Tessitore et al., 2002; Wang, Dapretto, Hariri, Sigman, & Bookheimer, 2004). This effect survived the more conservative threshold of $p < 0.05$, corrected for multiple comparisons.

2. Ventral striatal reactivity paradigm

Unexpectedly, this study failed to replicate the reward-related ventral striatal network previously shown to be engaged during the processing of positive and negative feedback relative to control blocks when examined at this conservative threshold. Whereas several studies in adult populations have replicated this pattern (Forbes, Brown et al., 2009; Hariri et al., 2006), only pilot data of this specific task had been collected in an adolescent sample prior to the present study. When the statistical threshold of analysis was lowered to a more liberal threshold of $p = 0.05$, using a small volume-correction procedure within the VS, the expected main effect pattern emerged. It appears, therefore, that in an adolescent population, these main effects are less robust than in adult populations, perhaps reflecting maturational differences in the development of the reward circuit in the brain. These may reflect neurobiological differences or potentially a difference in reward salience, with adolescents perceiving the feedback as less rewarding relative to adults. Moreover, a recent study of MDD and control adolescents employed a very similar

monetary guessing paradigm adapted for use with an event-related design. Although main effect of task is not reported collapsing across the two groups, within each group—control and depressed, a large region of the caudate body is reported but no ventral striatum (Forbes, Hariri et al., 2009). It may be that these monetary guessing paradigms (both the block and the event-related design) may be less effective at engaging the VS in adolescents than in similar studies in adult populations.

B. MAIN EFFECT OF RISK

1. Amygdala reactivity paradigm

At the conservative threshold correcting for multiple comparisons, the present study did not detect statistically significant differences in the perceptual processing of threat-related amygdala reactivity as a function of risk status. At the more liberal threshold of $p = 0.05$, using a small volume-correction procedure within the amygdala, adolescents at high-risk for depression displayed relatively greater bilateral amygdala reactivity relative to low-risk adolescents. These data suggest that if premorbid differences, as measured by this paradigm, exist prior to the onset of depression as a function of risk status, these differences may be more subtle than previously hypothesized. If premorbid differences exist, they may be masked by potential subtypes (as not all of the high-risk adolescents will develop depression) within these groups presently stratified by risk status. However, it may be that premorbid neurobiological differences, assessed by this paradigm, do not exist when examined by risk status. The majority of these children are free

from diagnoses, and neurobiological differences observed in prior studies of depressed adolescents may reflect the presence of concurrent depression.

The findings from this study do not necessarily diverge from data reported in the only other fMRI investigation of healthy adolescents at high-risk for depression due to familial loading, which reported higher amygdala activation in the high-risk relative to the low-risk group. This prior study used a small volume-correction procedure with a threshold of $p < 0.05$, a method similar to our more liberal threshold analysis where we did detect a difference in amygdala reactivity as a function of risk (Monk et al., 2008). Moreover, this previous study differed from the current study in a number of ways. First, the study was a much smaller dataset consisting of only 17 high-risk and 22 low-risk children and adolescents. Monk et al. defined high-risk as offspring of at least one depressed parent recruited from patients at a mood and anxiety disorder clinic; therefore, the parent's disorders were impairing enough for them to have sought treatment. The current study included offspring of parents who were not required to have been treated for their depression, and was therefore a potentially more heterogeneous sample. Further, the sample used by Monk et al. had a much higher percentage of children with anxiety disorders (59% of the high-risk group, 14% of the low-risk group) relative to the current study (18% high-risk, 0% low-risk). Additionally, differences in task design, for example, the inclusion of happy and neutral face stimuli rather than threat-only faces employed in the current task, and differences in analysis techniques (event-related versus block design) may also account for the diverging findings reported as a function of risk status. Finally, the differences as a function of risk status in the Monk et al. study were only detected in the passive viewing condition (subjects were asked only to view the faces) and not when subjects were asked either to (a) attend to their subjective fear of the face or (b) attend to a non-emotional feature of the

face (nose width) which Monk et al. referred to as "attention conditions." The task-design of the current dataset may engage cognitive attentional networks similar to either of the "attention conditions" of the Monk et al. study as subjects were instructed to match a target to a reference face (therefore necessitating some engagement, whether it be emotional or non-emotional attention). Similarly, these "attention" analyses in the Monk et al. study did not yield amygdala reactivity differences as a function of risk status; however, these analyses revealed greater prefrontal cortex activation in the high-risk relative to low-risk group. It is possible that the recruitment of prefrontal regions necessary in attention (even low-level attention necessary to perform the present study paradigm) may contribute to the lack of differences observed as a function of risk status by inhibiting a potential abnormal activation. A post-hoc analysis in the present study was conducted to investigate this potential explanation: results yielded greater prefrontal activation in the high-risk group relative to the low-risk group for the faces > shapes contrast. However, this difference was significant only at $p = 0.05$, uncorrected.

2. Ventral striatal reactivity paradigm

At the conservative threshold correcting for multiple comparisons, the present study did not detect statistically significant differences in reward-related ventral striatal reactivity as a function of risk status. However, when a more liberal threshold was applied ($p = 0.05$, (or $p = 0.005$) using a small volume-correction procedure within the VS), the hypothesized difference between risk groups emerges: adolescents at high-risk for depression display relatively blunted left VS reactivity relative to low-risk adolescents. As with the amygdala reactivity paradigm, premorbid differences, as measured by this task, may indeed vary as a function of risk, but these differences may be less pronounced than initially hypothesized. Because of the lack of significance for the

main effect of task for the reward > no reward contrast, however, interpretation of results related to risk status is difficult. It is unclear whether the lack of significant differences between groups, when analyzed at the conservative threshold, is due to an inadequacy of the task to engage the target reward-related VS regions or a genuine absence of risk difference. Prior studies of reward processing in children and adolescents have been predominantly employed event-related designs, allowing for separate analyses of anticipation and feedback. The current study, because of its block design, is unable to disambiguate VS response to these components of reward processing, therefore investigation of potential differences by risk of these facets was not possible. Comparison with the only other high-risk study of VS reactivity (Monk et al., 2008) is difficult because of the vastly different measures used to assess reward reactivity. The Monk et al. study used happy faces as the rewarding stimuli rather than the more concrete feedback of receiving a monetary reward based on performance in the current task. It may be that the blunted activity of the VS seen by Monk et al. in the high-risk group is related specifically to viewing faces engaged in positive emotions, a more interpersonal and biologically-salient stimuli. This finding may be highlighting the anhedonia related to interpersonal interactions seen in depression (Rudolph & Clark, 2001).

C.   FUNCTIONAL CONNECTIVITY

Functional connectivity between regions in both the threat-related amygdala reactivity network and the reward-related VS reactivity network was assessed by examining the coupling between predetermined reference and target regions within these networks in regression analyses assessing the existence of a differential effect based on risk. Of the 18 regression analyses

computed for the amygdala reactivity paradigm, only 2 analyses yielded significant results: the strength of the coupling between right BA 47 and right ventral amygdala was more significant in the low-risk group relative to the high-risk group; whereas, the strength of the coupling between left BA 47 and left total amygdala was more significant in the high-risk relative to the low-risk group (see Figure 7). BA 47 is a region within the orbitofrontal cortex with extensive connections with the amygdala. This region has been shown to be overactive in individuals with major depressive disorder (Brody, Barsom, Bota, & Saxena, 2001) as well as in normal controls during sadness induction and sadness suppression (Levesque et al., 2003). While speculative, it is interesting to consider possible explanations for the laterality difference observed in this analysis. Because the right hemisphere is typically associated with emotional processing relative to the left, and right hemisphere dysfunction is associated with depression, it may be that the right BA 47—amygdala coupling is less efficient in its connectivity in individuals at high-risk for depression relative to low-risk and that this inefficiency may contribute to an increased risk for depression. However, since these analyses did not survive a Bonferroni correction for multiple comparisons, these results should be interpreted with caution.

There were no statistically significant differences in the functional connectivity of regions engaged by the ventral striatal reactivity paradigm as a function of risk group status. Again, interpretation of findings related to the VS paradigm are difficult due to the lack of main effect of task.
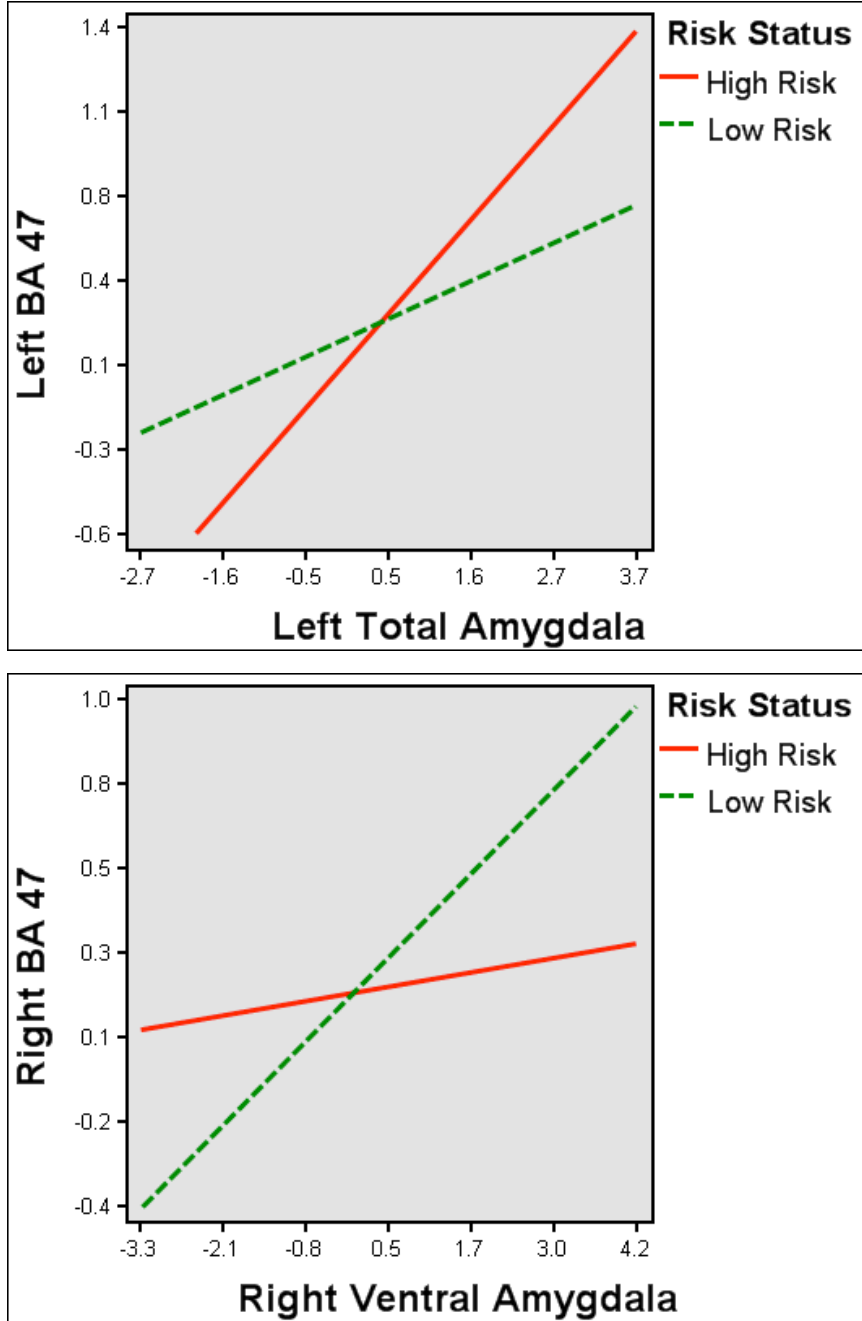
Figure 7: Functional Connectivity Analysis between BA 47 and Amygdala in the Threat-related Amygdala Reactivity Network. Connectivity between left BA 47 and left total amygdala: Interaction: $p = 0.019$, high risk simple slope: < 0.0001, low risk simple slope: 0.003. Connectivity between right BA 47 and right ventral amygdala: Interaction: $p = 0.05$, high risk simple slope: 0.326, low risk simple slope: < 0.0001

D. EXPLORATORY ANALYSES

1. Correlations with demographic data

After behavioral data were entered into a correlation analysis with risk status, sex, age, race, and parental education, several behavioral measures were correlated with these demographic measures. Interestingly, although the adolescents included in these analyses were predominantly free from DSM-IV diagnoses (only 35 children met criteria for an anxiety disorder), risk status was still correlated with several of the behavioral measures assessing negative symptomatologies. Specifically, MFQ-P, Total Internalizing score, and Total Externalizing score were associated with risk status: the high-risk group had a greater mean score relative to the low-risk group, suggesting the possible presence of a sub-clinical phenotype of depression that may contribute to risk for later depression. Additionally, race was correlated with parental education with White parents reporting more advanced highest levels of education. Moreover, sex was correlated with MFQ-C and SCARED-C, with females scoring higher than males, consistent with epidemiological findings reporting that females are at an increased risk for depression relative to males (Burt & Stein, 2002; Cyranowski, Frank, Young, & Shear, 2000). These results emphasize the need to have comparable race and sex distributions within risk groups of analysis. Finally, as expected, age was correlated with Tanner scores.

2. Correlations between behavioral measures

Not surprisingly, MFQ-C, MFQ-P, SCARED-C, SCARED-P, and Total Internalizing score were all correlated with each other, with the exception of SCARED-C and MFQ-P (which approached

significance). Total Externalizing total score was correlated with both parental measures of child mood as well as Total Internalizing score.

3.   Correlations between behavioral measures and extracted BOLD fMRI values

Of all of the behavioral measures investigated for correlations with extracted BOLD fMRI values derived from the main effects maps of both the amygdala reactivity paradigm (faces > shapes) and the VS reactivity paradigm (reward > no reward), only two correlations were statistically significant, but did not survive a Bonferroni correction for multiple comparisons. This lack of significant findings may be due to several possibilities. The method employed for this analysis is a conservative approach because behaviors were correlated with data extracted from main effect of task clusters, rather than examining correlations between behaviors and neurobiology directly within SPM, a method that increases the likelihood of obtaining false positives.   This conservative approach was chosen because of the lack of *a priori* hypotheses. Additionally, as mentioned previously, the assessments were not always administered concurrently with the fMRI scan. The date of assessment relative to the scan date are potentially critical in determining valid and meaningful relations between some of these measures and fMRI data, particularly for assessments such as the MFQ-C and MFQ-P that specifically probe symptom presence "in the past two weeks." This may have lead to a false lack of significance if the individual's scores changed during this time-lapse. Moreover, because of difficulties in coding the data, not all measures were available for all of the subjects, reducing the power to detect correlations.

E.   LIMITATIONS

The current study was designed to address the lack of familial high-risk studies of depression in adolescents, specifically the paucity of research investigating potential premorbid neurobiological markers of risk for depression. Moreover, because adolescents were assessed prior to depression onset, these data will help elucidate whether observed differences in brain activity seen in currently- or remitted-depressed individuals relative to never-depressed individuals may exist prior to depression onset or, rather, occur as a consequence of the depression.

The most significant limitation of these data is the failure to obtain a significant main effect of reward > no reward in the ventral striatal reactivity paradigm when examining the data at a conservative threshold corrected for multiple comparisons. This result severely limited the confidence with which we could make accurate conclusions regarding the results of the VS fMRI analyses because we could not disambiguate between task limitations and significant null findings. The development and administration of a more reliable VS paradigm for use in adolescent populations is an important next step in further investigations of premorbid markers of depression.

An additional limitation already mentioned is the lack of consistency in the timing of behavioral assessments. Because these data are part of a larger parent study, behavioral assessments were typically administered at the initial visit; however, MRIs were sometimes not scheduled for several months after the first visit. However, when the analyses were repeated with time lapse between assessment and scan entered as a covariate, the results did not change.

Further, this study was a concurrent design, with all assessments made within a few months of each other. Follow-up with these adolescents is an important step to determine who

goes on to develop depression. It may be that there are subtypes within both high risk and low risk groups who already show a neurobiological disposition to depression and that this effect is being muddled by the adolescents who will never develop depression. Finally, all of the adolescents assessed as part of this study were free from mood disorder diagnoses; therefore, similar studies of actively depressed adolescents are needed as a comparison group. Despite these limitations, the current study represents an important step in the investigation of adolescents at high- and low-risk for depression. Because the neurobiological differences between these groups was less pronounced than initially hypothesized, it may be that differences observed in currently depressed adolescents represent a consequence of depression. If this finding holds in replication studies, using these and other paradigms, risk-status may not convey neurobiological risk factors for depression at the group level; therefore, investigations of alternate mechanisms by which familial loading for depression may convey an increased susceptibility, such as environment, should be explored as potential risk factors for depression.

F.   CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

Familial risk for depression comprises both a genetic and environmental component since parents and siblings share both genes and environment. Disambiguating the varying degrees to which each of these factors contributes to risk for depression can inform intervention strategies, particularly in high-risk individuals, to mitigate the risk for future depression. Prior studies have investigated environmental factors such as mother-child interactions, parent-bonding, and family functioning in children at high- and low-risk for depression with compelling results (Dietz et al., 2008; D. Stein et al., 2000). Moreover, to address further the role of genes in risk for depression,

candidate genes implicated in risk for depression (e.g., 5HTTLPR, 5-HT-1A, TPH2) should be investigated as potential contributors to premorbid risk not only depression but for other mood disorders as well.

Additional studies are needed to replicate these findings, particularly because of the potential inconsistencies between these data and those of the only other neuroimaging study of adolescents at high-risk for depression. Longitudinal studies of at-risk populations are needed to understand the impact of differential emotion and reward function, if any, on emergence of depression in high-risk children and adolescents. Therefore, continued follow-up with these adolescents may yield promising trajectories of risk and resilience that may, in the future, aid the development and implementation of effective prevention and treatment studies.

VIII.   BIBLIOGRAPHY

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