NEURODEVELOPMENTAL CORRELATES AND PREDICTORS OF ALCOHOL USE AND ABUSE IN EARLY ADULTHOOD

by

Karen Elizabeth Muñoz

B.A., Haverford College in Pennsylvania, 2001

M.S., University of Pittsburgh, 2009

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This dissertation is presented

by

Karen Elizabeth Muñoz

It was defended on

September 28, 2012

and approved by

Erika E. Forbes, Ph.D., Associate Professor, Department of Psychiatry

Michael F. Pogue-Geile, Ph.D., Associate Professor, Department of Psychology

Daniel S. Shaw, Ph.D., Professor, Department of Psychology

Dissertation Advisor: Peter J. Gianaros, Ph.D., Associate Professor, Department of Psychology

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Alcohol use and abuse are significant concerns within adolescence and early adulthood. Although anxiety and depression frequently co-occur with alcohol use in adolescence and are postulated to be a pathway to alcohol problems (e.g., Chassin et al., 1993; Sher et al., 2005), prior work has predominantly focused on the so-called 'externalizing' pathway to alcohol use. One possibility is that the combination of both kinds of problems could be more pernicious than externalizing problems alone, but this has not been investigated. Furthermore, while the role of internalizing and/or externalizing problems on alcohol use could be exacerbated by the presence of neurobiological characteristics, the potential moderating role of factors such as the function of neural emotion-processing circuitry has been neglected. The current study examined the effects of both internalizing and externalizing disorders, symptom severity, and chronicity across development (between ages 8 to 17) on alcohol use and dependence at age 20. Also explored were the potential moderating effects of amygdala reactivity and functional connectivity measured during a face-processing paradigm at age 20. Study aims were tested within the context of a longitudinal study of 111 boys prospectively followed from early childhood to age 20. Although contrary to our hypotheses, results supported prior findings suggesting that

internalizing symptomatology alone may be protective against problematic alcohol use/dependence. However, in combination with high externalizing problems or early onset of alcohol intoxication, high internalizing problems were related to alcohol use and dependence. Across the majority of study observations, it was the comorbid internalizing/externalizing group that demonstrated the highest scores on alcohol outcome measures. Moreover, these effects were exacerbated for some of the alcohol use outcomes by the strength of the functional connectivity between the amygdala and the anterior cingulate cortex (BA 32 and BA 24), a limbic area involved in emotion regulation. Conceivably, the combination of both types of problems combined with difficulty controlling negative emotion creates a pattern of dysregulation that makes youth vulnerable to the effects of alcohol and leads them to problems with controlling their use. Differences between this study and prior literature are explored and limitations of the present study are discussed.

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PREFACE

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1.0 INTRODUCTION

Alcohol use and abuse are significant concerns in adolescents. It is estimated that by 8th grade, more than 40% of adolescents in the U.S. have consumed alcohol, and by 12th grade, approximately 80% have engaged in drinking behavior (Faden, 2006). Further, anxiety and depression are common psychological disorders in children and adolescents, with cumulative prevalence rates of approximately 10% for each of these disorders by age 16 (Costello, Mustillo, Erkanli, Keeler, & Angold, 2003). Importantly, these internalizing disorders (i.e., Depressive Disorders and Anxiety Disorders) frequently co-occur with alcohol use in adolescence, with approximately 20% to 40% of adolescents with alcohol use or dependence having a lifetime history of either depression or anxiety disorders (Grant & Dawson, 1997; Kandel et al., 1999; Rohde, Lewinsohn, & Seeley, 1996). Moreover, these prevalence rates approach 70% among adolescents seeking substance abuse treatment (Chan, Dennis, & Funk, 2008). In community samples, the comorbidity between co-occurring depression and substance use disorders is estimated to be between 11.1% and 32%, and between 7% and 40% for comorbid anxiety and substance use disorder, with particular percentages varying as a function of population demographics and diagnosis measures (O'Neil, Conner, & Kendall, 2011).

Extensive work has been conducted investigating the so-called 'externalizing' pathway (encompassing, for example, aspects of impulsivity, conduct problems, and attentional difficulties) to alcohol use. Recently, more studies have examined the link between depression and alcohol use disorders (AUD) in adolescents; however, examinations of the developmental

mechanisms by which such internalizing symptomatology associated with depression and anxiety (e.g., sadness, fears, worry, shyness) relate to risk for alcohol use/abuse remain scarce, as do studies directly comparing the internalizing pathway with the externalizing pathway to alcohol use within the same study. Recently, neuroimaging research has identified alterations in brain circuits known to be involved in the processing of emotional information and regulation of emotional states in both internalizing disorders (Forbes et al., 2009; Lau et al., 2009; Roberson-Nay et al., 2006; Thomas et al., 2001) and alcohol abuse (Clark, Thatcher, & Tapert, 2008; Heitzeg, Nigg, Yau, Zubieta, & Zucker, 2008; Hill et al., 2001). However, these studies have not yet systematically included the necessary clinical and control groups within the same study needed to directly test predictions about the relation between the two disorders and alterations in neurobiological reactivity. Rather, most have treated comorbidities as 'nuisance variables' or have not tested for differences between subgroups. Further, in order to clarify the existence of a relation between internalizing disorders and alcohol abuse, contributions of the externalizing pathway must also be explored and compared with effects of the internalizing pathway within the same study.

Prior research has established that high negative affect is a key component of both internalizing disorders and alcohol use disorders. Negative affect in child and adolescent populations has been associated with both depressive disorders and anxiety disorders and is considered to be a common aspect of internalizing disorders generally (Anderson, Veed, Inderbitzen-Nolan, & Hansen, 2010; Cannon & Weems, 2006; Chorpita, Plummer, & Moffitt, 2000). Negative affect has also been considered as a risk factor for and characteristic of AUD. Several studies have reported positive associations between high negative affect and substance

use in adolescence (Chassin, Pillow, Curran, Molina, & Barrera, 1993; Desrichard & Denarie, 2005; Hussong & Hicks, 2003; Wills, Sandy, Shinar, & Yaeger, 1999). Specifically, the negative affect-regulation or "self-medication" model suggests that individuals engage in substance use to reduce negative affect in order to cope with life stressors (Sher, Grekin, & Williams, 2005). In light of this prior work, further exploration of the role of negative affect and its neurobiological correlates is warranted.

One approach to examine the contributions of negative affect to internalizing disorders and AUD has been to investigate the neurobiological factors associated with the processing of emotion, and negative emotion in particular. By this approach, a specific emphasis has been placed on understanding the threat processing systems of the brain, namely the amygdala and networked regions known to regulate its activity (sub-regions of the prefrontal cortex (PFC), including the orbitofrontal cortex (OFC)). Therefore, because affective disorders are considered to involve 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 the functioning of the amygdala—given that this brain region plays a significant role in both emotional processes.

Accordingly, as part of the present study, blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI), a technique used to indirectly study neural activity within the brain, was used to investigate the functioning of the amygdala, which mediates behavioral and physiological arousal in response to salient or evocative environmental stimuli and challenges. Amygdala reactivity to emotional facial expressions was

assayed using a well-characterized challenge paradigm that robustly engages the amygdala and networked corticolimbic regions (Hariri et al., 2005). Importantly, versions of this task have 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). By this approach, the present study was created to investigate the relation between 1) internalizing symptomatology during childhood/adolescence (ages 8 to 17) and 2) alcohol use in early adulthood (at age 20), as well as the contributions of externalizing symptomatology to alcohol use. Further, the potential moderating effects of amygdala reactivity during emotional face processing in early adulthood (at age 20) on the link between both early internalizing and externalizing symptomatology and alcohol use were explored.

Additionally, altered interactions between the amygdala and regions of the PFC with direct inhibitory connections to the limbic regions have been detected in affective disorders, which may contribute to the deficits in affect regulation commonly seen in these individuals. In adults, neuroimaging studies have shown significant differences in these brain regions associated with emotion processing in individuals with affective disorders, specifically the amygdala and extended limbic regions (Fales et al., 2008; Fu et al., 2008; Grimm et al., 2008; Harvey et al., 2005; Sheline et al., 2001). Moreover, the literature in depressed and anxious children and adolescents has also found support for limbic network dysfunction (Forbes et al., 2009; Lau et al., 2009; Roberson-Nay et al., 2006; Thomas et al., 2001). Few studies have utilized functional magnetic resonance imaging (fMRI) to investigate the affective circuitry in alcohol use in adolescence;

however, structural MRI studies have shown differences in these limbic regions, although the direction of effect has varied (e.g., Clark et al., 2008). Therefore, the present study also seeks to determine the influence of functional connectivity between the amygdala and interconnected regions of the PFC during the same face processing paradigm on the relation between early (ages 8 to 17) internalizing and externalizing symptomatology and alcohol use at age 20.

The present study sought to explore primarily the effect of early internalizing markers (between ages 8 and 17) on alcohol use at age 20, and to investigate the potential contributions of neurobiological correlates of negative affect, operationalized as amygdala reactivity and connectivity within a face processing fMRI paradigm, on this association. In order to obtain a more complete picture of the role of early problematic symptomatology on later alcohol use, the role of early externalizing markers (between ages 8 and 17) on alcohol use at age 20 was also explored.

It was predicted that higher levels of either internalizing or externalizing symptomatology would be associated with higher levels of quantity and frequency of alcohol consumed and higher scores of alcohol dependence. Further, it was predicted that this relation would be moderated by amygdala reactivity, such that individuals with higher internalizing symptomatology would demonstrate higher amygdala reactivity and low connectivity and individuals with higher levels of externalizing symptomatology would demonstrate lower amygdala reactivity and connectivity. Similarly, it was predicted that a comparable pattern would be observed when individuals were separated according to diagnostic group, with those individuals meeting criteria for either an internalizing or externalizing disorder between ages 8 and 17 scoring the highest on the alcohol outcome measures, with a similar internalizing versus externalizing moderating effect of

amygdala reactivity and connectivity. Finally, it was hypothesized that those individuals with more chronic patterns of internalizing and externalizing diagnoses across childhood and adolescence would have higher scores on the alcohol outcome measures relative to those individuals with less chronic patterns, and that this difference would also be moderated by amygdala reactivity and connectivity.

2.0 LITERATURE SUPPORTING STUDY HYPOTHESES

Adolescents who begin drinking before age 15 are four times more likely to develop alcohol dependence relative to individuals who begin drinking at age 21 (Grant & Dawson, 1997), and prior work has demonstrated that peak years of alcohol-use onset are between ages 13-14, when most adolescents are in 7^{th}-8^{th} grades (Harford, Grant, Yi, & Chen, 2005). Further, initiation of binge drinking in early adolescence predicts poor rates of high school completion, poor pro-social functioning, and low parental bonding (Hill, White, Chung, Hawkins, & Catalano, 2000). Similarly, the onset of a depressive or anxiety disorder during childhood/adolescence confers risk for adult affective disorders (Lewinsohn, Rohde, Klein, & Seeley, 1999; Lewinsohn, Rohde, Seeley, Klein, & Gotlib, 2000; Pine, Cohen, Gurley, Brook, & Ma, 1998; Rao et al., 1995; Stein et al., 2001). Prior work has indicated that the early onset of either internalizing symptomatology or alcohol use may contribute to the later onset of the other (Kuo, Gardner, Kendler, & Prescott, 2006). Therefore, the early onset of these disorders can have long-term consequences; however, studies have not investigated these consequences at the neurobiological level in terms of disruptions in emotion processing within the same study. Nonetheless, prior work suggests that understanding the development of early affective and alcohol use disorders can increase our understanding of the factors contributing to the onset and course of alcohol use problems and affective disorders in later life. In this regard, longitudinal study designs, such as the present study, provide a critical methodological advantage for studying the sequence and time course of comorbidity between internalizing disorders and alcohol use in adolescence. There is a need, therefore, to investigate

risk factors for alcohol use disorders so that effective interventions can be employed to aid in risk reduction. Studying the neural correlates and neurobiological mechanisms linked to early affective disorders and an early onset of alcohol use will also expand our knowledge of predictive risk markers and guide the development of biologically-grounded and individually-tailored intervention and prevention strategies.

2.1 ADOLESCENCE IS A RISK PERIOD FOR BOTH INTERNALIZING DISORDERS AND AUD

Adolescence is the peak age of onset for both internalizing disorders and initiation of alcohol use. One method to understand their interrelation is to examine the contribution of affective processes during adolescence. Even in typically-developing individuals, adolescence is a time of rapid change—a period of increased experimentation, risky behavior, and mood changes—during which emotional problems in adolescence relate to difficulties with regulating emotions and impulsive behaviors (Dahl, 2004). These difficulties may arise because of developmental differences in the rate of maturation in functional neural circuits that are critical for mediating arousal, attention, and affect (e.g., amygdala), as well as those necessary for monitoring and regulating the drive of these regions to shape behavior adaptively and avoid negative consequences (e.g., PFC) (Casey, Jones, & Hare, 2008). Importantly, the amygdala serves as a relay between afferent sensory and visceral information, and it supports the generation of efferent physiological (e.g., autonomic and neuroendocrine) responses that are coordinated with behavioral and subjective arousal states associated with processing emotionally-salient information (LeDoux, 2000). In contrast, the PFC, which is one of the last regions to develop fully

in the brain, plays a vital role in higher cognitive functions, such as reasoning, planning, and adaptive social behavior, as well as in regulating subcortical (e.g., limbic) regions. Hence, self-control, the ability to regulate one's behaviors and emotions in the service of achieving long-term goals, and related processes supported by the slowly developing PFC, crystallizes gradually and well into early adulthood (Casey, Giedd, & Thomas, 2000; Gogtay et al., 2004; Gogtay et al., 2007; Rapoport & Gogtay, 2008; Spear, 2000).

2.2 VULNERABILITIES OF BRAIN DEVELOPMENT

By age 6, the human brain reaches approximately 90% of adult volume (Lenroot & Giedd, 2006); however, it undergoes tremendous restructuring during adolescence and into early adulthood. Advances in neuroimaging have allowed researchers to investigate more fully the changes that occur across brain development. Evidence from studies using structural magnetic resonance imaging (sMRI), a neuroimaging technique employed to investigate the regional volume and morphology of brain matter, has demonstrated that grey matter maturation tracks an inverted parabolic curve, with cortical grey matter reaching peak volumes followed by subsequent decreases in grey matter volume as the PFC continues to develop and mature into early adulthood (Giedd et al., 1999), a process thought to involve selective pruning and reduction in glial cells as a consequence of use/inactivity (Huttenlocher & Dabholkar, 1997; Tamnes et al., 2010). Within the medial temporal lobe (MTL), which encompasses the amygdala and hippocampus, grey matter generally increases during adolescence, peaking at approximately age 16 (Giedd et al., 1996; Yurgelun-Todd, Killgore, & Cintron, 2003). This developmental trend places

subcortical maturation on a different (accelerated) trajectory relative to prefrontal regions known to regulate subcortical regions. Definitive conclusions regarding the association between structural changes and behavior have not been established, but it is generally regarded that structural maturation corresponds to the developmental capacity for higher order cognitive control (Sowell et al., 2003). Casey and colleagues have proposed a neurobiological model of adolescence; whereby, early maturation of subcortical limbic regions (e.g., amygdala) in conjunction with protracted PFC development contributes to the adolescent tendency to recruit primarily bottom-up brain regions (subcortical limbic structures) during emotionally-salient situations, leading them to poor decision-making (Casey et al., 2008). Further, data from diffusion tensor imaging (DTI) studies, an sMRI method used to quantify morphological aspects of white matter fiber tracts, indicate that white mater, which is composed of myelinated axons that connect various grey matter areas, continues to increase with age during adolescence and into early-to-mid adulthood (Giedd et al., 1999; Reiss, Abrams, Singer, Ross, & Denckla, 1996). An increase in white matter would presumably lead to more efficient communication of information between and within frontal and cortical and subcortical regions.

Taken together, the pattern of grey and white matter development has been characterized as maturing from inferior to superior and posterior to anterior (Shaw et al., 2008; Sowell, Trauner, Gamst, & Jernigan, 2002); thus, the frontal regions of the brain known to be responsible for higher order processes such as regulation of emotion, planning, organizing, and strategizing, are the last to be fully developed. Similarly, developmental DTI studies indicate that the temporal-frontal connections are also slow to mature relative to other tracts in the brain (Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008; Tamnes et al., 2010). This restructuring of the

brain that occurs within adolescence has implications for rapid changes in cognitive, behavioral, emotional, and social development. These changes occurring in adolescence also leave the brain vulnerable to adverse changes, such as neuronal assembly alterations resulting from or contributing to mood disorders and alcohol use. Such changes, in turn, can affect the developmental risk trajectories of these individuals, potentially leading to long-term difficulties with emotional information processing and regulation throughout life. These potential changes within the brain may persist into adulthood and may be detectable through neuroimaging techniques. Neuroimaging data for the present study represent scans obtained when the individuals were age 20. This developmental time frame has been referred to in the literature as both late adolescence and early adulthood. Given the maturation rates of subcortical and cortical regions, it is likely that, as a group, this age will be neurobiological more mature than a "true"' adolescent group but will be immature relative to an age group assessed firmly in adulthood, thus representing the transition from adolescence into adulthood. Therefore, it is important to understand prior neuroimaging work conducted in both adolescent and adult populations.

Major consequences of adolescent drinking include the potential for neurobiological damage while the brain is still developing. In rats, binge drinking behavior is associated with damage to areas of the brain that correspond to the OFC and medial temporal lobe in humans (Monti et al., 2005). This may result in deficiencies in both emotion reactivity and emotion regulation—aspects of emotionality that may interact with depressive and anxious symptomatology with long-term consequences. Thus, deficits in the development of and functional dynamics between limbic areas such as the amygdala and regions of PFC as a

consequence of adolescent disorders may contribute to potential alterations in emotion processing in early adulthood.

2.3 NEGATIVE AFFECT AND NEURAL CORRELATES

One factor contributing to the comorbidity between internalizing disorders and AUD in adolescence may be the general increase in negative affect among individuals during this time period (e.g., as a consequence of more frequent exposures to social stressors, and threats to self-esteem). Depressed individuals tend to display negative affect information processing biases that contribute 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 ambiguous events as negative (Dearing & Gotlib, 2009), and ruminating about negative life events in an abstract, not concrete, manner (Nolen-Hoeksema, 2000). As such, negative affect is considered to be a commonality within affective disorders, and studies have implicated a role for negative affect across populations. (Anderson et al., 2010; Cannon & Weems, 2006; Chorpita et al., 2000).

Similarly, an additional mechanism by which negative affect may influence internalizing disorders is via deficits in emotion regulation, which can otherwise serve to quell negative emotions once initiated. Regulation may be established by various techniques, such as cognitive reappraisal, distraction, or suppression (for review, see Gross, 1998). Few neuroimaging studies appropriately measure emotion regulation in its strictest sense, which necessitates the experience of a negative emotion before regulation can be assessed. Therefore, here the term

"emotion regulation" is more broadly defined by the degree to which neural regions implicated in emotion processing and regulation are functionally integrated or coupled during the processing of emotion (e.g., as reflected by an increase in inhibitory PFC activity that is directionally correlated with a decrease in amygdala activity during an emotional event). A family of measures, referred to as "functional connectivity," can be used to quantify fMRI alterations in the neural systems and circuits presumptively involved in regulating negative emotional processes. Hence, deficits in emotion regulation can be characterized by alterations in the functional coupling (correlated fMRI BOLD signal activity) between 1) limbic regions involved in the processing of emotion and 2) regions of the prefrontal cortex with direct connections to limbic regions. The specific methods to be used in the present study are outlined in the method section below.

Similarly, negative affect has additionally been investigated in studies of AUD, highlighting an association between high negative affect and substance use in adolescent populations (Chassin et al., 1993; Desrichard & Denarie, 2005; Hussong & Hicks, 2003; Wills et al., 1999). In particular, the "self-medication" model suggests that individuals engage in substance use to reduce negative affect for the purpose of coping with life stressors (Sher et al., 2005). A review of adolescent personality factors suggests that there are two personality groups, each associated with different self-reported motivations. Adolescents who reported "enhancement" motivations (e.g., drinking for pleasure, to get drunk, or for its own sake) demonstrated characteristics associated with extraversion, impulsivity, and aggression and were more likely to be characterized as sensation seekers, have low inhibitory control, and low levels of responsibility. Conversely, adolescents who reported "coping" motivations (e.g., drinking to cope with bad feelings, to relieve stress, or to avoid social rejection) tended to have higher levels of neuroticism,

low levels of agreeableness, and a negative self view (Kuntsche, Knibbe, Gmel, & Engels, 2006). These personality characteristics have interesting implications due to the similarity between these factors and those of internalizing and externalizing symptoms and disorders. Similarly, studies investigating the comorbidity with anxiety, specifically, and AUD have also supported the self-medication hypothesis with analogous motivations, such as drinking to cope with fear or physiological hyper-arousal. As such, the effects of alcohol intoxication can lead to the lowering of anxiety by distracting an individual from these physical feelings (Steele & Josephs, 1988). These rewarding effects of alcohol—physical, psychological, or social—may reinforce continued use as a coping mechanism. Further, once this feed-forward cycle (reduction in negative affect—anxiety, sadness, etc. due to the immediate effects of alcohol use) is established, it can contribute to the maintenance of the comorbidity and increase the likelihood of relapse (Kushner, Abrams, & Borchardt, 2000). Similarly, individuals with problem drinking (e.g., negative consequences as a result of alcohol use, treatment history, formal diagnosis, excessive alcohol consumption) demonstrate higher levels of drinking to cope with negative affect motivations (Carpenter & Hasin, 1999).

Prior work has established the relation between negative affect and internalizing disorders, and there is sufficient evidence that negative affect also plays a role in AUD. It is likely that negative affect alone is not responsible for the development of AUD, but it is a potential pathway that merits further exploration. Since there is evidence to support an association between negative affect and both internalizing disorders and AUD, this may serve as an underlying common factor that may help explain the comorbidity between the two disorders, with long-term consequences.

Exploration of the threat processing system within the brain (i.e., amygdala and interconnected regions within the PFC) is one method for studying the contributions of negative affect to internalizing disorders and AUD. The amygdala is structurally connected to the OFC via GABAergic inhibitory connections. Therefore, OFC connections to the amygdala can directly modulate its activity in a regulatory manner. In particular, specific regions of the OFC, such as BA 47, have dense connections with the amygdala. This bilateral region shows functional alterations in individuals with major depressive disorder (Brody, Barsom, Bota, & Saxena, 2001) and in normal controls during sadness induction and sadness suppression (Levesque et al., 2003). Additionally, the cingulate cortex, specifically the anterior portion (anterior cingulate cortex (ACC)) has also been implicated in emotion processing, and the dorsal portion of the ACC (dACC; BA 32), above the genu of the corpus callosum, has been generally implicated in attention, decision-making, and motor initiation but is also known to be important for appraisal and expression of emotion. Functional subdivisions of the rostral/ventral ACC, encompassing supragenual/ventral (BA 24/32) and subgenual ACC regions (sgACC; BA 25), are thought to be involved in regulation of emotion, fear extinction, affect labeling, and self-distraction (Etkin, Egner, & Kalisch, 2011). In addition to the amygdala, these specific regions of the PFC will serve as the focal points for the present analyses.

2.4 NEUROIMAGING STUDIES OF ADULT INTERNALIZING DISORDERS

Major Depressive Disorder: In adults, previous neuroimaging studies of internalizing disorders 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 (e.g., Fales et al., 2007; Fu et al., 2007; Grimm et al., 2007; Harvey et al., 2005; Sheline et al., 2001). Individuals with major depressive disorder (MDD) have repeatedly exhibited increased amygdala reactivity to negative stimuli across studies, and these differences have been frequently associated with increases in attention to negative information (e.g., better recall of negative words), which is characteristic of a negative emotion processing bias. This demonstrated hyperactivity of the amygdala is seen for emotionally-salient information that is processed both consciously and unconsciously, assessed for example, through presentation of masked stimuli (information presented for short time periods so that conscious awareness is not viable) (for review, see Savitz & Drevets, 2009). Much of this research in adults, however, 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 or depression treatment. Additionally, these populations are frequently confounded by current or prior psychotropic medication use and psychiatric comorbidity, which both can bias amygdala activity (Breiter et al., 1996; Hariri & Fisher, 2007; Perez-Edgar et al., 2007; Rauch et al., 2000).

Functional connectivity between frontal and subcortical limbic regions has also been shown to be disrupted in adult MDD, which may contribute to the deficits in negative affect regulation attributed to depression. Previous studies of functional connectivity 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; Dannlowski et al., 2009; Mayberg et al., 1999). Regions of the PFC that have been shown to be

functionally-coupled with the amygdala have varied across studies, but have included the anterior cingulate cortex (ACC), dorsolateral PFC (DLPFC), ventrolateral PFC (vlPFC) (for review, see Savitz & Drevets, 2009).

Anxiety Disorders: Data from adults with primary anxiety disorders have reported similar data to MDD (for reviews, see Freitas-Ferrari et al., 2010; Martin, Ressler, Binder, & Nemeroff, 2010). Amygdala hyper-activation during the processing of emotionally-salient information (e.g., processing emotionally-valenced faces) has been demonstrated in fMRI studies of social anxiety disorder (SAD) and generalized anxiety disorder (GAD), although bilaterality of amygdala activation has been inconsistent and varies by paradigm used and sample demographics (e.g., Birbaumer et al., 1998; Cooney, Atlas, Joormann, Eugene, & Gotlib, 2006; Gentili et al., 2008; Straube, Mentzel, & Miltner, 2005). Timing of neural responses during cognitive appraisals of negative self-beliefs in SAD patients has been shown to be altered relative to a control group, with the SAD group demonstrating a delay of amygdala response reduction during the appraisal, suggesting that SAD individuals may have difficulty dampening the initial anxiety-provoking response to negative self evaluation (Goldin, Manber-Ball, Werner, Heimberg, & Gross, 2009). Increased amygdala responses relative to control groups have been seen not only for emotionally-valenced faces, but also for neutral faces (right amygdala reactivity), suggesting a negative emotion bias toward ambiguous stimuli (Cooney et al., 2006).

Conclusions and Limitations: Overall, adult neuroimaging data of both anxiety and depression have reported similar results across studies. Increases in amygdala reactivity are consistently reported in MDD and anxiety adult groups relative to controls, in both conditions of conscious and unconscious processing. Further, in anxious individuals, increases in amygdala

reactivity in response to neutral faces have also been demonstrated. Functional connectivity has been assessed predominantly only in populations with primary MDD diagnoses, and studies tend to report diminished functional coupling between subcortical limbic and PFC regions. Specific limitations of the adult literature include frequent comorbidities with other psychiatric disorders, differences in age range, severity, and duration of illnesses, as well as additional confounds such as psychotropic medication exposure.

2.5 NEUROIMAGING STUDIES OF ADOLESCENT INTERNALIZING DISORDERS

MDD and Anxiety Disorders: Relative to adults studies, there are significantly fewer fMRI studies of adolescent internalizing disorders, although the number of studies has increased dramatically within the last few years. Similarly to adults, fMRI studies of internalizing disorders in adolescents have focused predominantly on eliciting an amygdala response, typically through the presentation of emotionally-valenced faces. Subjects are asked to complete relatively easy tasks to maximize task differences as a function of group characteristics rather than performance. For example, subjects may be asked to passively view or match angry, fearful, or neutral faces (Killgore & Yurgelun-Todd, 2005, 2006; Thomas et al., 2001; Yang et al., 2010), or to engage in simple tasks to elicit varying attentional states (e.g., focusing attention on the bridge of the nose, rating how threatening the participant perceives the face to be) (Beesdo, Pine, Lieb, & Wittchen, 2010; Lau et al., 2009; Lau et al., 2010; McClure, Adler, et al., 2007; McClure, Monk, et al., 2007; Monk et al., 2008).

There is high comorbidity between depression and anxiety disorders in neuroimaging studies of adolescents; however, amygdala hyper-activation relative to controls is consistently reported in general, with a few exceptions. Decreased amygdala reactivity relative to controls in a sample of adolescents with a primary diagnosis of MDD (but that was 50% comorbid for anxiety) was observed while participants passively viewed emotionally-valenced faces; however, hyper-activation was seen when individuals were asked to rate how afraid they were of each face presented (Beesdo et al., 2009). Additionally, one other study conducted with a primary MDD group with comorbid anxiety reported amygdala hypo-activation in response to passively viewing faces relative to controls. However, this study should be interpreted with caution, as there were only 5 participants (all female), two of whom had comorbid GAD (Thomas et al., 2001). One additional study reported no difference in the amygdala for primary GAD adolescents with comorbid MDD relative to controls during a more complicated dot probe detection task (Monk et al., 2006). This study differed from similar studies in that the task stimuli were presented in an event-related manner, resulting in a more complicated task that robustly engaged ventrolateral PFC (vlPFC). These GAD participants did demonstrate hyper-activation of the vlPFC (BA 47) relative to controls, which may account for the lack of amygdala reactivity. Only one study reported directly comparing an MDD group (without comorbid anxiety) to an anxious group (without comorbid depression) (Roberson-Nay et al., 2006). In this study, the anxious group exhibited greater left amygdala activation relative to the MDD group when comparing trials of forgotten faces relative to faces successfully remembered. It may be, therefore, that the anxiety group (some with social anxiety) may interpret forgotten faces (interpretable as potentially novel) as more threatening than MDD subjects; however, replication is necessary to test this hypothesis.

Healthy Samples: There have also been a few studies conducted in psychiatrically healthy adolescents, which have assessed depression and anxiety scores via measures such as the Beck Depression Inventory (BDI), the State-Trait Anxiety Inventory for Children (STAI), and the Multidimensional Anxiety Scale for Children (MASC) (Killgore, Gruber, & Yurgelun-Todd, 2007; Killgore & Yurgelun-Todd, 2005, 2006; Telzer et al., 2008). These studies failed to demonstrate an overall correlation of depression symptoms scores with amygdala reactivity; however, one study reported a positive correlation with measures of social/interpersonal aspects of anxiety (Killgore & Yurgelun-Todd, 2005). Further, depressed mood was positively correlated with regions of the left PFC (DLPFC—BA 9, 44, 46, rostral ACC—BA 32, and left BA 10, 11) during a Stroop task (Killgore et al., 2007) and with left OFC (BA 10), right OFC (BA 11), and rostral ACC (BA 32) while viewing fearful faces (Killgore & Yurgelun-Todd, 2006), which may be indicative of a compensatory response in these individuals. Further, trait anxiety scores were positively correlated with right vlPFC (BA 10) when viewing angry faces and with right DLPFC (BA 46) on a contrast representing attention bias to angry faces (Telzer et al., 2008). These studies are partially consistent with data observed in adult studies. It should be noted, however, that the symptom scores in these studies were all within the nonclinical range.

Functional Connectivity: There have also been a few studies assessing functional connectivity in depressed and anxious adolescents. The only study of resting state connectivity did not observe any differences in amygdala connectivity between a medicated comorbid MDD group relative to controls. Prior work suggests that medication may mitigate the effects of depression at a neuronal level (Arce, Simmons, Lovero, Stein, & Paulus, 2008; Fu et al., 2004; Sheline et al., 2001). Decreases in functional connectivity were observed between dorsal ACC (BA

32) and connected brain regions (Cullen et al., 2009). Three additional studies of adolescents with a primary diagnosis of anxiety reported a strong functional connectivity between the amygdala and vlPFC (Guyer et al., 2008; McClure, Monk, et al., 2007; Monk et al., 2008); however, only two studies suggest alterations in this circuit as a function of anxiety (Guyer et al., 2008; Monk et al., 2008).

Conclusions and Limitations: Similar to adults, the fMRI data of emotion processing in adolescents with internalizing disorders suggest that there is increased amygdala reactivity in response to processing negatively-valenced stimuli in patient groups relative to psychiatrically healthy controls. Functional connectivity studies suggest that there are alterations in the functional coupling between amygdala and vlPFC in patient populations relative to controls. These data suggest, therefore, that internalizing disorders are represented in the brain by increased amygdala reactivity in response to threat and decreases in functional coupling between amygdala and vlPFC.

There are several limitations of the extant literature. First, although comorbidity was frequently reported within these studies, predominantly between anxiety disorders and MDD, but also with additional disorders such as attention-deficit/hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD), there were only a few studies that allowed the inclusion of subjects with comorbid AUD. Most studies reported that they specifically excluded for alcohol and drug abuse or dependence. Additionally, because these were patient populations, many of these samples included subjects with either current or prior psychotropic medicine exposure (Caetano et al., 2007).

Moreover, the studies reviewed had very broad age ranges of inclusion. Frequently, these studies spanned 7 years (e.g., 8 to 17, 11 to 19), with some as many as 9 years (8 to 17). Many of the studies did not report age ranges at all. As discussed previously, the transition from childhood to adolescence and into adulthood is dramatic in terms of social restructuring, and as previously emphasized, adolescents undergo dramatic neurobiological restructuring at this time. Although many of the studies reported that they controlled statistically for effects of both age and sex or used case-matched controls, future studies should focus on obtaining more narrow age ranges of subjects. Few longitudinal studies incorporating neuroimaging of internalizing disorders and AUD have been conducted. Therefore, there is a need to explore the neurodevelopmental consequences of early illness.

2.6 NEUROIMAGING STUDIES OF ADULT AUD

Functional neuroimaging of AUD in adults has predominantly focused on characterizing disinhibition, executive function deficits, attention impairments, and reward dysfunction—components thought to be related to the externalizing pathway. In general, alcohol use disorders in adults are typically characterized by varying degrees of neuropsychological impairment in attention, visuo-motor coordination, short-term memory loss, alterations in executive functioning, working memory deficits, difficulty with decision making, impulsivity, and cognitive inflexibility (for review, see Yucel, Lubman, Solowij, & Brewer, 2007).

Until recently, prior neuroimaging studies have focused primarily on externalizing aspects of alcohol problems; therefore, emotional face processing as a function of AUD has not been fully

evaluated. Further, there have been no studies of functional connectivity of affective processing that have been reported in the literature. Nonetheless, there have been a few recent studies assessing threat processing in AUD: one in a population of adults high in disinhibition with a positive family history for alcoholism (Glahn, Lovallo, & Fox, 2007), one in a population of social drinkers (Gilman, Ramchandani, Davis, Bjork, & Hommer, 2008), and two in adults with alcoholism (Marinkovic et al., 2009; Salloum et al., 2007). In the first study of young adults with a positive family history for alcoholism, the high-risk group (also high on a measure of disinhibition) demonstrated amygdala hypo-responsiveness when matching angry and fearful faces relative to controls (Glahn et al., 2007). A second study administered alcohol intravenously to social drinkers who were viewing threatening and nonthreatening facial stimuli. These data demonstrated increased reactivity in striatal reward regions of the brain (ventral striatum), and increased reactivity in the nucleus accumbens and caudate with subjective feelings of intoxication. Further, alcohol modulated the emotional processing in limbic regions by decreasing neuronal response differences between threatening and nonthreatening stimuli. These findings have implications for both the anxiolytic properties of alcohol and the propensity to make risky decisions as a result of alcohol intoxication (Gilman et al., 2008). In the two fMRI studies of adult alcoholics, hypo-activation in threat regions of the brain was seen in response to negatively-valenced stimuli in both alcoholic populations relative to control groups. In one study, abstinent alcoholic participants demonstrated an undifferentiated amygdala and hippocampal response to negative versus neutral faces, which was inversely correlated with lateral PFC (BA 9, 10) reactivity (Marinkovic et al., 2009). Similarly, in the other study of adult inpatient alcoholics (with a high degree of comorbid psychopathology), reductions within the supragenual/ventral ACC (BA 32, 24)

in alcoholics relative to controls were observed when decoding fear, sadness, and disgust. It was only in the processing of angry faces that alcoholics did not demonstrate an aberrant ACC (BA 25) response relative to controls (Salloum et al., 2007). These alterations may underlie increases in disinhibition frequently seen in alcoholics, as well as interpersonal difficulties. However, due to the prolonged duration of alcoholism in these participants, it is unclear whether these deficits represent a risk factor or consequence of alcoholism, nor is it apparent what role potential neurotoxicity on grey matter in these regions plays.

Conclusions and Limitations: These preliminary adult fMRI studies of threat processing indicate neurobiological alterations in the processing of negatively-valenced facial stimuli, which may have implications for potential interpersonal difficulties, disinhibition, and risk-taking behavior. One limitation of these data is that the typical populations of these samples tend to be older adults with chronic histories of alcohol use. The effects reported may be a result of neurotoxic damage to the structural integrity of these regions due to prolonged abuse of alcohol. Therefore, conducting studies in adolescent and/or early adult populations would be beneficial to address this issue.

2.7 NEUROIMAGING STUDIES OF ADOLESCENT AUD

Similar to the adult literature, there have been only a few fMRI studies that have assessed negative emotion processing in AUD or AUD high-risk adolescents. To date, there are no fMRI tasks interrogating the threat circuit specifically; however, there have been a few studies conducted that have implications for understanding emotion processing in these populations.

These were conducted in adolescents at high risk for AUD (due to high familial loading for alcoholism). In the first study, high-risk participants were defined as either vulnerable or resilient based on a composite score assessing potential drinking problems (Heitzeg et al., 2008). During the paradigm, participants were asked to passively view positive, negative, and neutral words in a block design task, and activation to negative and happy words were compared to the neutral condition. Results indicated that the resilient group demonstrated increased bilateral orbital frontal gyrus reactivity and left insula/putamen relative to control and vulnerable groups during the processing of emotional words. In contrast, the vulnerable group demonstrated increased dorsomedial PFC (dmPFC; BA 9, 10) and decreased VS, bilateral extended amygdala and OFC (BA 11). It should be noted that the vulnerable group in this study was highly comorbid with conduct disorder (CD), ADHD, and SUD, and an exclusion criteria for this study was comorbid depression or anxiety. This vulnerable group was also higher in externalizing scores, which were correlated with increased dmPFC (BA 8, 9) and decreased VS and extended amygdala activation. These data suggest that the vulnerable group may have engaged in the active suppression of emotion processing (due to increases in PFC regions); whereas, the resilient group may have processed the stimuli as more actively emotionally engaged. These differences in the neural circuitry between groups may represent neural correlates of risk and resilience; however, it would be necessary to continue following these subjects into adulthood to determine AUD outcomes.

An additional study of adolescents at high risk for AUD assessed a relatively young population (mean age = 13) who were not comorbid for any psychiatric diagnoses at the time of the study, but who were selected for having either a parent or grandparent with an AUD (Silveri, Rogowska, McCaffrey, & Yurgelun-Todd, 2011). This study used a Stroop Color-Word Interference

Test and demonstrated that within the challenging Color-Word naming condition, high-risk adolescents more strongly recruited BA 6, 8, 9, left insula, right supragenual ACC (BA 32), and ventral ACC (BA 24) relative to adolescents at low risk for alcoholism, suggesting that the high-risk group is potentially overcompensating to accomplish the task (response inhibition). Finally, a study of high-risk and low-risk adolescents was conducted using a theory of mind task in which faces were presented and subjects were asked to determine socially relevant information, the gender, or the inferred mental state from photographs of faces (Hill et al., 2007). Results from this study indicated that high-risk adolescents demonstrated decreased right middle temporal gyrus (BA 21), right superior frontal gyrus (BA 10) and left inferior frontal gyrus (BA 46) relative to controls, suggesting aberrant activity in regions known to be active during traditional theory of mind tasks. This finding may represent a neurobiological correlate of social processing difficulties frequently seen in alcoholic individuals, but replication is necessary. With respect to functional connectivity, the only study of high-risk individuals demonstrated reduced functional connectivity between bilateral cerebellar regions and contralateral anterior PFC (BA 9, 10, 21) and posterior ACC (BA 23, 24), putamen, cuneus, and insula in high-risk relative to low-risk adolescents (Herting, Fair, & Nagel, 2011). Alterations in these data may implicate a dysregulation of executive control functioning in high-risk adolescents. Taken together, these studies indicate that there may already be disruptions in emotion processing and executive control systems in the brain in high-risk groups, regardless of the presence of a current AUD.

Conclusions and Limitations: Overall, studies of alcohol use disorders or of high-risk adults and adolescents suggest alterations in both bottom-up regions of the brain known to be engaged in emotion processing and regions known to regulate these regions (regions of the PFC). Studies

of both fMRI and functional connectivity in adolescence implicate a dysfunctional executive function system, with hypoactivity in regulatory regions in high-risk adolescents. Since there have been very few fMRI studies specifically designed to interrogate the threat system in the brain, more studies similar to those previously conducted in internalizing populations need to be conducted in order to determine potential alterations in regions such as the amygdala, which were not specifically targeted in the fMRI studies reviewed here.

The limitations of the alcohol literature are similar to those enumerated in the section of internalizing disorders, in that the reviewed studies tended to focus on regions implicated in the externalizing pathway to alcohol use, namely executive function and disinhibition. Further, these studies were also highly comorbid for externalizing disorders, and comorbid internalizing disorders were generally excluded. This has potentially biased these results toward a subset of the AUD or AUD-risk population that displays more aggressive and disinhibited tendencies rather than individuals high in negative affect and anxiety. Further, there have been no studies specifically designed to systematically assess the contributions of internalizing disorders relative to AUD. Such studies are key to further understanding these preliminary conclusions. As previously noted, longitudinal designs are imperative as they may help to disentangle potential risk and resiliency factors that may potentially be currently diluting the results, if there are, in fact, protective factors present.

2.8 SUMMARY AND COMORBIDITY MODEL

Internalizing symptoms of emotional distress, particularly symptoms of depression and anxiety, are related to adolescent substance use (Newcomb & Bentler, 1989). Such internalizing symptoms are also disproportionately prevalent among children of parents with a substance abuse disorder, particularly alcoholism (Colder & Chassin, 1993). Further, increased negative affectivity—defined as a predisposition to aversive emotional states—is associated with adolescent alcohol use (Colder & Chassin, 1993; White, Xie, Thompson, Loeber, & Stouthamer-Loeber, 2001) and dependence (Rohde et al., 1996), and negative affectivity is considered a key component of clinical vulnerability to depression and anxiety (Anderson et al., 2010; Cannon & Weems, 2006; Chorpita et al., 2000). The above review summarized evidence that negative affect may link internalizing disorders and AUD, and it critiqued the limited neuroimaging work identifying the brain circuits instantiating this presumptive link.

The literature summarized here is broadly consistent with the view that adolescence increases risk for comorbid internalizing disorders and AUD, in part, as a consequence of early maturation of limbic regions combined with a protracted PFC development. This developmental sequence hence results in a slowly developing PFC-limbic regulatory mechanism. By this view of adolescent development and in light of prior neuroimaging findings, the following networked brain regions may thus be involved in internalizing and AUD comorbidity: the amygdala and interconnected subcortical regions (e.g., hippocampus) and regulatory sub-regions of the PFC involved in 'top-down' subcortical control processes. However, research that includes the necessary clinical and control groups within one study are needed to directly test the model proposed here. In this way, the contributing factors and neurobiological phenotypes of each

disorder can be further disentangled and potential differences between subgroups can be assessed. Additionally, longitudinal studies with long follow-up periods of adolescents targeting the threat circuit would further serve to answer questions about early internalizing disorders and AUD, and the potential consequences of these syndromes on the brain.

3.0 STATEMENT OF PURPOSE

In order to understand fully the development of Alcohol Use Disorders, it is necessary to explore the existence of potential early risk factors. Adolescence is considered a time period of rapid change with transformations occurring both at the social/interpersonal and neurobiological levels. This combination leaves adolescents vulnerable to negative outcomes, which are manifested by the increased rates of both internalizing disorders and AUD at this time. This comorbidity is at least partly a result of shared high levels of negative affect present in these disorders. Despite an increase in the recent number of studies exploring the neural correlates of these disorders, there is still a paucity of studies that have adequately explored the role of comorbidity in adolescence and the long-term affective consequences of these illnesses at the neurobiological level. Prior work has instead focused predominantly on the externalizing pathway and consequent executive functioning deficits.

The current study aims to address some of these outstanding needs and advance our understanding of the development of AUD by examining the effects of both internalizing and externalizing symptomatology across adolescence on alcohol use at age 20 and to explore potential moderating effects of neurobiological correlates of negative affect, defined as amygdala reactivity and functional connectivity in response to the processing of emotionally-valenced faces, in early adulthood in order to understand dysfunctions in emotion-related neural circuitries related to these disorders. This was accomplished by using a longitudinal study of 111 boys prospectively followed from early childhood to age 20 (current assessment). First, a continuous

measures approach was used to assess the effects of both internalizing and externalizing symptomatology on alcohol use and dependence at age 20. Second, a more stringent approach was taken with the data to assess the effects of ever meeting criteria for a DSM-IV internalizing or externalizing diagnosis across ages 8 to 17 on alcohol use and dependence at age 20. Third, the effects of chronicity (defined by either severe internalizing or externalizing symptomatology or DSM diagnosis) between early (ages 8 to 12) and late (15 or 17) onset were assessed for differences in alcohol use and dependence at age 20 relative to those meeting criteria at either time point (non-chronic). For all of these analyses, amygdala reactivity and connectivity will be tested as moderators.

4.0 QUESTIONS AND HYPOTHESES

Based on the previous research on internalizing disorders and AUD reviewed above, the following questions were addressed and the following hypotheses were tested:

Question 1: Are associations between levels of childhood/adolescence internalizing and externalizing symptomatology (ages 10 to 17) and alcohol use at age 20 moderated by differential amygdala reactivity patterns at age 20? (See Figure 1, Appendix 1)

Hypotheses bearing on Question 1: It was predicted that higher levels of either early internalizing or early externalizing symptomatology, defined by both severity and chronicity of early symptomatology, would predict to increased drinking behavior at age 20, relative to individuals with lower levels of symptomatology. In the presence of these associations, potential neural moderators of this correlation were hypothesized to be significant. Given prior work with adolescent and adult depression and anxiety, it was predicted that individuals high on internalizing measures would demonstrate increased amygdala reactivity and decreased connectivity relative to those with low internalizing scores. In contrast, given evidence from studies of individuals high on externalizing behaviors (e.g., Heitzeg et al., 2008), it was predicted that individuals high on externalizing measures would demonstrate decreased amygdala reactivity and decreased connectivity. It was predicted that individuals who were high on early measures of internalizing behaviors and high on measures of age 20 alcohol use would display the highest amygdala reactivity and lowest connectivity between the amygdala and regulatory prefrontal regions, regardless of comorbid externalizing disorders. It was predicted that those

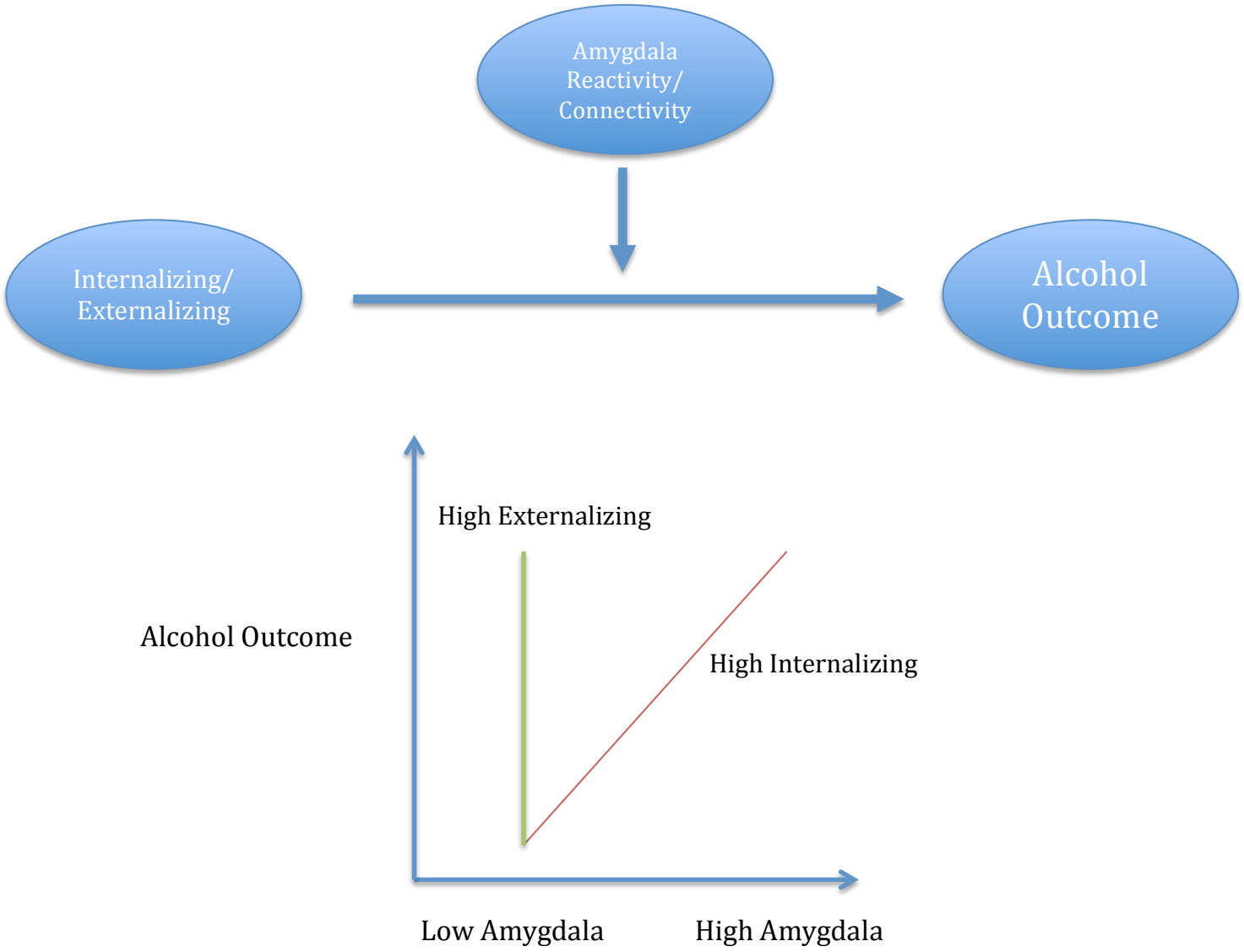
Amygdala Reactivity/ Connectivity
Internalizing/ Externalizing
Alcohol Outcome
High Externalizing
Alcohol Outcome
High Internalizing
Low Amygdala
High Amygdala

Figure 1. Hypotheses bearing on Question 1: Are associations between levels of childhood/adolescence internalizing and externalizing symptomatology (ages 10 to 17) and alcohol use at age 20 moderated by differential amygdala reactivity patterns at age 20?

individuals who were high on early measures of externalizing behaviors would demonstrate lower amygdala reactivity and lower amygdala-prefrontal connectivity regardless of level of alcohol use at age 20.

Question 2: Do individuals with early history of or high-chronicity of internalizing and externalizing disorders demonstrate more severe patterns of drinking at age 20, and is this relation moderated by amygdala reactivity/connectivity? (See Figure 2, Appendix 2)

Hypotheses bearing on Question 2—Severity (Figure 2A): It was predicted that individuals with a history of internalizing disorders would demonstrate a higher level of drinking relative to a group of individuals who never met criteria for any disorder (comparison group). Additionally, it was hypothesized that these individuals with a history of internalizing disorders would demonstrate the highest amygdala reactivity and lowest amygdala-prefrontal connectivity relative to the comparison group. Further, it was predicted that individuals with a history of externalizing disorders would also demonstrate a level of drinking greater than the comparison group and that these individuals would demonstrate decreased amygdala reactivity and decreased amygdala-prefrontal connectivity relative to the comparison group. It was hypothesized that individuals with a history of both internalizing and externalizing disorders would demonstrate patterns similar to those with severe internalizing disorders. *Chronicity (Figure 2B):* It was predicted that individuals with a persistent course of internalizing behaviors, defined by either severe internalizing symptomatology or DSM diagnosis during at least one time point in childhood (ages 8, 11, 12) and at least one time point in adolescence (ages 15, 17),

would demonstrate a more severe pattern of alcohol use at age 20 relative to both a group of individuals with a non-chronic history of internalizing disorders (e.g., individuals meeting these criteria at only one time point), and to the comparison group. It was hypothesized that these individuals would demonstrate increased amygdala reactivity and decreased amygdala-prefrontal connectivity relative to both the non-chronic group and the comparison group. It was predicted that individuals with chronic externalizing symptomatology (defined similarly to the chronic internalizing group) would demonstrate decreased amygdala reactivity and decreased amygdala-prefrontal connectivity relative to individuals with non-chronic externalizing disorders and to the comparison group.

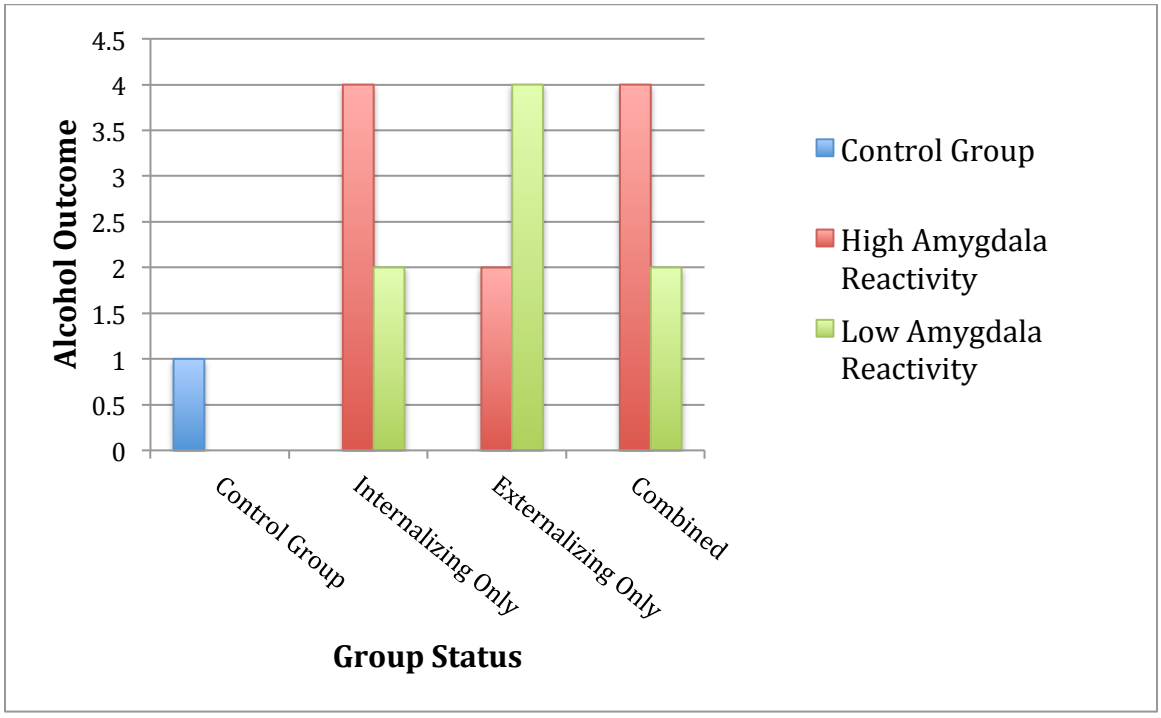

Figure 2A. Hypotheses bearing on Question 2: Do individuals with early history of or high-chronicity of internalizing and externalizing disorders demonstrate more severe patterns of drinking at age 20, and is this relation moderated by amygdala reactivity/connectivity? (Severity)

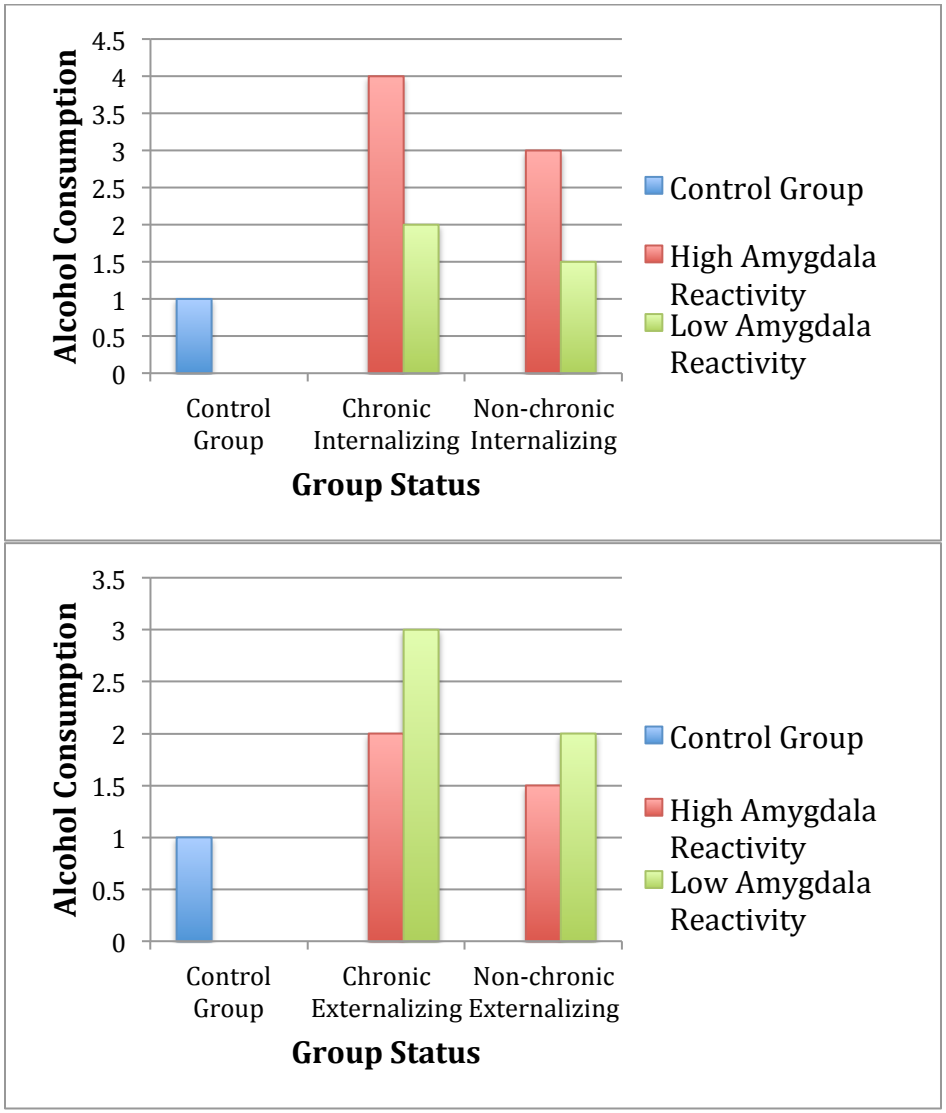

Figure 2B. Hypotheses bearing on Question 2: Do individuals with early history of or high-chronicity of internalizing and externalizing disorders demonstrate more severe patterns of drinking at age 20, and is this relation moderated by amygdala reactivity/connectivity? (Chronicity)

5.0 METHOD AND EXPERIMENTAL DESIGN

5.1 PARTICIPANTS

The participants in the study were males recruited as part of the Pitt Mother and Child Project (PMCP), an ongoing longitudinal study of child development in low-income families in Pittsburgh, Pennsylvania (e.g., Feng, Shaw, & Silk, 2008; Shaw, Gilliom, Ingoldsby & Nagin, 2003). These individuals were initially recruited when they were between 6 and 17 months old from the Allegheny County's Women, Infants, and Children (WIC) Program, an organization that provides nutritional food supplements for income-eligible mothers. Of these 311 participants initially recruited, 53% were Caucasian, 36% were African-American, 5% were biracial, and 6% were characterized as other race (e.g., Hispanic, Asian). Mean per capita income for the families was $241 dollars per month ($2,892 per year), and the mean Hollingshead socioeconomic status was 24.8, which is indicative of a working-class sample (Shaw, Gilliom, Ingoldsby, & Nagin, 2003).

5.2 PROCEDURES

5.2.1 Visit Procedures

Target children and their mothers participated in two- to three-hour assessments beginning when the child was age 1.5 years old. The present study included data collected at ages 8, 10,

11, 12, 15, 17, and 20 years old. These data were collected in the laboratory and/or the target child's home. At ages 16 and 18, participants were interviewed by phone. At age 20, participants were assessed without their mothers in the laboratory. Participants were reimbursed for their time at the end of each assessment. The parent protocol from which these participants were selected was approved by the University of Pittsburgh Institutional Review Board. Informed consent was obtained from parents prior to their child's enrollment in the study.

5.2.2 Neuroimaging Procedures

Amygdala reactivity paradigm. In this paradigm, four blocks of a perceptual face-processing task are interleaved with five blocks of a sensorimotor control task (Figure 3). During the face task, subjects viewed a trio of faces (expressing one of four emotions—anger, fear, surprise, neutral) 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 of the four face blocks consisted of six trios, three of each sex, randomly assigned, with blocks counterbalanced between subjects. Each image was presented for 4 seconds, with a variable interstimulus interval (ISI = 2-6 seconds) for a total block length of 48 seconds. The presentation of stimuli for 4 seconds has previously been shown to allow for the hemodynamic response 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 (circles, vertical and horizontal ellipses) 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 seconds, with a fixed ISI of 2 seconds for a block length of 36 seconds. All blocks were preceded by brief instructions (‘‘Match Faces’’ or ‘‘Match Shapes’’) lasting 2 seconds. The total scan time is 390 seconds. Subject performance (accuracy and reaction time) was monitored during all scans.

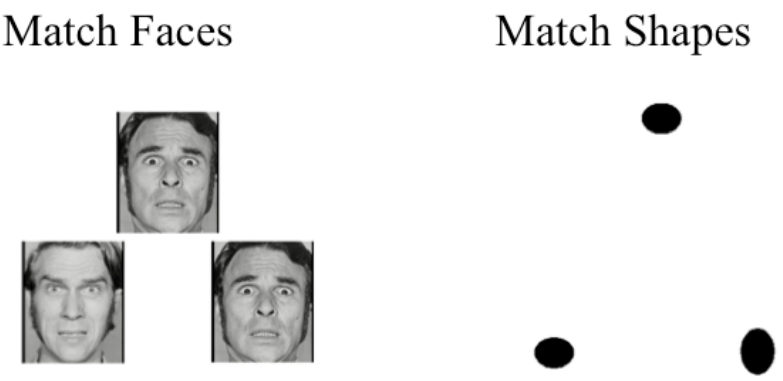

Figure 3. Emotion face-processing paradigm

BOLD fMRI acquisition parameters. The fMRI scans were performed at the Magnetic Resonance Research Center (MRRC) of Presbyterian University Hospital of Pittsburgh. Data were collected using a Siemens 3T Tim Trio scanner (Siemens Medical Solutions, Erlangen, Germany). BOLD functional images were acquired using a gradient-echo echoplanar imaging (EPI) sequence to obtain 34 interleaved axial slices (3 mm slice thickness). The middle slice was aligned to the AC-PC line to maximize coverage of the limbic regions (TE = 25 milliseconds, TR = 2000 milliseconds, acquisition matrix = 64 × 64, field of view = 20 cm). All scanning parameters were selected to optimize the quality of the BOLD signal while maintaining a sufficient number of slices to acquire whole-brain data. 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. Data from all of the subjects included in the analyses were cleared of such problems.

Image processing and analyses. Analysis of the fMRI data was completed using Statistical Parametric Mapping (SPM8) software (Wellcome Department of Imaging Neuroscience, London, England). Images for each subject were grey matter segmented, realigned to the mean volume in the time series and unwarped to correct for head motion, co-registered to each subject's high resolution structural scan (MPRAGE scan), 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. After preprocessing, the ARtifact detection Tools (ART) software package (MIT, Boston, MA, USA) was used to detect global mean intensity and translation or rotational motion outliers (> 4.5 *SD* from the mean global brain activation) within each participant's data and omitted them from subsequent statistical analyses. These preprocessed data sets were then analyzed using second level random-effects models that accounted for both scan-to scan and participant-to-participant variability to determine task-specific regional responses.

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 (i.e., faces > shapes). These individual contrast images were then used to determine task-specific regional responses using predetermined regions of interest (including bilateral amygdala) at the group level for the entire sample (main effects of task) and direct comparisons between groups.

All analyses were conducted with a threshold of $p < 0.05$, family-wise error (FWE) corrected for multiple comparisons. In addition to whole brain analyses in SPM8, anatomically-based 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. Further, BOLD contrast estimates were extracted from functional clusters based on main effect of task to delineate anatomy-specific effects without risk of double correlation when these clusters are extracted and used in regression and structural models (Vul, Harris, Winkielman, & Pashler, 2009).

Connectivity processing and analyses. Functional connectivity was assessed by conducting a psychophysiological interaction (PPI) analysis using SPM8. PPI analysis allows for the specification of a particular region of interest to examine other regions' connectivity with this region and whether connectivity changes with cognitive or perceptual task demands (Friston et al., 1997). This method of connectivity was used to examine the degree to which early internalizing and externalizing symptoms and alcohol abuse relate to the change in the functional connectivity of the right and left amygdala with regions of the OFC/ACC (BA 11, 24, 25, 32 and 47) when viewing faces versus shapes. These regions of interest were selected because of previous findings reporting significant recruitment of these areas when subjects are processing emotionally salient stimuli compared with control conditions.

For the PPI analyses, the right and left amygdala were selected as seed regions. The time series representing the first eigenvariate for each seed region for each subject was extracted. The BOLD signal time series was mean-centered, submitted through a high-pass filter to remove

low-frequency signal drifts, and deconvolved using the canonical SPM8 hemodynamic response function (HRF). An interaction variable was then constructed representing the interaction between the time series of the seed regions (i.e., right and left amygdala composite seed) and the psychological variable (faces versus shapes), which was reconvolved with the HRF. This interaction term was entered as a regressor for each subject in a first level model with the time series of seed regions and the vector coding for task effect. The individual contrast images were then explored in a second level analysis to determine relative differences in the connectivity with group status specified as regressors in the model. Specifically, the connectivity between the right and left amygdala regions, entered as composite score, and regions of the OFC were explored. For these analyses, a small volume correction (SVC) was applied using predetermined anatomically-based ROI masks from the WFU Pickatlas.

5.3 MEASURES

The primary measures of early internalizing/externalizing symptomatology and age 20 alcohol use are specified below. Measures that were used as covariates are also specified. Specific uses for each measure are outlined in the Data Analytic Plan and Results sections (See Figure 4 for collection timeline of measures).

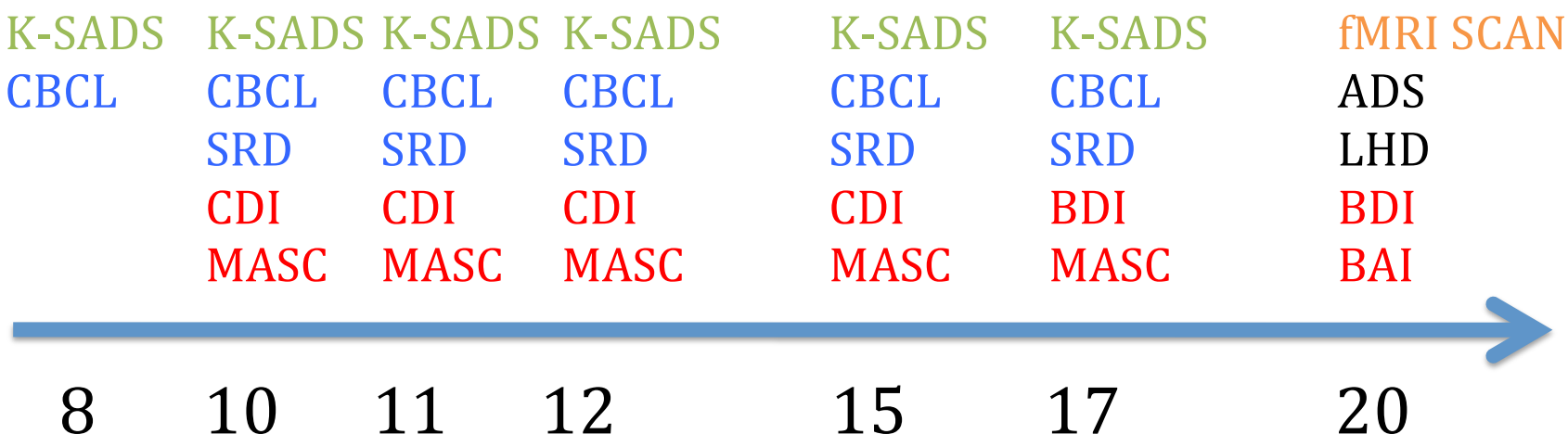
K-SADS
CBCL
K-SADS
CBCL
SRD
CDI
MASC
K-SADS
CBCL
SRD
CDI
MASC
K-SADS
CBCL
SRD
CDI
MASC
K-SADS
CBCL
SRD
CDI
MASC
K-SADS
CBCL
SRD
BDI
MASC
fMRI SCAN
ADS
LHD
BDI
BAI
8
10
11
12
15
17
20
Age data collected

Figure 4. Timeline of variable collection
(internalizing measures shown in red, externalizing measures shown in blue, measures used in both shown in green, outcome measures shown in black, moderator shown in orange)

5.3.1 Early internalizing/externalizing behavior measures

The Schedule for Affective Disorders and Schizophrenia for School-age Children (K-SADS). Diagnoses DSM-IV (1994) were determined at each assessment through the administration of a modified version of the Schedule for Affective Disorders and Schizophrenia in School-Age Children (K-SADS; Kaufman et al., 1997) to the target child and/or their primary caregiver. The K-SADS is a semi-structured interview that assesses DSM child psychiatric symptoms over the last year. The K-SADS was administered only to the primary caregiver at age 8, and to both child and primary caregiver at ages 10, 11, 15, and 17 (interviewed separately). When both the child and the parent were interviewed, the examiner made a clinical judgment about the presence or absence of each symptom. At age 12, the internalizing modules of the K-SADS were administered only to the child, and the externalizing modules were administered only to the primary caregiver. To establish reliability, interviewers participated in an intensive training program at Western Psychiatric Institute and Clinic or were trained by a doctoral-level clinical psychology student who attended the training. Every case in which the participant approached or met diagnostic criteria was discussed by the research team, which included additional interviewers and the principal investigators of the study.

Participants were considered to have an internalizing disorder if they received a diagnosis of major depressive disorder (MDD), dysthymic disorder, or generalized anxiety disorder (GAD), social phobia/social anxiety disorder, or separation anxiety disorder. GAD and

social phobia/social anxiety disorder are known to have high comorbidity rates with depression. Indeed, epidemiological studies of adolescents and young adults have reported comorbidity rates of 25% to 31% between social anxiety disorder and depression (Wittchen, Stein, & Kessler, 1999) and an odds ratio of 4.44 for GAD and depression (Beesdo et al., 2010). Further, factor analysis studies demonstrate a stronger relation between GAD and depression, relative to other anxiety disorders (Krueger, 1999; Lahey et al., 2008). Participants were considered to have an externalizing disorder if they received a diagnosis of ADHD, ODD, or CD. As internalizing symptoms are highly correlated with externalizing symptoms (for review, see Angold & Costello, 1993; also Chan, Dennis, & Funk, 2008; Garnefski & Diekstra, 1997; Reitz, Dekovic, & Meijer, 2005), a comorbid group of individuals with both internalizing and externalizing disorders was created for use in the analyses. Due to the differences between the subtypes of anxiety disorders and the present focus only on internalizing and externalizing disorders, individuals were excluded if they had diagnoses outside of the prescribed definitions outlined here for internalizing and externalizing disorders. K-SADS diagnoses were used to explore question 2 using a categorical/diagnostic approach.

Child Behavior Checklist (CBCL). The CBCL (Achenbach, 1991) is a measure administered to the primary caregiver to assess their child's behavioral problems and social competence. The CBCL was acquired at ages 8, 10, 11, 12, 15, and 17 and is composed of Likert-scale items that ask the primary caregiver 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. T-scores for the Internalizing and Externalizing Factor scores were created using SPSS syntax based on calculations outlined in the CBCL manual (Achenbach, 1991). These Factor T-scores were used to create composite scores to answer question 1 and as one of the criteria to determine chronicity group status for question 2.

Self Report of Delinquency (SRD). The SRD (Elliot, Huizinga, & Ageton, 1985) is a semi-structured interview that contains 33 items (at age 10, 11, 12) or 62 items (at age 15, 16, 17, 18) that assess the frequency with which an individual has engaged in aggressive and delinquent behavior, alcohol and drug use, and related offenses. Using a 3-point rating scale (1 = never, 2 = once/twice, 3 = more often), children were asked to rate the extent to which they engaged in different types of antisocial activities (e.g., stealing, throwing rocks at people, drug use). When the SRD was administered at ages 10, 11, and 12, a version designed for younger youth was used, eliminating more serious forms of drug use and physical assaults. To compensate for the different number of items used at ages 10-12 versus 15-17, a mean averaged from both versions was used in the analyses. Total score was used in the creation of externalizing composite scores for question 1.

Multidimensional Anxiety Scale for Children (MASC). The short form of the MASC (March, 1998) is a 10-item measure of anxiety symptoms for children ages 8 to 18 (e.g., "The idea of going away to camp scares me"). This measure was administered to the target child at

ages 10, 11, 12, 15, and 17. Higher scores are indicative of higher anxiety. The total score was used in the creation of internalizing composite scores for question 1.

Children's Depression Inventory (CDI). The short form of the CDI (Kovacs, 1992) is a 10-item self-report questionnaire widely used to assess depressive symptoms in children (e.g., "I am sad once in a while," "I am sad many times," "I am sad all the time.") Participants were asked how they had been feeling in the past 2 weeks and to rate each item based on severity on a 4-point scale (0 to 3). This measure was administered to the target child at ages 10, 11, 12, and 15. Higher scores are indicative of greater depression. The total score was used in the creation of internalizing composite scores for question 1.

Beck Depression Inventory (BDI). The BDI-IA (Beck & Sheer, 1993b) is a 21-item self-report questionnaire used to assess symptoms of depression in the last 6 months. Scores above 18 are considered to be indicative of moderate depression. The BDI was collected at ages 17 and 20. Total score at age 17 was used in the creation of the internalizing composite score for question 1.

5.3.2 Age 20 alcohol use outcome measures

Alcohol Dependence Scale (ADS). The ADS (Skinner & Horn, 1984) is a 25-item self-report questionnaire that assesses the severity of alcohol dependence symptoms over the past year. The ADS assesses alcohol withdrawal symptoms, impaired control over drinking, awareness of a compulsion to drink, increased tolerance to alcohol, and salience of drink-seeking behavior. A score of 9 or greater is highly predictive of a DSM diagnosis of alcohol dependence (Ross et al., 1990).

Lifetime History of Drinking. The lifetime history of drinking (Skinner, 1982) is an interview-based questionnaire, administered at age 20, that assesses an individual's patterns of alcohol consumption from the first year of drinking to the present time. Specifically, this interview determines an individual's age of first alcohol use, age of first alcohol intoxication, and age of first significant alcohol use. Beginning with the age of significant use, drinking averages and most drinks per day are recorded until the present day in a year-to-year pattern. This interview was used to determine the participant's pattern of alcohol consumption (e.g., average days per month drinking, average quantity consumed, maximum quantity of alcohol used at one time, and maximum number of days using maximum quantity of alcohol) at age 20. For this measure, a standard drink (quantity unit) was defined as 12 ounces of beer (5% alcohol) or wine cooler, 8 ounces of malt liquor (6-7% alcohol), 5 ounces of dinner wine/champagne (12-14% alcohol), 3 ounces of liqueur (40 proof) or fortified wine (20% alcohol), 1.5 ounces of hard liquor or liqueur (80 proof), or 1.2 ounces of hard liquor (100 proof).

5.3.3 Covariates

Socioeconomic Status

Socioeconomic status was designated as the highest level of maternal education reported between ages 8 and 20 for each subject, self-reported by the mother.

Early Alcohol Use

Lifetime History of Drinking (LHD). The LHD (Skinner, 1982) was used to determine age of first intoxication and age of first significant alcohol use.

Age 20 Internalizing Behavior Measures

Beck Anxiety Inventory (BAI). The BAI (Beck & Steer, 1993) is a 21-item self-report questionnaire, collected at age 20, assessing symptoms of anxiety during the past month. Scores above 15 are considered to be indicative of moderate anxiety. Total score was used as a covariate in the analyses.

Beck Depression Inventory (BDI). The BDI-IA (Beck & Sheer, 1993b), administered at age 20, was used to assess symptoms of depression in the previous 6 months at age 20. Total score was used as a covariate in the analyses.

5.4 DATA ANALYTIC PLAN

To address the questions in these analyses, two approaches were taken to define severity and chronicity. To answer question 1 (see Appendix A), a continuous variable approach was used. Every participant with data was included in these analyses regardless of clinical-cutoff diagnosis or level of severity and chronicity. To categorize the most severe and chronic groups and determine differences between them, a categorical/diagnostic approach was used to answer question 2 (See Appendix B). Details of each analysis are described below, separated by question.

Question 1: Are associations between levels of childhood/adolescence internalizing and externalizing symptomatology and alcohol use in early adulthood moderated by differential amygdala reactivity patterns at age 20?

To investigate the existence of two differential pathways to alcohol use problems in early adulthood, internalizing and externalizing behaviors were assessed using aggregate scores

developed to represent severity and chronicity of early internalizing/externalizing behaviors. The following continuous measures were outlined *a priori* to represent level of early internalizing behaviors across ages 10 to 17: 1) CDI (total score), 2) MASC (total score), and 3) CBCL (Internalizing Factor T score). A correlation analysis between these three measures at each time point was conducted to determine their inclusion in the composite score. The Internalizing T score was not significantly correlated with either CDI or MASC at any time point except age 17 and was therefore not included in the analyses. CDI and MASC were significantly correlated at all ages except age 15 (see Table 1A for correlation statistics). Therefore, the internalizing composite scores comprised only CDI and MASC total scores.

The following measures were outlined *a priori* to represent early externalizing behaviors across ages 10 to 17: 1) SRD (total score) and 2) CBCL (Externalizing Factor score). As with the internalizing measures, a correlation analysis between the two measures at each time point was conducted to determine the appropriateness of a composite score. The SRD and CBCL Externalizing T Scores were significantly correlated at each time point (see Table 1B for correlation statistics). The scores for the internalizing measures (CDI, MASC) were standardized and combined into a Z score for each time point the participant attended (See Table 2 for descriptive statistics of composite variables). Similarly, the scores for the externalizing measures (SRD, CBCL) were standardized and combined. Chronicity was defined as the mean of the Z scores across all of the time points each person attended, therefore taking into account data encompassing all available time points. Two scores were determined for each individual: one for internalizing chronicity and one for externalizing chronicity. Severity was measured using the highest Z score among all of the time points for each person (e.g., Z score for age 10

for internalizing, Z score for age 12 for externalizing), therefore using only the most severe time point, separately for internalizing and externalizing. Similarly, one score was determined for internalizing severity and one score for externalizing severity. Using these measures, a continuous variable was used so that each person received four scores: internalizing severity, externalizing severity, internalizing chronicity, and externalizing chronicity. Separate analyses were conducted to determine the effects of both severity and chronicity and amygdala reactivity and connectivity.

Table 1A. Correlations between internalizing measures at each age

AGE 10	**CDI**	**MASC**
CDI		
MASC	0.23*	
CBCL Internalizing T Score	0.00	0.06
AGE 11	**CDI**	**MASC**
CDI		
MASC	0.35***	
CBCL Internalizing T Score	0.15	0.01
AGE 12	**CDI**	**MASC**
CDI		
MASC	0.27**	
CBCL Internalizing T Score	0.13	0.07
AGE 15	**CDI**	**MASC**
CDI		
MASC	0.13	
CBCL Internalizing T Score	0.17 +	0.17 +
AGE 17	**BDI**	**MASC**
BDI		
MASC	0.47***	
CBCL Internalizing T Score	0.24**	0.28***

+ p < .10, * p < .05, ** p < .01, *** p < .001

Note: CDI: Child Depression Inventory (Kovacs, 1992), BDI: Beck Depression Inventory (Beck & Steer, 1993), MASC: Multidimensional Anxiety Scale for Children (March, 1998), CBCL: Child Behavioral Checklist (Achenbach, 1991)

Table 1B. Correlations between externalizing measures at each age

AGE 10	**SRD**
SRD	
CBCL Externalizing T Score	0.24*
AGE 11	**SRD**
SRD	
CBCL Externalizing T Score	0.29***
AGE 12	**SRD**
SRD	
CBCL Externalizing T Score	0.29***
AGE 15	**SRD**
SRD	
CBCL Externalizing T Score	0.36***
AGE 17	**SRD**
SRD	
CBCL Externalizing T Score	0.36***

* $p < .05$, *** $p < .001$

Note: SRD: Self Report of Delinquency (Elliot et al., 1985), CBCL: Child Behavioral Checklist (Achenbach, 1991)

Alcohol use at age 20 was defined two ways. The first was a more severe measure, the Alcohol Dependence Scale, which assesses level of dependence on alcohol by asking such questions as "do you get physically sick as a result of drinking," "have you had blackouts as a result of drinking" (No, Sometimes, Almost every time I drink). The second was chosen as a less severe measure to assess quantity and frequency of drinking as assessed by the LHD. Alcohol use and dependence data were not available for all participants (see Table 2). Notably, data from the LHD were only available for approximately half of the total sample due to ongoing

data coding and cleaning at the time of analysis. Using the LHD, “average days per month” (n = 53), “average quantity” (n = 51), “maximum quantity” (n = 50), and “maximum number of days using maximum quantity of alcohol” (n = 52) were calculated. The total ADS score was used to assess severity of alcohol dependence (n = 105). A correlation analyses revealed that these 5 outcome measures were highly correlated (between 0.575 to 0.741, $p < 0.001$).

For this and all subsequent analyses, separate analyses were conducted for amygdala reactivity and connectivity. Amygdala reactivity was extracted from the main effect of Faces > Shapes analysis in SPM for each person, and amygdala connectivity was extracted from the connectivity map generated using the method explained above. Moderation was tested using a hierarchical multiple regression analysis with highest maternal education (between ages 8 and 17) entered as covariate. Multiplicative interaction terms were created for Internalizing X Externalizing scores, Reactivity (Connectivity) X Internalizing score, Reactivity (Connectivity) X Externalizing Score and Internalizing X Externalizing X Reactivity (Connectivity)) with alcohol use as the outcome variable. These analyses were conducted separately for severity and chronicity of early internalizing and externalizing behaviors.

Table 2. Means and standard deviations of study variables and composite variables (Z scores*)*

Variable	*N*	Range	*M*	*SD*
CDI 10	102	0 to 12	1.29	1.79
CDI 11	106	0 to 6	1.08	1.56
CDI 12	104	0 to 6	0.78	1.19
CDI 15	110	0 to 10	1.19	1.85
BDI 17	109	0 to 35	5.34	5.93
MASC 10	101	2 to 24	11.80	5.02
MASC 11	107	0 to 20	10.37	4.56
MASC 12	105	0 to 24	9.32	5.37
MASC 15	110	0 to 21	6.39	4.93
MASC 17	110	0 to 24	6.04	5.09
CBCL Internalizing T score 10	99	34 to 73	47.95	10.24

CBCL Internalizing T score 11	107	34 to 75	47.07	10.08
CBCL Internalizing T score 12	103	32 to 73	46.15	9.30
CBCL Internalizing T score 15	110	32 to 79	43.81	9.91
CBCL Internalizing T score 15	111	32 to 65	43.29	9.75
CBCL Externalizing T score 10	99	30 to 75	48.51	10.95
CBCL Externalizing T score 11	107	30 to 74	47.09	10.15
CBCL Externalizing T score 12	103	32 to 67	48.37	9.77
CBCL Externalizing T score 15	110	32 to 74	46.07	10.34
CBCL Externalizing T score 15	111	32 to 71	44.20	10.17
SRD 10	105	0 to 0.48	0.085	0.09
SRD 11	108	0 to 0.45	0.097	0.09
SRD 12	105	0 to 0.52	0.095	0.10
SRD 15	110	0 to 0.73	0.12	0.13
SRD 16	107	0 to 0.95	0.13	0.15
SRD 17	110	0 to 0.85	0.19	0.17
ADS 20	105	0 to 20	4.30	4.08
LHD: Average days per month using alcohol 20	53	0 to 20	1.49	3.48
LHD: Average quantity of alcohol used 20	51	0 to 24	2.17	4.40
LHD: Maximum quantity of alcohol used 20	50	0 to 24	2.54	5.01
LHD: Maximum number of days using maximum amount of alcohol 20	52	0 to 24	1.73	4.22
Variable Composite	***N***	**Range (Z score)**	***M (Z)***	***SD (Z)***
Internalizing composite 10	100	-1.34 to 3.50	-0.0001	0.7823
Internalizing composite 11	106	-1.48 to 2.31	-0.0069	0.8189
Internalizing composite 12	104	-1.20 to 2.44	0.0030	0.7995
Internalizing composite 15	110	-0.97 too 2.51	0.0000	0.7529
Internalizing composite 17	109	-1.04 to 3.58	0.0045	0.8557
Externalizing composite 10	99	-1.27 to 3.63	-0.0157	0.7848
Externalizing composite 11	107	-1.37 to 2.48	-0.0022	0.8059
Externalizing composite 12	103	-1.28 to 2.59	0.0023	0.8072
Externalizing composite 15	110	-1.15 to 3.17	0.0000	0.8259
Externalizing composite 17	110	-1.10 to 2.05	0.0055	0.8243
Internalizing mean (Chronicity score)	111	-0.92 to 2.05	0.0050	0.5775
Externalizing mean (Chronicity score)	111	-1.10 to 2.08	-0.0004	0.6611
Highest Internalizing mean (Severity score)	111	-0.82 to 3.58	0.7675	0.8454
Highest Externalizing mean (Severity score)	111	-0.97 to 3.63	0.5564	0.8939

Note: CDI: Child Depression Inventory (Kovacs, 1992), BDI: Beck Depression Inventory (Beck & Steer, 1993), MASC: Multidimensional Anxiety Scale for Children (March, 1998), CBCL: Child Behavioral Checklist (Achenbach, 1991), SRD: Self Report of Delinquency (Elliot et al., 1985), ADS: Alcohol Dependency Scale (Skinner & Horn, 1984), LHD: Lifetime History of Drinking (Skinner, 1982)

Question 2: Do individuals with early history of or high-chronicity of internalizing and externalizing disorders demonstrate more severe patterns of drinking at age 20, and is this relation moderated by amygdala reactivity/connectivity?

2A: Severity/Early History of Disorders Analysis:

To determine the relation between the presence of early internalizing disorders and/or externalizing disorders and age 20 patterns of drinking, four groups were defined: Internalizing Only, Externalizing Only, Combined, and Comparison. The Internalizing Only group comprised individuals who met diagnostic criteria (between ages 8 and 17) for an internalizing disorder (in the absence of externalizing disorders) as determined by the K-SADS. The Externalizing Only group comprised individuals who met criteria for an externalizing disorder (in the absence of internalizing disorders) as determined by the K-SADS. The Combined group comprised individuals who met criteria for at least one of each type of disorder (e.g., MDD and ADHD) at least once between ages 8 and 17 as determined by the K-SADS. The Comparison group was composed of individuals who did not meet criteria for any disorders based on the K-SADS. However, when these groups were calculated, the Internalizing Only group comprised only 8 individuals, which when broken down further to assess potential three-way interactions would yield very small cell sample sizes, and was therefore merged with the Combined Group, as the hypotheses predicted that these two groups would yield the same results. The dependent measure, alcohol consumption at age 20, was determined by drinking averages as defined by the LHD and level of dependence (total score) as measured by the ADS. Similar to Question 1 analyses, moderation was tested in a hierarchical multiple regression analysis, with diagnostic group dummy coded. Moderation was tested using a hierarchical multiple regression analysis with highest maternal education (between ages 8 and 17) entered as covariate. Multiplicative interaction terms were created for Reactivity (Connectivity) X Combined Group Status and Reactivity (Connectivity) X Externalizing Group Status with alcohol use as the outcome variable. These analyses were conducted separately for amygdala reactivity and connectivity.

2B: Chronicity Analysis:

To determine the relation between high-chronicity of both internalizing and externalizing symptomatology and age 20 patterns of drinking, two separate analyses were initially planned: one for the internalizing pathway and one for the externalizing pathway. The following groups were defined for the internalizing analysis: Chronic Internalizing, Non-chronic Internalizing, and Comparison. The following groups were defined for the externalizing analysis: Chronic Externalizing, Non-chronic Externalizing, and Comparison. Within each pathway, a person was considered to have a chronic/persistent disorder (either internalizing or externalizing) if he met one of the following criteria in both childhood (ages 8 to 12) and adolescence (ages 15, 17): 1) He met criteria for either an internalizing or externalizing disorder as indicated by the K-SADS, 2) he had a T score in the borderline clinical or greater range (> 63) on the Total Factor score (internalizing/externalizing) or 3) he was greater than 1 SD above the nonclinical mean for the CDI or MASC. (Note: This criterion was not used for the externalizing component because there are no nonclinical norms for the SRD). These criteria were used to represent those individuals with a persistent disorder across development. Individuals were members of the non-chronic groups if they met these criteria only in childhood (ages 8 to 12) or only in adolescence (ages 15, 17). The dependent measure, alcohol consumption at age 20 was determined by current drinking averages as defined by the LHD and level of dependence as measured by the ADS. Levels of alcohol consumption were to be compared between the Chronic Internalizing, Non-chronic Internalizing, and Comparison groups separately for internalizing and externalizing. Potential moderation was then to be tested in the same manner as described above with amygdala reactivity/connectivity as the moderator. When the data

were analyzed, however, the cell sizes of each of the groups were very small, therefore, internalizing and externalizing group status was collapsed, and the data were investigated as chronic (both internalizing and externalizing) versus non-chronic (both internalizing and externalizing) status.

Prior to addressing the two study questions, the variables were first examined with descriptive statistics and histograms to determine availability of data/missing data, distribution, and skewness. Data that were positively skewed (CDI/BDI, SRD, and alcohol measures) were transformed with the natural log function (ln(n+1)). Data were then transformed into Z scores for use in the analyses. Descriptive data of the untransformed values of the variables are presented for ease of interpretation in Table 2.

6.0 RESULTS

6.1 Sample demographics and preliminary analyses

Overlapping useable behavioral and neuroimaging data were available for 111 subjects. See Table 3 for summary of available data. Of the 111 subjects, 54.5% of the sample self-categorized as Caucasian/White, 38.2% as Black/African-American, 4.5% as biracial, 1.8% as other, 0.9% as Native Hawaiian/Pacific Islander, and one subject's race was left blank. For simplicity of analysis purposes, these groups were combined into three groups: Caucasian/White, Black/African-American, and Other. Socioeconomic status was designated as the highest level of maternal education reported between ages 8 and 20 for each subject (See Table 4). Of the 111 subjects, 43.2% reported mother's highest level of education as high school graduate, 27.9% as partial high school (10^{th} or 11^{th} grade), 19.8% as partial college (at least one year or specialized training), 5.4% as standard college or university graduate, and 3.6% as junior high school (9^{th} grade) (see Table 4). These groups were collapsed into three groups: Less then High School, High School Graduate, and At Least Some College. The relation between these covariates and the study variables was assessed using one-way analyses of variance (ANOVAs) to compare the means between the groups. Unexpectedly, there was a significant difference in both externalizing mean ($F(1, 107) = 5.85$, $p = 0.004$) and externalizing severity ($F(1, 107) = 6.33$, $p = 0.003$) by highest level of maternal education, such that individuals whose mothers were high school graduates were higher on levels of externalizing measures relative to individuals

whose mothers had less than a high school education. The relation between externalizing severity and race was significant at trend ($F(1, 106) = 2.37$, $p = 0.099$), such that individuals who self-identified as Caucasian/White had lower levels of externalizing severity scores relative to those individuals who were either Biracial, Native Hawaiian/Pacific Islander, or Other. Additionally, there was a significant correlation between maternal education and two of the alcohol outcome measures. Maternal education was significantly negatively correlated with "average days per month" using alcohol ($r = -0.27$, $p < 0.05$) and "maximum number of days using maximum quantity of alcohol" ($r = -0.31$, $p < 0.05$). Given the significance of highest level of maternal education, this was entered as a covariate into the regression analyses outlined below, dummy coded as two groups ("less than high school education" and "high school graduate and above.") (See Table 5 for *r* values between covariates and study variables).

Analyses were also conducted both with and without the following covariates: age 20 internalizing symptomatology as measured by the BAI and BDI, and early alcohol use as measured by "age at first intoxication" and "age of first significant alcohol use" determined by the LHD. In order to assess "intoxication," the subject was asked about the effects of alcohol experienced, such as slurred speech and blurred vision. "Significant use" was defined as approximately 10 times in a 12-month period. BAI ($r = 0.21$, $p < 0.05$) and BDI ($r = 0.20$, $p < 0.05$) scores were significantly positively correlated with ADS; and age at first intoxication ($r = -0.36$, $p < 0.001$) and age of first significant alcohol use ($r = -0.22$, $p < 0.05$) were significantly negatively correlated with ADS. Additionally, BAI and BDI were significantly positively correlated with both internalizing chronicity (BAI: $r = 0.25$, $p < 0.01$; BDI: $r = 0.43$, $p < 0.001$) and severity (BAI: $r =$

0.29, $p < 0.01$; BDI: $r = 0.45$, $p < 0.001$). Age at first alcohol intoxication was significantly negatively correlated with externalizing severity ($r = -0.23$, $p < 0.05$) (See Table 5 for statistics).

Table 3. Summary of available data for analyses

	Number lost	Participants with data
Original sample		**311**
Sample with behavioral data at age 20		**249**
- Parent requested drop out	11	
- Target youth requested drop out	3	
- Incarcerated	10	
- In the military	5	
- Deceased	1	
- Unable to locate	11	
- Hard to contact	5	
- Probable drop outs	6	
- On the schedule but not yet visited	1	
- Data collected but not yet available	7	
- Data collection error/permanently missing	2	
Total lost	62	
Sample with imaging data at age 20		**182**
- Concussion/head injury	24	
- Bullets/metal fragments	15	
- Braces	2	
- Phone interviews (out of the area)	5	
- MRI portion refused	7	
- Living at home/treatment facility (too ill to participate – schizophrenia, autism, car accident)	4	
- Claustrophobic	6	
- Left before scanning portion or wanted to stop scan	2	
- Did not physically fit in the bore	1	
- Reported being currently on drugs/rescheduled	1	
Total Lost	67	
Sample with usable imaging data at age 20		**160**
- Incidental findings on sMRI	2	
- Poor amygdala coverage (< 90%) or visual overlap	15	
- Poor performance on task (< 75%)	1	
- No amygdala reactivity or processing errors	1	
- Excessive movement/outliers	1	
- Psychosis	1	
- Appeared to be on drugs and not responding to task	1	

Total Lost	22	
Sample with usable behavioral data ages 8-17		**111**
- Excluded for diagnosis (e.g., PTSD, OCD, Specific Phobia)	25	
- Missed time points—could not determine diagnosis age 8-17	14	
- Excluded for diagnosis and missed time points	10	
Total Lost	49	

Table 4. Demographic data

		N	**Percent of Total**
Race			
	Caucasian/White	60	54.5
	Black/African-American	42	38.2
	Biracial	5	4.5
	Native Hawaiian/Pacific Islander	1	0.9
	Other	2	1.8
	Missing	1	0.9
Total		111	100
Highest level of maternal education			
	Junior high school (9^{th} grade)	4	3.6
	Partial high school (10^{th} or 11^{th} grade)	31	27.9
	High school graduate	48	43.2
	Partial college (at least one year or specialized training)	22	19.8
	Standard college or university graduate	6	5.4
Total		111	100

Table 5. Correlations of selected study variables with covariates

Variable	Maternal Education	BAI Age 20	BDI Age 20	Age at first intoxication	Age of first significant alcohol use
Maternal education (1 = < high school, 2 = high school or above)				-0.094	-0.023
BAI age 20	-0.52			-0.076	-0.057
BDI age 20	0.052	0.52***		-0.15	-0.13
ADS	-0.021	0.212*	0.20*	-0.36***	-0.22*
Alcohol avg days per month	-0.27*	-0.018	-0.17	-0.13	0.055
Alcohol avg quantity	-0.22	-0.032	-0.20	-0.22	0.14
Alcohol max quantity	-0.26	0.042	-0.24	-0.25	0.12
Alcohol max day max quantity	-0.31*	-0.059	-0.17	-0.23	0.056
Int chronicity	0.054	0.25**	0.43***	0.013	-0.045
Ext chronicity	0.24**	-0.38	0.094	-0.18	-0.13
Int severity	0.11	0.29**	0.45***	-0.008	0.001
Ext severity	0.25**	-0.32	0.065	-0.23*	-0.16
Amygdala reactivity	-0.18	0.079	0.11	0.12	0.007
Amygdala-BA 24 connectivity	0.046	0.14	0.070	0.15	0.113
Amygdala-BA 32 connectivity	-0.038	0.001	-0.066	-0.022	-0.008

* $p < .05$, ** $p < .01$, *** $p < .001$

6.2 Main effects of the neuroimaging task

Consistent with previous findings, BOLD fMRI revealed robust amygdala, hippocampal, fusiform, and PFC reactivity in response to the perceptual processing of novel faces relative to control blocks of shapes when applying a statistical threshold of $p < 0.05$ FWE (Family-Wise Error) corrected for multiple comparisons (Figure 5A). To examine amygdala reactivity, each individual subject's value was extracted from SPM for the contrast of all faces > shapes using an ROI generated from the WFU PickAtlas (using the procedure outlined above in the method section) for the left and right amygdala. Because amygdala reactivity was highly correlated between the left and right amygdala ($r = 0.76$, $p < 0.01$), these values were averaged together and used as a composite amygdala reactivity variable for all reactivity analyses. The size of each cluster and the coordinates of the peak voxel within each cluster are presented in Table 6.

To examine functional connectivity effects, a PPI analysis was conducted to generate a connectivity map of brain regions correlated with a combined left and right amygdala seed (combined because of the high correlation). Each individual subject's values were extracted for each of the following ROIs within the OFC/ACC: BA 11, 24, 25, 32, and 47 using the WFU PickAtlas for both positive and negative connectivity with a statistical threshold of $p < 0.05$ FWE. There were no clusters that survived FWE correction. However, when the threshold was lowered to $p < 0.05$ at false discovery rate (FDR) correction, two regions of the ACC (BA 32 and 24) demonstrated significant negative connectivity with the amygdala seed (Figure 5B). It should be noted that negative connectivity here represents a reciprocal relation between the amygdala and ACC, with one region's activation related to the other region's deactivation (negative connectivity). Although this statistical threshold is lower than was initially planned,

FDR correction is thought to be an appropriate and principled approach allowing for good ability to detect meaningful signal while only slightly increasing the probability of false positives (Bennett, Wolford, & Miller, 2009). Connectivity values from these two structures were extracted and used in the analyses. The size of each cluster and coordinates of the peak voxel within each cluster are presented in Table 6.

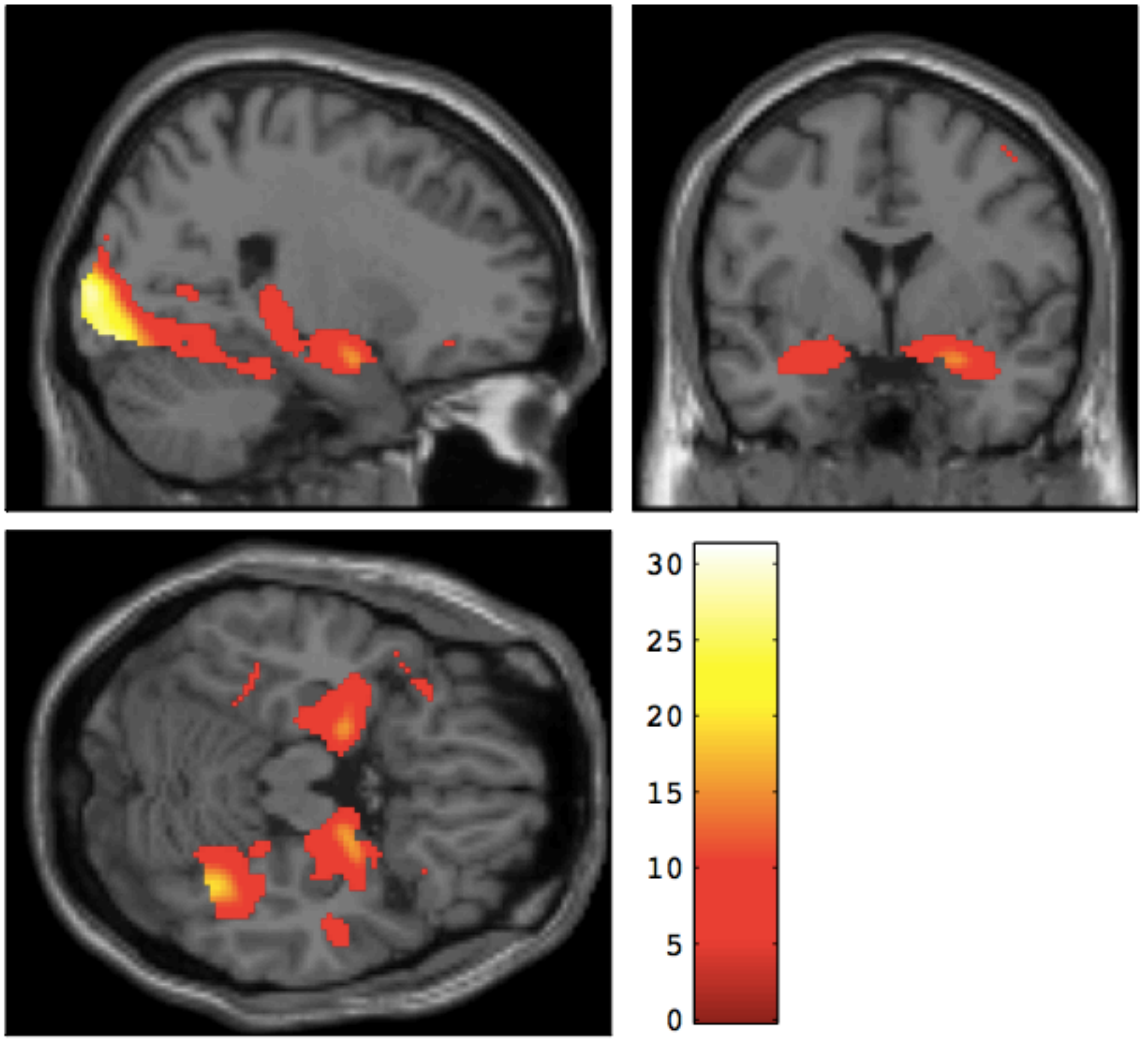

Figure 5A. Main effect of task
Statistical parametric map of brain activation during the perceptual processing of fearful and threatening faces across all 160 subjects with useable imaging data. Activations are shown overlaid onto an averaged structural magnetic resonance image. Color bar represents t scores for activations.

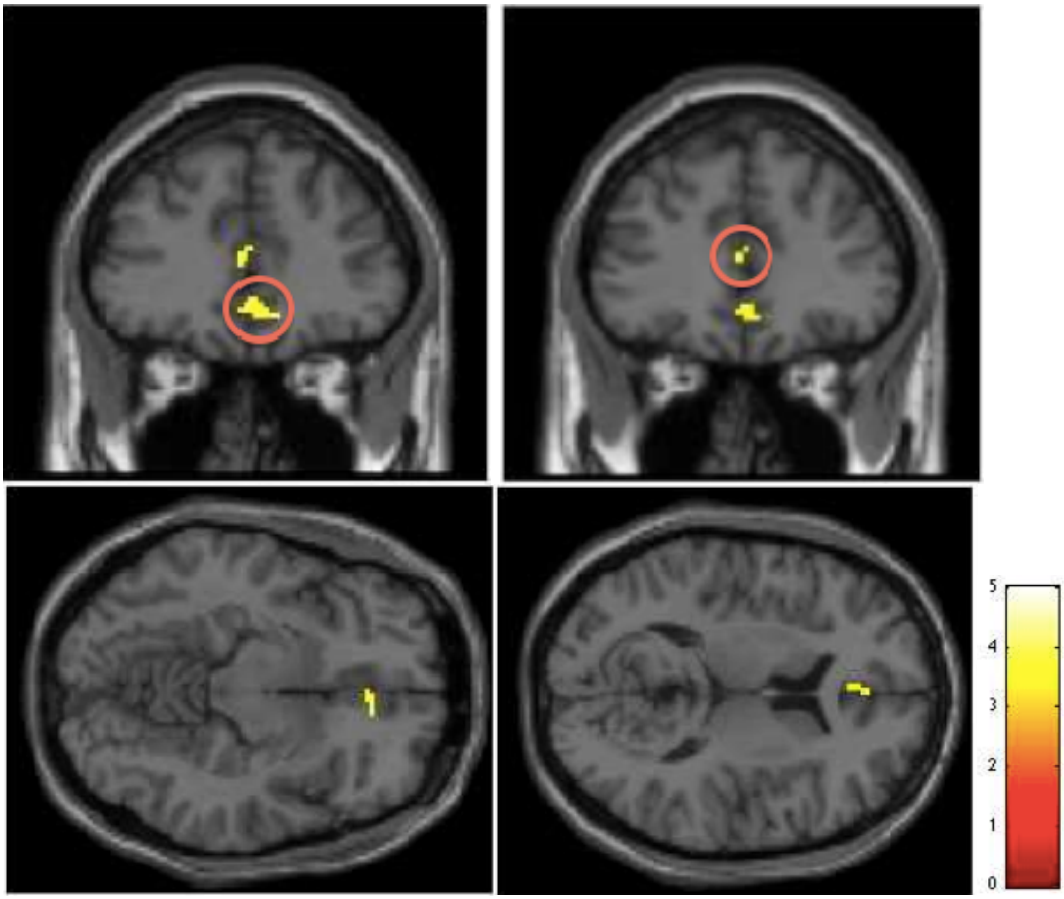

Figure 5B. *Connectivity map of BA 32 (left figure) and BA 24 (right figure) negatively correlated with the composite amygdala seed*
Activations are shown overlaid onto an averaged structural magnetic resonance image. Color bar represents t scores for activations.

Table 6. Amygdala reactivity and connectivity clusters included in the analyses

	Coordinates of peak voxel (Talairach)	Number of Voxels	T	Significance (*p* = FWE)	Significance (*p* = FDR)
Right Amygdala	22 -3 -15	367	3.45	< 0.001	< 0.001
Left Amygdala	-20 -5 -15	394	3.45	< 0.001	< 0.001
Negative Connectivity BA 32	8 36 -10	40	3.71	0.186	0.005
Negative Connectivity BA 24	-2 35 9	37	3.43	0.384	0.009

Note: FWE: Family-wise error, FDR: False discovery rate

6.3 Correlational analyses

Prior to testing the question hypotheses, correlations were conducted between the variables to be used in the analyses. Table 7 presents correlations for the main study variables for Question 1. Not surprisingly, the 5 alcohol outcome measures were significantly correlated, as were chronicity and severity scores. Interestingly, externalizing chronicity (r = 0.28, p < 0.01) and severity (r = 0.23, p < 0.05) were correlated with ADS; whereas internalizing chronicity was negatively correlated with three of the LHD alcohol outcomes, significant at trend (r =-0.25 p < 0.10). Amygdala reactivity was significantly positively correlated with internalizing chronicity and severity (r = 0.19, p < 0.05). Additionally, negative amygdala-BA 32 connectivity was positively correlated with both average quantity and maximum quantity of alcohol consumed (r = 0.28, p < 0.05). Finally, both connectivity scores were significantly positively correlated (r = 0.42, p < 0.001). To reduce the probability of Type I errors across these analyses presented below, only data that were significant at p < 0.05 were interpreted as significant.

Table 7. Correlations of selected study variables

Variable	1	2	3	4	5	6	7	8	9	10	11
1. Alcohol Dependence Scale total score											
2. Alcohol avg days per month	0.578***										
3. Alcohol avg quantity	0.741***	0.093***									
4. Alcohol max quantity	0.72***	0.95***	0.997***								
5. Alcohol max day max quantity	0.73***	0.91***	0.93***	0.93***							
6. Int chronicity	0.10	-0.20	-0.25 +	-0.26 +	-0.25 +						
7. Int severity	0.13	-0.16	-0.18	-0.20	-0.21	0.88***					
8. Ext chronicity	0.28**	0.12	0.18	0.12	0.18	0.12	0.13				
9. Ext severity	0.23*	0.044	0.13	0.078	0.10	0.074	0.11	0.922***			
10. Amygdala reactivity	-0.083	0.013	-0.045	-0.079	-0.038	0.19*	0.19*	-0.065	-0.14		
11. Negative Amygdala-BA 24 connectivity	-0.025	-0.037	-0.029	-0.067	0.014	0.14	0.070	-0.066	-0.13	0.11	
12. Negative Amygdala-BA 32 connectivity	0.019	0.221	0.283*	0.289*	0.238	-0.080	-0.159	0.016	-0.016	0.009	0.42***

+ p < .10, * p < .05, ** p < .01, *** p < .001

Table 8. Summary table for significant overall results for Question 1

Variable	Chronicity Amygdala	Severity Amygdala	Chronicity Amyg-BA 32	Severity Amyg-BA 32	Chronicity Amyg- BA 24	Severity Amyg-BA 24
ADS	Externalizing		Externalizing		Externalizing Amyg-BA 24 X Int X Ext	Amyg-BA 24 X Int X Ext
ADS with age 20 BAI/BDI	Externalizing	Externalizing	Externalizing		Externalizing Amyg-BA 24 X Int X Ext	Int X Ext Amyg-BA 24 X Int X Ext
ADS with early alcohol	1st intoxication Internalizing	1st intoxication	1st intoxication Internalizing	1st intoxication Int X Ext	1st intoxication Internalizing	1st intoxication Int X Ext
Avg quantity			Int Ext Int X Ext Amyg-BA 32 X Int X Ext			
Avg quantity with age 20 BAI/BDI			Ext Int X Ext Amyg-BA 32 X Int X Ext			
Avg days	Maternal Ed	Maternal Ed	Maternal Ed	Maternal Ed	Maternal Ed	Maternal Ed
Maximum quantity with age 20 BAI/BDI			Externalizing Int X Ext			
Maximum quantity maximum days	Maternal Ed Externalizing	Maternal Ed	Externalizing	Maternal Ed	Amyg-BA 24 X Int X Ext	Maternal Ed
Maximum quantity maximum days with age 20 BAI/BDI					Amyg-BA 24 X Int X Ext	

Note: Analyses with the following outcomes were not significant and are therefore not reported here: Average days per month consuming alcohol with age 20 covariates and maximum quantity of alcohol consumed. ADS: Alcohol Dependence Scale, Maternal Ed: Level of maternal education, Int: Internalizing, Ext: Externalizing, 1st intoxication: Age at first intoxication

6.4 Question 1 Analyses (See Table 8 for overall significant findings)

6.4.1 Chronicity —Amygdala reactivity (Table 9)

Z scores of the variables were entered into a hierarchical multiple regression analysis with highest level of maternal education (covariate) entered first, followed by internalizing and externalizing chronicity scores and amygdala composite scores (main effects model), and finally the interaction terms outlined above (full model). Five regression analyses were conducted, varying the alcohol outcome: one with ADS total score and four with the LHD measure. These data were analyzed with and without the age 20 internalizing covariates (BAI and BDI). Additionally, for the analysis using ADS as the outcome measure, the regression was conducted with and without early alcohol use measures as the covariates (age at first intoxication, age at first significant use). Due to the unavailability of some of the data and the resulting small sample cell sizes, these early alcohol use covariates were not used with the LHD outcome measures.

Overall, there were no significant effects of the interaction terms (full model); therefore, only data from the main effects model are presented (See Table 9 for chronicity analyses statistics). Additionally, within the main effects model, there were no significant effects of amygdala reactivity. Within the ADS analysis (R^2_{adj} = 0.064, $F(3, 103) = 2.77$, p = 0.031), only externalizing chronicity scores significantly predicted alcohol dependence at age 20 (B = 0.43, SE = 0.15, t = 2.90, p = 0.005, $r^2_{partial}$ = 0.28), such that the higher the mean externalizing score between ages 10 and 17, the higher the level of alcohol dependence at age 20. With the age 20

internalizing covariates entered into the model (R^2_{adj} = 0.096, F(5, 103) = 2.84, p = 0.014), externalizing chronicity score remained significant (B = 0.43, SE = 0.15, t = 2.96, p = 0.004, $r^2_{partial}$ = 0.29). When the measures of early alcohol use were entered as covariates (R^2_{adj} = 0.22, F(5, 80) = 4.82, p < 0.001), age at first intoxication (B = -0.31, SE = 0.10, t = -3.13, p = 0.002, $r^2_{partial}$ = -0.34) and internalizing chronicity (B = 0.54, SE = 0.18, t = 3.02, p = 0.003, $r^2_{partial}$ = 0.33) significantly predicted alcohol dependence at age 20, such that younger ages of first intoxication and greater mean internalizing scores were associated with higher ADS scores.

With "average days per month using alcohol" as the outcome measure, the main effects model was not significant. Only the covariate, maternal level of education, was significantly associated with average days per month using alcohol (R^2_{adj} = 0.054, F(1, 51) = 3.97, p = 0.052), such that individuals whose mother's highest level of education was less than high school reported higher average days per month using alcohol (B = -0.55, SE = 0.28, t = -1.99, p = 0.052, $r^2_{partial}$ = -0.27). This relation was no longer significant when the age 20 internalizing measures were included in the analysis (R^2_{adj} = 0.025, F(2, 51) = 1.44, p = 0.24).

With "maximum number of days using maximum quantity of alcohol" entered as the outcome, the main effects model was significant (R^2_{adj} = 0.15, F(3, 50) = 3.16, p = 0.022). Within this model, maternal level of education was significantly associated with the alcohol outcome measure, (B = -.66, SE = 0.28, t = -2.34, p = 0.02, $r^2_{partial}$ = -0.32), such that individuals whose mother's highest level of education was less than high school reported a greater maximum number of days using maximum quantity of alcohol. Additionally, externalizing chronicity scores were significantly associated with maximum days using maximum amount of alcohol at age 20 (B = 0.44, SE = 0.21, t = 2.09, p = 0.04, $r^2_{partial}$ = 0.29), such that the higher the mean externalizing

score between ages 10 and 17, the higher the maximum days using maximum amount of alcohol was reported. There were no significant regressions when the age 20 internalizing measures were included in the models using the LHD outcomes. The analyses with "average quantity of alcohol used" and "maximum quantity of alcohol used" were not significant.

Table 9. Regression results for chronicity and amygdala reactivity

Variable	B	SE B	*B*	*t*	*p*
ADS					
R^2_{adj} = 0.064, *F*(3, 103) = 2.77, *p* = 0.031					
Maternal Education	-0.230	0.212	-0.107	-1.084	0.281
Internalizing (Int)	0.152	0.166	0.090	0.916	0.362
Externalizing (Ext)	0.429	0.148	0.284	2.898	0.005 **
Amygdala Reactivity	-0.103	0.097	-0.104	-1.060	0.292
ADS with age 20 covariates					
R^2_{adj} = 0.096, *F*(5, 103) = 2.84, *p* = 0.014					
Maternal Education	-0.192	0.209	-0.089	-0.917	0.361
BAI Age 20	0.164	0.109	0.164	1.504	0.136
BDI Age 20	0.110	0.118	0.109	0.935	0.352
Internalizing (Int)	0.004	0.179	0.002	0.021	0.983
Externalizing (Ext)	0.433	0.146	0.287	2.964	0.004 **
Amygdala Reactivity	-0.111	0.096	-0.112	-1.160	0.249
ADS with early alcohol covariates					
R^2_{adj} = 0.221, *F*(5, 80) = 4.82, *p* < 0.001					
Maternal Education	-0.155	0.216	-0.074	-0.718	0.475
Age of first intoxication	-0.313	0.100	-0.587	-3.130	0.002 **
Age of first significant alcohol use	0.207	0.120	0.321	1.727	0.088
Internalizing (Int)	0.536	0.177	0.306	3.021	0.003 **
Externalizing (Ext)	0.211	0.147	0.150	1.433	0.156
Amygdala Reactivity	-0.020	0.100	-0.021	-0.205	0.838
Average days per month drinking					
R^2_{adj} = 0.054, *F*(1, 51) = 3.97, *p* = 0.052					
Maternal Education	-0.549	0.276	-0.269	-1.991	0.052 *
Average days per month drinking with age 20 covariates (regressions not significant)					

Maximum quantity maximum days R^2_{adj} = 0.145, F(3, 50) = 3.16, p = 0.022					
Maternal Education	-0.657	0.280	-0.323	-2.344	0.023 *
Internalizing (Int)	-0.394	0.229	-0.238	-1.720	0.092
Externalizing (Ext)	0.442	0.212	0.280	2.087	0.042 *
Amygdala Reactivity	-0.096	0.133	-0.101	-0.720	0.475
Maximum quantity maximum days with age 20 covariates (regressions not significant)					

* $p < .05$, ** $p < .01$, *** $p < .001$

6.4.2 Severity — Amygdala reactivity (Table 10)

Similar to the chronicity analyses, Z scores of the variables were entered into a hierarchical multiple regression analysis with highest level of maternal education (covariate) entered first, followed by internalizing and externalizing severity scores and amygdala composite scores (main effects model), and finally the interaction terms outlined above (full model), with ADS or LHD question as the outcome variable. Again, five regression analyses were conducted, varying the alcohol outcome: one with ADS total score and four with the LHD measure. Similar to the chronicity analyses, these data were analyzed with and without the age 20 internalizing covariates (BAI and BDI), and for the ADS regression, with and without early alcohol use measures as the covariates (age at first intoxication, age at first significant use).

Similarly to the chronicity analyses, there were no significant effects of either amygdala reactivity or any of the interaction terms. For the ADS analysis, there were no significant regression results. The main effects model became significant when the age 20 internalizing measures were entered as covariates (R^2_{adj} = 0.065, F(5, 103) = 2.21, p = 0.049). Within this model, externalizing severity score was positively associated with ADS at age 20 (B = 0.26, SE = 0.12, t = 2.23, p = 0.028, $r^2_{partial}$ = 0.22). When the measures of early alcohol use were entered as covariates, the full model was significant (R^2_{adj} = 0.23, F(9, 80) = 3.35 p < 0.001).

Within this model, age at first intoxication (B = -0.29, SE = 0.10, t = -2.82, p = 0.006, $r^2_{partial}$ = -0.32) significantly predicted alcohol dependence at age 20, such that younger ages of first intoxication were associated with higher ADS scores.

For the analyses using the LHD alcohol outcome measures, the main effects models were not significant. Only the covariate, highest level of maternal education, was associated with drinking levels at age 20. With "average days per month using alcohol" as the outcome (R^2_{adj} = 0.054, F(1, 51) = 3.97, p = 0.052), individuals whose mother's highest level of education was less than high school reported a higher average days per month using alcohol (B = -.55, SE = 0.28, t = -2.00, p = 0.052, $r^2_{partial}$ = -0.27). Similarly, with "maximum days using maximum amount of alcohol" as the outcome variable (R^2_{adj} = 0.075, F(1, 50) = 5.13, p = 0.028), individuals whose mother's highest level of education was less than high school reported a higher number of maximum number of days using maximum amount of alcohol (B = -.62, SE = 0.28, t = -2.26, p = 0.028, $r^2_{partial}$ = -0.31). The analyses with "average quantity of alcohol used" and "maximum quantity of alcohol used" were not significant. Additionally, LHD analyses with the age 20 internalizing covariates were not significant.

Table 10. Regression results for severity and amygdala reactivity

Variable	B	SE B	*B*	t	*p*
ADS (regressions not significant)					
ADS with age 20 covariates R^2_{adj} = 0.065, F(5, 103) = 2.21, p = 0.049					
Maternal Education	-0.175	0.214	-0.081	-0.816	0.416
BAI Age 20	0.140	0.105	0.147	1.330	0.186
BDI Age 20	0.107	0.120	0.106	0.896	0.372

Internalizing (Int)	0.061	0.150	0.044	0.406	0.685
Externalizing (Ext)	0.262	0.118	0.220	2.225	0.028 *
Amygdala Reactivity	-0.099	0.099	-0.100	-1.006	0.317
ADS with early alcohol covariates					
R^2_{adj} = 0.23, $F(9, 80) = 3.35$ $p < 0.001$					
Maternal Education	-0.172	0.217	-0.082	-0.793	0.431
Age of first intoxication	-0.287	0.102	-0.539	-2.818	0.006 **
Age of first significant alcohol use	0.148	0.124	0.230	1.202	0.233
Internalizing (Int)	0.235	0.163	0.169	1.437	0.155
Externalizing (Ext)	-0.044	0.171	-0.040	-0.256	0.799
Amygdala Reactivity	-0.025	0.144	-0.026	-0.175	0.862
Int X Ext	0.260	0.145	0.290	1.800	0.076
Amygdala X Int	-0.013	0.144	-0.013	-0.088	0.930
Amygdala X Ext	0.126	0.178	0.144	0.710	0.480
Amygdala X Int X Ext	-0.163	0.164	-0.199	-0.999	0.321
Average days per month drinking					
R^2_{adj} = 0.054, $F(1, 51) = 3.97$, $p = 0.052$					
Maternal Education	-0.549	0.276	-0.269	-1.991	0.052 *
Average days per month drinking with age 20 covariates (regressions not significant)					
Maximum quantity maximum days					
R^2_{adj} = 0.075, $F(1, 50) = 5.13$, $p = 0.028$					
Maternal Education	-0.621	0.274	-0.305	-2.264	0.028 *
Maximum quantity maximum days with age 20 covariates (regressions not significant)					

* $p < .05$, ** $p < .01$, *** $p < .001$

6.4.3 Chronicity — Amygdala-BA 32 connectivity (Table 11)

Connectivity analyses were specified using the same method outlined above for the reactivity analyses (See Table 11 for chronicity analyses statistics). For the ADS analysis, only the main effects model was significant (R^2_{adj} = 0.054, F(3, 103) = 2.48, p = 0.049) but connectivity scores were not significant. Within this model, only externalizing chronicity score significantly predicted alcohol dependence at age 20 (B = 0.43, SE = 0.15, t = 2.92, p = 0.004, $r^2_{partial}$ = 0.28), such that the higher the highest externalizing score between ages 10 and 17, the higher the level of alcohol dependence at age 20. With the age 20 internalizing covariates included, the full model became significant (R^2_{adj} = 0.10, F(9, 103) = 2.20, p = 0.024), but there were no significant effects of the interaction terms or connectivity scores. Externalizing chronicity score remained significant (B = 0.33, SE = 0.15, t = 2.16, p = 0.033, $r^2_{partial}$ = 0.22). When the measures of early alcohol use were entered as covariates, again the full model was significant (R^2_{adj} = 0.25, F(9, 80) = 3.68, p = 0.001) with no significant effects of the interaction terms or connectivity scores. Within this model, age at first intoxication (B = -0.25, SE = 0.10, t = -2.51, p = 0.015, $r^2_{partial}$ = -0.29) and internalizing chronicity scores (B = 0.65, SE = 0.19, t = 3.41, p = 0.001, $r^2_{partial}$ = 0.38) significantly predicted alcohol dependence at age 20, such that younger ages of first intoxication and higher internalizing chronicity scores were associated with higher ADS scores.

Table 11. Regression Results for Chronicity and Amygdala-BA 32 Connectivity

Variable	B	SE B	*B*	t	*p*
ADS					
R^2_{adj} = 0.054, F(3, 103) = 2.475, p = 0.049					

Maternal Education	-.0187	0.210	-0.087	-0.892	0.375
Internalizing (Int)	0.119	0.164	0.071	0.730	0.467
Externalizing (Ext)	0.435	0.149	0.288	2.923	0.004 **
Amygdala-BA 32 Connectivity	0.024	0.095	0.024	0.249	0.804
ADS with age 20 covariates					
R^2_{adj} = 0.100, F(9, 103) = 2.20, p = 0.024					
Maternal Education	-0.168	0.206	-0.078	-0.813	0.419
BAI Age 20	0.161	0.111	0.160	1.445	0.152
BDI Age 20	0.130	0.120	0.128	1.088	0.279
Internalizing (Int)	-0.039	0.183	-0.023	-0.214	0.831
Externalizing (Ext)	0.331	0.153	0.219	2.162	0.033 *
Amygdala-BA 32 Connectivity	0.017	0.098	0.017	0.170	0.865
Int X Ext	0.285	0.209	0.134	1.362	0.176
Amygdala-BA 32 X Int	0.271	0.182	0.149	1.486	0.141
Amygdala-BA 32 X Ext	-0.111	0.152	-0.078	-0.728	0.468
Amygdala-BA 32 X Int X Ext	0.169	0.241	0.072	0.703	0.483
ADS with early alcohol covariates					
R^2_{adj} = 0.249, F(9, 80) = 3.68, p = 0.001					
Maternal Education	-0.081	0.213	-0.039	-0.382	0.704
Age of first intoxication	-0.253	0.101	-0.475	-2.505	0.015 *
Age of first significant alcohol use	0.109	0.124	0.169	0.884	0.380
Internalizing (Int)	0.647	0.190	0.370	3.412	0.001 ***
Externalizing (Ext)	0.141	0.162	0.100	0.870	0.387
Amygdala-BA 32 Connectivity	-0.152	0.120	-0.142	-1.269	0.208
Int X Ext	0.366	0.253	0.188	1.448	0.152
Amygdala-BA 32 X Int	-0.214	0.238	-0.106	-0.896	0.373
Amygdala-BA 32 X Ext	-0.254	0.229	-0.172	-1.109	0.271
Amygdala-BA 32 X Int X Ext	0.121	0.376	0.051	0.323	0.748
Average quantity					
R^2_{adj} = 0.213, F(7, 49) = 2.69, p = 0.017					
Maternal Education	-0.322	0.265	-0.159	-1.214	0.231
Internalizing (Int)	-0.501	0.253	-0.303	-1.984	0.054 *
Externalizing (Ext)	0.634	0.251	0.405	2.525	0.015 *
Amygdala-BA 32 Connectivity	0.171	0.162	0.182	1.055	0.298
Int X Ext (Figure 3)	0.830	0.412	0.321	2.017	0.050 *
Amygdala-BA 32 X Int	0.402	0.275	0.222	1.464	0.151
Amygdala-BA 32 X Ext	0.198	0.283	0.126	0.699	0.488
Amygdala-BA 32 X Int X Ext (Figure 4)	0.974	0.458	0.370	2.126	0.039 *

Average quantity with age 20 covariates
R^2_{adj} = 0.244, $F(9, 49) = 2.61$, $p = 0.015$

Maternal Education	-0.153	0.275	-0.076	-0.557	0.581
BAI Age 20	0.199	0.156	0.196	1.277	0.209
BDI Age 20	-0.328	0.175	-0.355	-1.875	0.068
Internalizing (Int)	-0.356	0.280	-0.216	-1.273	0.210
Externalizing (Ext)	0.812	0.263	0.520	3.083	0.004 **
Amygdala-BA 32 Connectivity	0.156	0.161	0.166	0.971	0.338
Int X Ext	0.934	0.418	0.361	2.238	0.031 *
Amygdala-BA 32 X Int	0.376	0.271	0.208	1.387	0.173
Amygdala-BA 32 X Ext	0.252	0.279	0.161	0.903	0.372
Amygdala-BA 32 X Int X Ext	1.019	0.452	0.387	2.254	0.030 *

Average days per month drinking
R^2_{adj} = 0.054, $F(1, 51) = 3.97$, $p = 0.052$

Maternal Education	-0.549	0.276	-0.269	-1.991	0.052 *

Average days per month drinking with age 20 covariates
(regressions not significant)

Maximum quantity
R^2_{adj} = 0.18, $F(7, 48) = 3.34$, $p = 0.036$

Maternal Education	-0.378	0.272	-0.187	-1.387	0.173
Internalizing (Int)	-0.466	0.261	-0.285	-1.781	0.082
Externalizing (Ext)	0.514	0.276	0.317	1.863	0.070
Amygdala-BA 32 Connectivity	0.186	0.169	0.199	1.095	0.280
Int X Ext	0.757	0.421	0.295	1.801	0.079
Amygdala-BA 32 X Int	0.402	0.281	0.224	1.429	0.161
Amygdala-BA 32 X Ext	0.211	0.293	0.135	0.720	0.475
Amygdala-BA 32 X Int X Ext	0.864	0.476	0.331	1.817	0.077

Maximum quantity with age 20 covariates
R^2_{adj} = 0.122, $F(9, 48) = 2.37$, $p = 0.027$

Maternal Education	-0.203	0.281	-0.100	-0.720	0.476
BAI Age 20	0.219	0.160	0.217	1.373	0.178
BDI Age 20	-0.345	0.178	-0.376	-1.939	0.060
Internalizing (Int)	-0.316	0.286	-0.193	-1.103	0.277
Externalizing (Ext)	0.694	0.286	0.428	2.432	0.020 *
Amygdala-BA 32 Connectivity	0.171	0.167	0.183	1.024	0.312
Int X Ext (Figure 5)	0.860	0.425	0.335	2.024	0.050 *
Amygdala-BA 32 X Int	0.375	0.276	0.209	1.357	0.183
Amygdala-BA 32 X Ext	0.271	0.288	0.174	0.942	0.352
Amygdala-BA 32 X Int X Ext	0.902	0.469	0.346	1.926	0.061

Maximum quantity maximum days R^2_{adj} = 0.17, F(7, 50) = 2.30, p = 0.038					
Maternal Education	-0.526	0.271	-0.258	-1.937	0.059
Internalizing (Int)	-0.445	0.250	-0.269	-1.778	0.083
Externalizing (Ext)	0.544	0.257	0.345	2.118	0.040 *
Amygdala-BA 32 Connectivity	0.156	0.160	0.166	0.973	0.336
Int X Ext	0.684	0.421	0.262	1.624	0.112
Amygdala-BA 32 X Int	0.283	0.272	0.158	1.040	0.304
Amygdala-BA 32 X Ext	0.239	0.288	0.151	.829	0.412
Amygdala-BA 32 X Int X Ext	0.607	0.468	0.228	1.296	0.202
Maximum quantity maximum days with age 20 covariates (regressions not significant)					

* $p < .05$, ** $p < .01$, *** $p < .001$

With "average quantity of alcohol used" as the outcome measure, the full model was significant (R^2_{adj} = 0.21, F(7, 49) = 2.69, p = 0.017). Within this model, internalizing chronicity score (B = -0.50, SE = 0.25, t = -1.98, p = 0.054, $r^2_{partial}$ = -0.29) and externalizing chronicity score (B = 0.63, SE = 0.25, t = 2.53, p = 0.015, $r^2_{partial}$ = 0.36) predicted average quantity of alcohol used at age 20, such that higher externalizing and lower internalizing chronicity scores were associated with higher quantities of alcohol use at age 20. Additionally, there was a significant interaction between internalizing and externalizing chronicity scores (B = 0.83, SE = 0.41, t = 2.02, p = 0.05, $r^2_{partial}$ = 0.30), such that those individuals who were high on both internalizing and externalizing chronicity reported consuming the highest average quantities of alcohol and those high on internalizing and low on externalizing consuming the least amount (Figure 6).

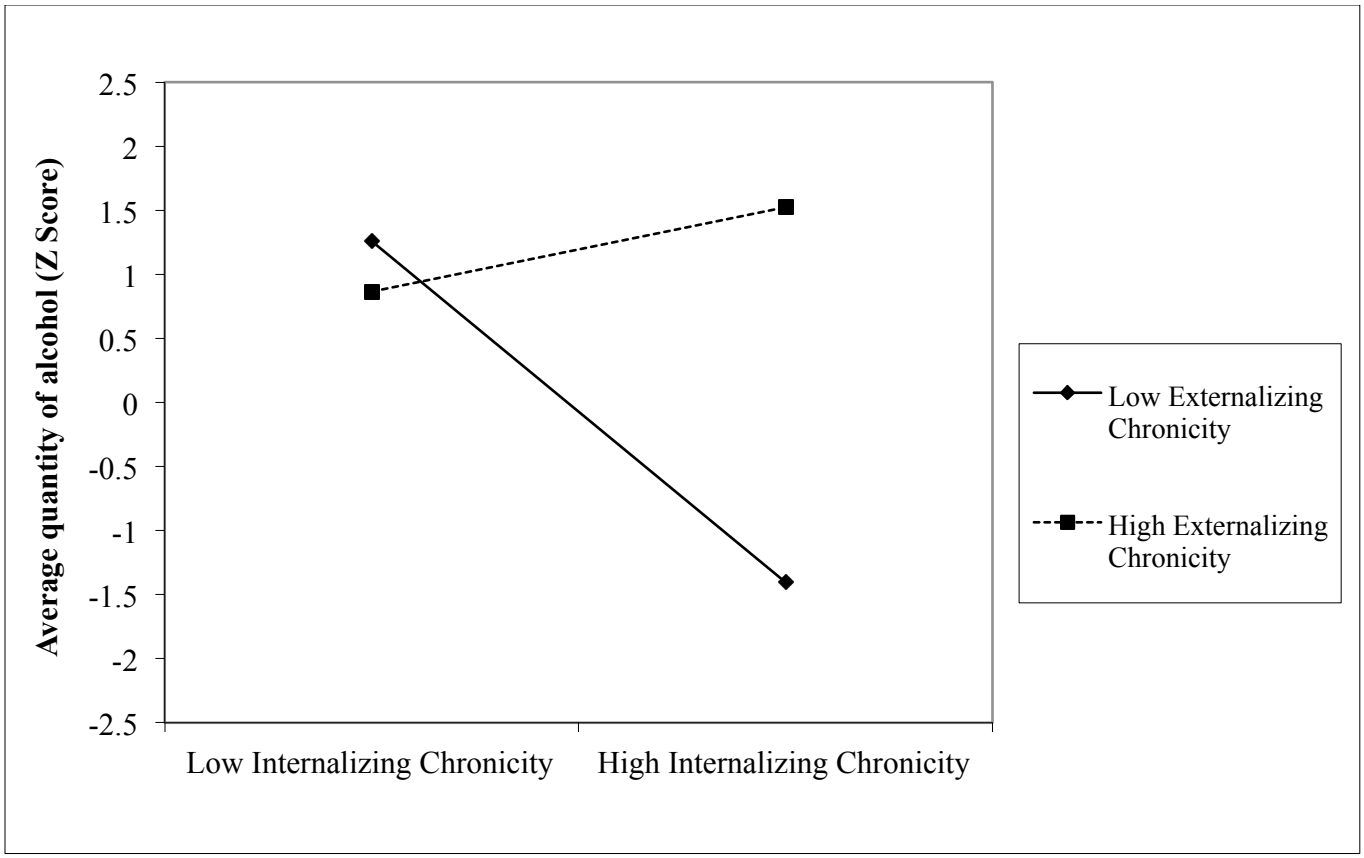

Figure 6. Interaction between internalizing and externalizing chronicity scores predicts to average drinking quantity of alcohol at age 20 (chronicity and amygdala-BA 32 connectivity analysis)

Additionally, the three-way interaction (Figure 7) between connectivity, internalizing, and externalizing chronicity scores was significant (B = 0.97, SE = 0.46, $t = 2.13$, $p = 0.039$, $r^2_{partial} = 0.31$). Analyses of the simple slopes revealed that there was a significant difference between those individuals high on both internalizing and externalizing chronicity (Line 1, Figure 7) and those high on internalizing and low on externalizing (Line 2, Figure 7), with those individuals high on both chronicity measures and high on negative amygdala-BA 32 connectivity reporting the highest average quantity of alcohol consumed and those individuals who were high internalizing/low externalizing and high negative amygdala-BA 32 connectivity reporting the lowest ($p = 0.03$). Additionally, there was a significant difference ($p = 0.033$) between those

individuals high on both internalizing and externalizing (Line 1, Figure 7) and those individuals low on internalizing and high on externalizing (Line 3, Figure 7), with those individuals high on both internalizing/externalizing chronicity demonstrating a positive correlation between negative amygdala-BA 32 connectivity and average quantity of alcohol consumed versus those individuals in the low internalizing/high externalizing group who demonstrated a negative relation between connectivity and average quantity of alcohol consumed. With the age 20 internalizing covariates included in the model (R^2_{adj} = 0.24, F(9, 49) = 2.61, p = 0.015), the effect of externalizing chronicity score remained significant (B = 0.81, SE = 0.26, t = 3.08, p = 0.004, $r^2_{partial}$ = 0.44) as did the two-way internalizing and externalizing interaction (B = 0.93, SE = 0.42, t = 2.24, p = 0.031, $r^2_{partial}$ = 0.30) and the three-way interaction (B = 1.02, SE = 0.45, t = 2.25, p = 0.030, $r^2_{partial}$ = 0.34).

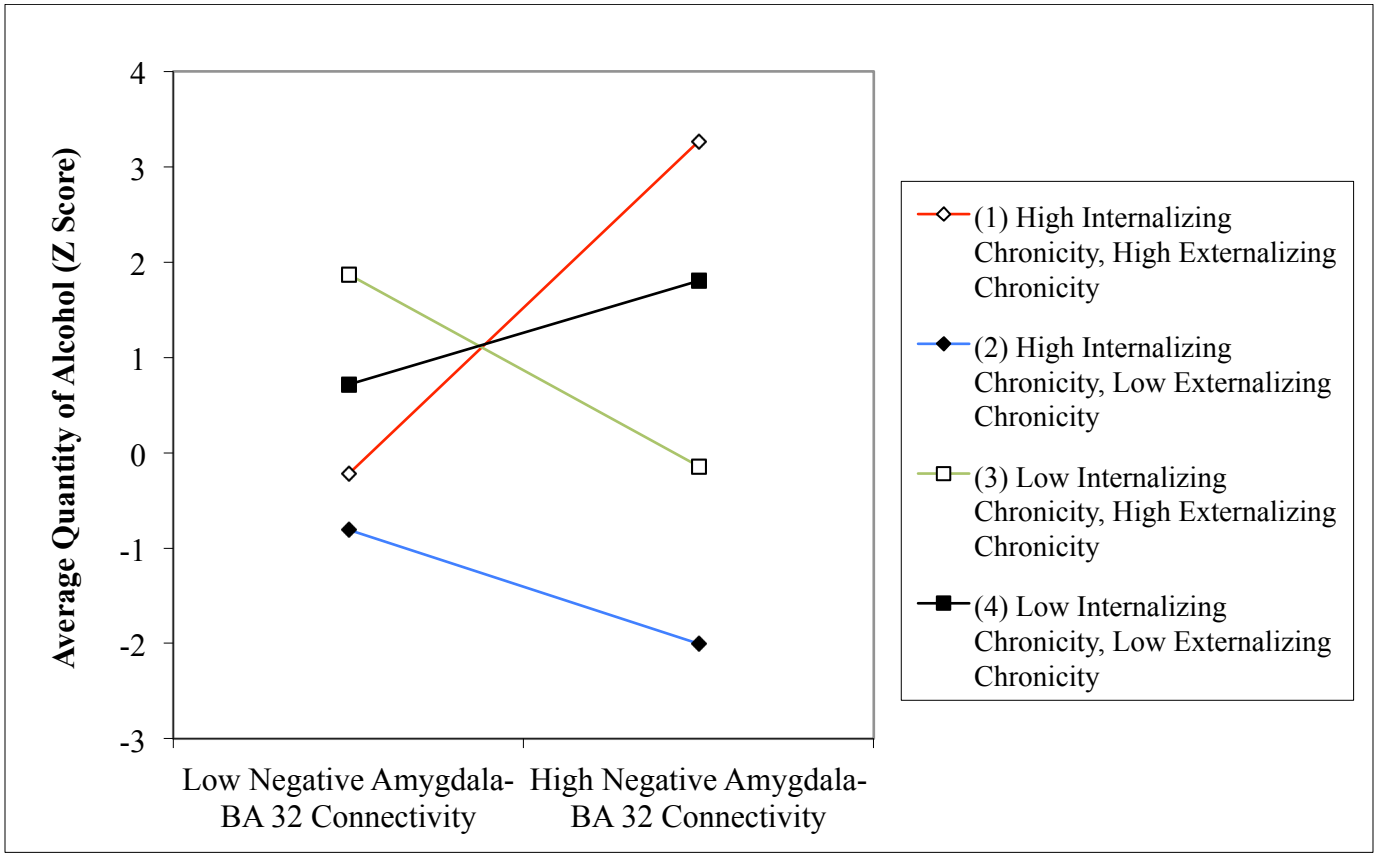

Figure 7. Three-way interaction between internalizing and externalizing chronicity scores and negative amygdala-BA 32 connectivity and average drinking quantity of alcohol at age 20

With "average days per month using alcohol" as the outcome measure, the main effects model was not significant. Only the covariate, maternal level of education was significant (R^2_{adj} = 0.054, F(1, 51) = 3.97, p = 0.052), such that individuals whose mother's highest level of education was less than high school reported a higher "average days per month using alcohol," (B = -.55, SE = 0.28, t = -1.99, p = 0.052, $r^2_{partial}$ = -0.27). This association became non-significant when the age 20 internalizing covariates were entered into the regression (R^2_{adj} = 0.025, F(2, 51) = 1.44, p = 0.243).

With "maximum quantity of alcohol used" as the outcome measure, the full model was significant (R^2_{adj} = 0.18, F(7, 48) = 3.34, p = 0.036); however, neither the main effect nor interaction terms were significant. When the age 20 internalizing covariates were entered into the regression, the full model remained significant (R^2_{adj} = 0.12, F(9, 48) = 2.37, p = 0.027). Within this model, higher externalizing chronicity scores (B = 0.69, SE = 0.29, t = 2.43, p = 0.020, $r^2_{partial}$ = 0.36) were associated with greater maximum quantity of alcohol used at age 20. Additionally, there was a significant interaction between internalizing and externalizing chronicity score within this model (B = 0.86, SE = 0.43, t = 2.02, p = 0.05, $r^2_{partial}$ = 0.31), with the same pattern present as demonstrated with average quantity of alcohol, such that those individuals that were high on both internalizing and externalizing chronicity reported consuming the highest maximum quantities of alcohol (Figure 8).

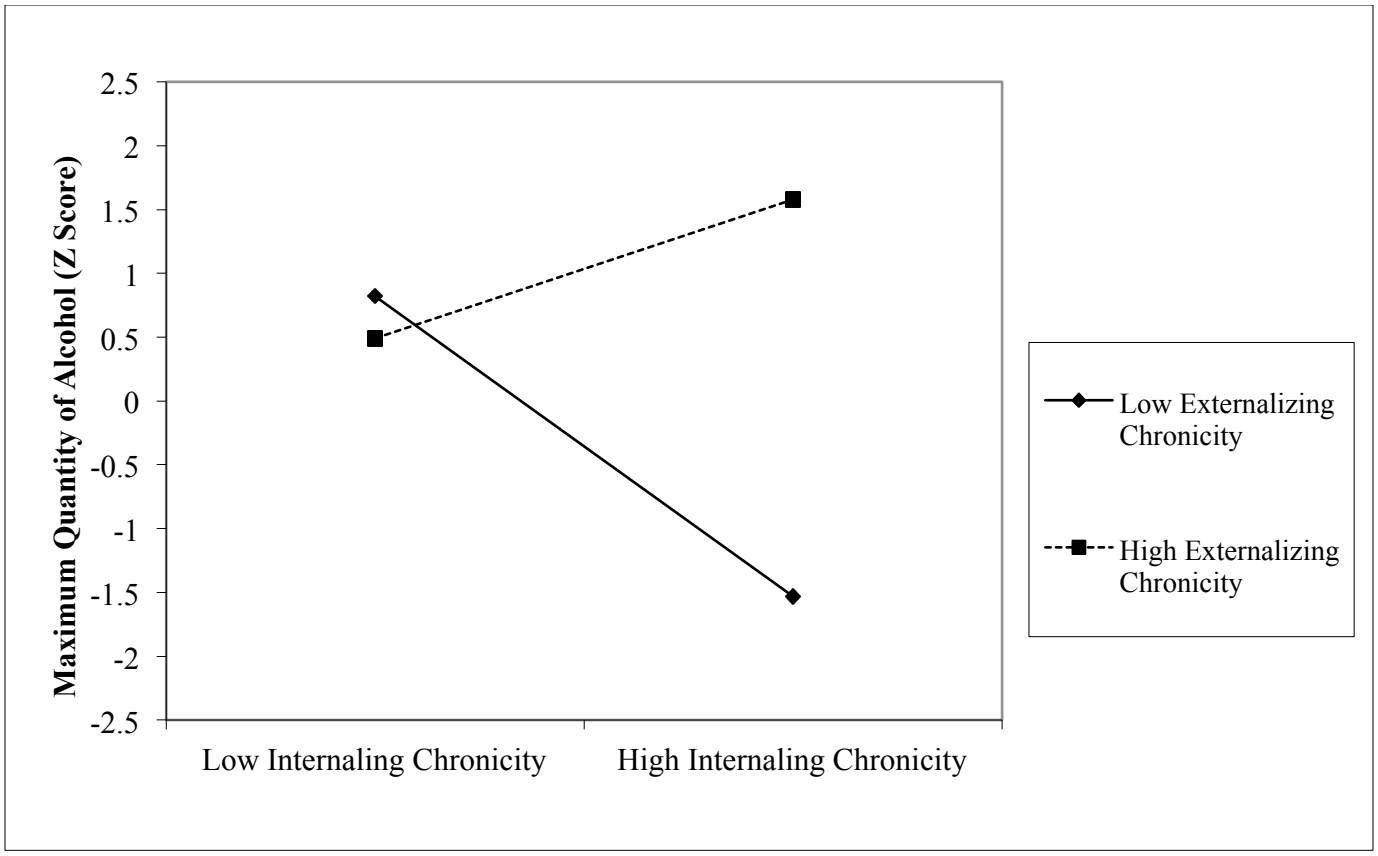

Figure 8. Interaction between internalizing and externalizing chronicity scores predicts to maximum drinking quantity of alcohol at age 20 (chronicity and amygdala-BA 32 connectivity analysis with age 20 internalizing covariates)

With "maximum number of days using maximum quantity of alcohol" as the outcome, the full model was significant (R^2_{adj} = 0.17, $F(7, 50)$ = 2.30, p = 0.038). Within this model, externalizing chronicity scores (B = 0.54, SE = 0.26, t = 2.12, p = 0.04, $r^2_{partial}$ = 0.31) were significantly associated with the maximum number of days using maximum amount of alcohol, such that higher externalizing scores predicted greater maximum number of days using maximum amount of alcohol at age 20. With the age 20 internalizing covariates entered into the analysis, the regressions were not significant.

6.4.4 Severity—Amygdala BA 32 Connectivity (Table 12)

The regression analyses involving the severity scores were specified in the same way as the chronicity regressions specified above (See Table 12 for overall statistics). For the regressions with ADS as the outcome measure, only the regression that included the early alcohol measures as covariates was significant (R^2_{adj} = 0.25, F(9, 80) = 3.63, p = 0.001). Within this model, age at first intoxication (B = -0.24, SE = 0.10, t = -2.36, p = 0.021, $r^2_{partial}$ = -0.27) significantly predicted alcohol dependence at age 20, such that younger ages of first intoxication were associated with higher ADS scores. Additionally, the Internalizing X Externalizing interaction term (Figure 9) was also significant (B = 0.32, SE = 0.14, t = 2.23, p = 0.029, $r^2_{partial}$ = 0.26), with a positive association between internalizing severity and ADS in individuals also high on externalizing severity.

For the analyses using the LHD alcohol outcome measures (average days per month and maximum quantity maximum days), only the covariate, highest level of maternal education, was associated with drinking levels at age 20. These statistics are reported above (See Table 12). The analyses with "average quantity of alcohol used" and "maximum quantity of alcohol" were not significant. None of the analyses with the age 20 internalizing covariates entered into the regression were significant.

Table 12. Regression results for severity and amygdala-BA 32 connectivity

Variable	B	SE B	*B*	t	*p*
ADS (regressions not significant)					
ADS with age 20 covariates (regressions not significant)					

ADS with early alcohol covariates					
R^2_{adj} = 0.25, F(9, 80) = 3.63, p = 0.001					
Maternal Education	-0.122	0.210	-0.058	-0.579	0.564
Age of first intoxication	-0.237	0.101	-0.445	-2.355	0.021 *
Age of first significant alcohol use	0.088	0.122	0.136	0.722	0.473
Internalizing (Int)	0.280	0.155	0.201	1.802	0.076
Externalizing (Ext)	-0.137	0.162	-0.125	-0.847	0.400
Amygdala-BA 32 Connectivity	0.068	0.182	0.063	0.371	0.712
Int X Ext (Figure 6)	0.320	0.144	0.357	2.226	0.029 *
Amygdala-BA 32 X Int	-0.035	0.222	-0.028	-0.159	0.874
Amygdala-BA 32 X Ext	-0.226	0.168	-0.239	-1.349	0.182
Amygdala-BA 32 X Int X Ext	0.028	0.191	0.028	0.149	0.882
Average days per month drinking					
R^2_{adj} = 0.054, F(1, 51) = 3.97, p = 0.052					
Maternal Education	-0.549	0.276	-0.269	-1.991	0.052 *
Average days per month drinking with age 20 covariates (regressions not significant)					
Maximum quantity maximum days					
R^2_{adj} = 0.075, F(1, 50) = 5.13, p = 0.028					
Maternal Education	-0.621	0.274	-0.305	-2.264	0.028 *
Maximum quantity maximum with age 20 covariates (regressions not significant)					

* $p < .05$, ** $p < .01$, *** $p < .001$

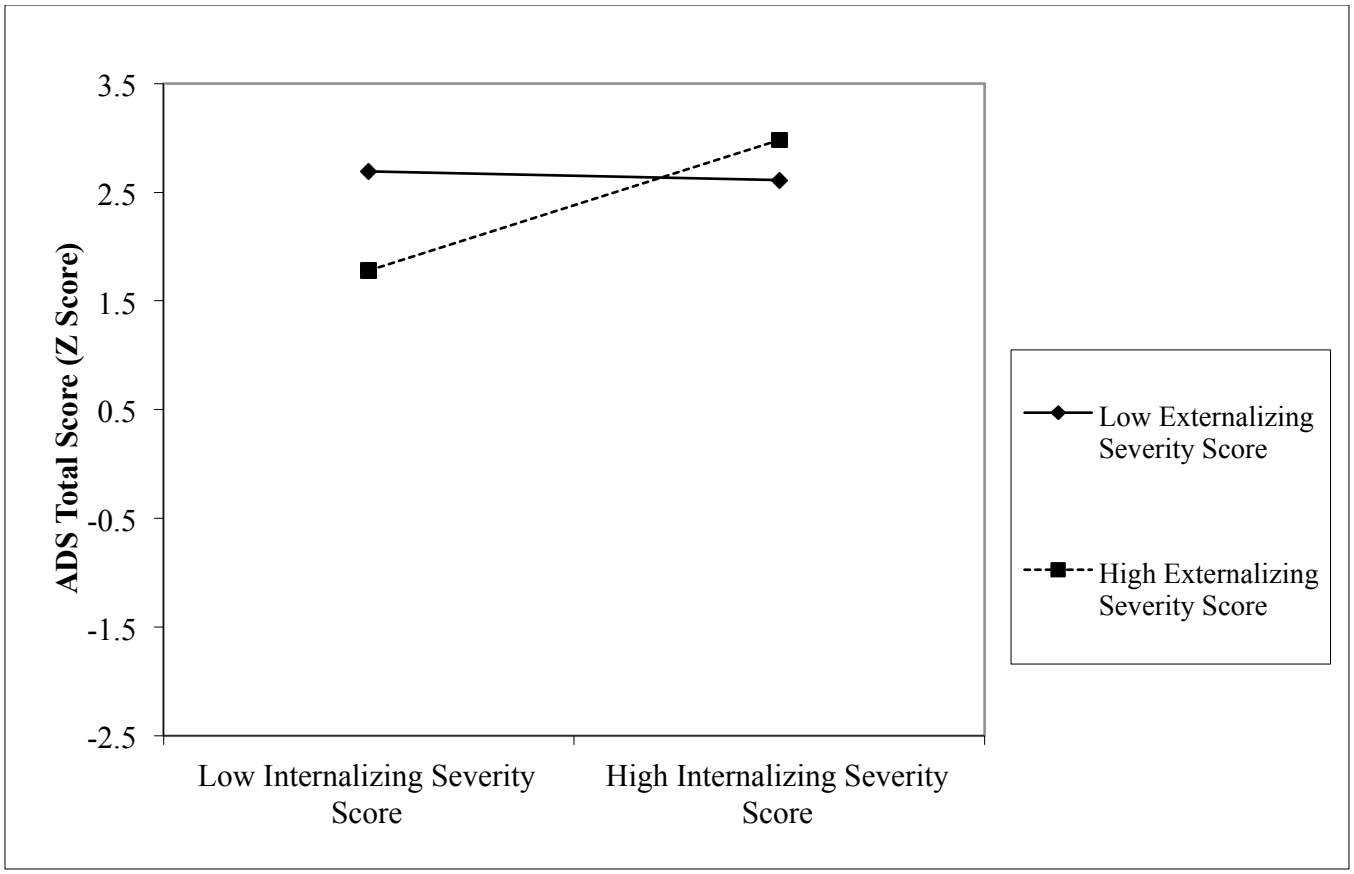

Figure 9. Interaction between internalizing and externalizing severity scores predicts to ADS total score at age 20 (severity and amygdala-BA 32 connectivity analysis with early alcohol covariates)

6.4.5 Chronicity—Amygdala BA 24 Connectivity (Table 13)

For the ADS analysis, the full model was significant (R^2_{adj} = 0.077, F(7, 103) = 2.08, p = 0.045), with externalizing chronicity positively associated with ADS total score at age 20 (B = 0.45, SE = 0.15, t = 2.99, p = 0.004, $r^2_{partial}$ = 0.29). Additionally, the three-way interaction between internalizing and externalizing chronicity and amygdala-BA 24 connectivity was significant (B = -0.60, SE = 0.31, t = -1.95, p = 0.054, $r^2_{partial}$ = -0.20, see Figure 10). Analyses of the simple slopes revealed that there was a significant difference between those individuals high on both

internalizing and externalizing chronicity (Line 1, Figure 10) and those high on internalizing and low on externalizing (Line 2, Figure 10), such that there was a negative association between level of negative amygdala-BA 24 connectivity and ADS total score in individuals high on both chronicity measures, and a positive association between connectivity and ADS in those individuals who were high internalizing/low externalizing (p = 0.018). When the age 20 internalizing covariates were added to the analysis, the full model remained significant (R^2_{adj} = 0.12, $F(9, 103) = 2.42$, p = 0.013), with the effects of externalizing chronicity (B = 0.45, SE = 0.15, t = 3.01, p = 0.003, $r^2_{partial}$ = 0.30) and the three-way interaction (B = -0.65, SE = 0.30, t = -2.15, p = 0.034, $r^2_{partial}$ = -0.22) also remaining significant. With the early alcohol measures entered as covariates (R^2_{adj} = 0.21, $F(9, 80) = 3.13$, p = 0.002), age of first intoxication (B = -0.30, SE = 0.10, t = -2.93, p = 0.005, $r^2_{partial}$ = -0.33) and internalizing chronicity (B = 0.48, SE = 0.19, t = 2.48, p = 0.016, $r^2_{partial}$ = 0.28) were significant, such that younger ages of first intoxication and higher internalizing chronicity scores were associated with higher ADS scores at age 20.

Table 13. Regression results for chronicity and amygdala-BA 24 connectivity

Variable	B	SE B	*B*	t	*p*
ADS R^2_{adj} = 0.077, $F(7, 103) = 2.08$, p = 0.045					
Maternal Education	-0.131	0.209	-0.061	-0.626	0.533
Internalizing (Int)	0.111	0.164	0.066	0.680	0.498
Externalizing (Ext)	0.449	0.150	0.298	2.989	0.004 **
Amygdala-BA 24 Connectivity	-0.011	0.099	-0.011	-0.108	0.914
Int X Ext	0.296	0.203	0.139	1.456	0.149
Amygdala-BA 24 X Int	0.005	0.223	0.002	0.022	0.983
Amygdala-BA 24 X Ext	-0.175	0.141	-0.121	-1.240	0.218
Amygdala-BA 24 X Int X Ext	-0.601	0.308	-0.190	-1.952	0.054 *

ADS with age 20 covariates					
R^2_{adj} = 0.12, F(9, 103) = 2.42, p = 0.013					
Maternal Education	-0.080	0.205	-0.037	-0.391	0.696
BAI Age 20	0.177	0.110	0.176	1.603	0.112
BDI Age 20	0.123	0.118	0.122	1.050	0.296
Internalizing (Int)	-0.054	0.177	-0.032	-0.303	0.762
Externalizing (Ext)	0.450	0.147	0.298	3.061	0.003 **
Amygdala-BA 24 Connectivity	-0.031	0.097	-0.031	-0.317	0.752
Int X Ext	0.336	0.199	0.158	1.690	0.094
Amygdala-BA 24 X Int	0.023	0.218	0.010	0.106	0.916
Amygdala-BA 24 X Ext	-0.173	0.139	-0.120	-1.243	0.217
Amygdala-BA 24 X Int X Ext (Figure 7)	-0.647	0.301	-0.204	-2.146	0.034 *
ADS with early alcohol covariates					
R^2_{adj} = 0.21, F(9, 80) = 3.13, p = 0.002					
Maternal Education	-0.072	0.220	-0.034	-0.326	0.746
Age of first intoxication	-0.302	0.103	-0.568	-2.929	0.005 **
Age of first significant alcohol use	0.197	0.124	0.306	1.593	0.116
Internalizing (Int)	0.479	0.193	0.273	2.480	0.016 *
Externalizing (Ext)	0.193	0.158	0.137	1.219	0.227
Amygdala-BA 24 Connectivity	0.080	0.138	0.068	0.581	0.563
Int X Ext	0.268	0.208	0.137	1.289	0.202
Amygdala-BA 24 X Int	-0.069	0.245	-0.031	-0.282	0.778
Amygdala-BA 24 X Ext	-0.218	0.187	-0.134	-1.170	0.246
Amygdala-BA 24 X Int X Ext	-0.054	0.315	-0.018	-0.172	0.864
Average days per month drinking					
R^2_{adj} = 0.054, F(1, 51) = 3.97, p = 0.052					
Maternal Education	-0.549	0.276	-0.269	-1.991	0.052 *
Average days per month drinking with age 20 covariates (regressions not significant)					
Maximum quantity maximum days					
R^2_{adj} = 0.20, F(7, 50) = 2.63, p = 0.019					
Maternal Education	-0.424	0.267	-0.208	-1.586	0.120
Internalizing (Int)	-0.287	0.221	-0.174	-1.303	0.200
Externalizing (Ext)	0.329	0.210	0.209	1.566	0.125
Amygdala-BA 24 Connectivity	0.172	0.129	0.195	1.331	0.190
Int X Ext	0.425	0.351	0.163	1.211	0.232
Amygdala-BA 24 X Int	-0.155	0.310	-0.072	-0.498	0.621
Amygdala-BA 24 X Ext	0.116	0.182	0.095	0.639	0.526
Amygdala-BA 24 X Int X Ext (Figure 8)	-1.054	0.462	-0.351	-2.280	0.028 *

Maximum quantity maximum days with age 20 covariates

R^2_{adj} = 0.17, $F(9, 50) = 2.05$, $p = 0.053$

Maternal Education	-0.379	0.286	-0.186	-1.327	0.192
BAI Age 20	0.045	0.161	0.044	0.279	0.782
BDI Age 20	-0.091	0.175	-0.098	-0.518	0.607
Internalizing (Int)	-0.240	0.260	-0.146	-0.925	0.360
Externalizing (Ext)	0.372	0.229	0.235	1.619	0.113
Amygdala-BA 24 Connectivity	0.166	0.133	0.188	1.246	0.220
Int X Ext	0.439	0.368	0.168	1.191	0.241
Amygdala-BA 24 X Int	-0.150	0.320	-0.070	-0.469	0.642
Amygdala-BA 24 X Ext	0.116	0.186	0.095	0.622	0.537
Amygdala-BA 24 X Int X Ext	-1.040	0.475	-0.346	-2.192	0.034 *

* $p < .05$, ** $p < .01$, *** $p < .001$

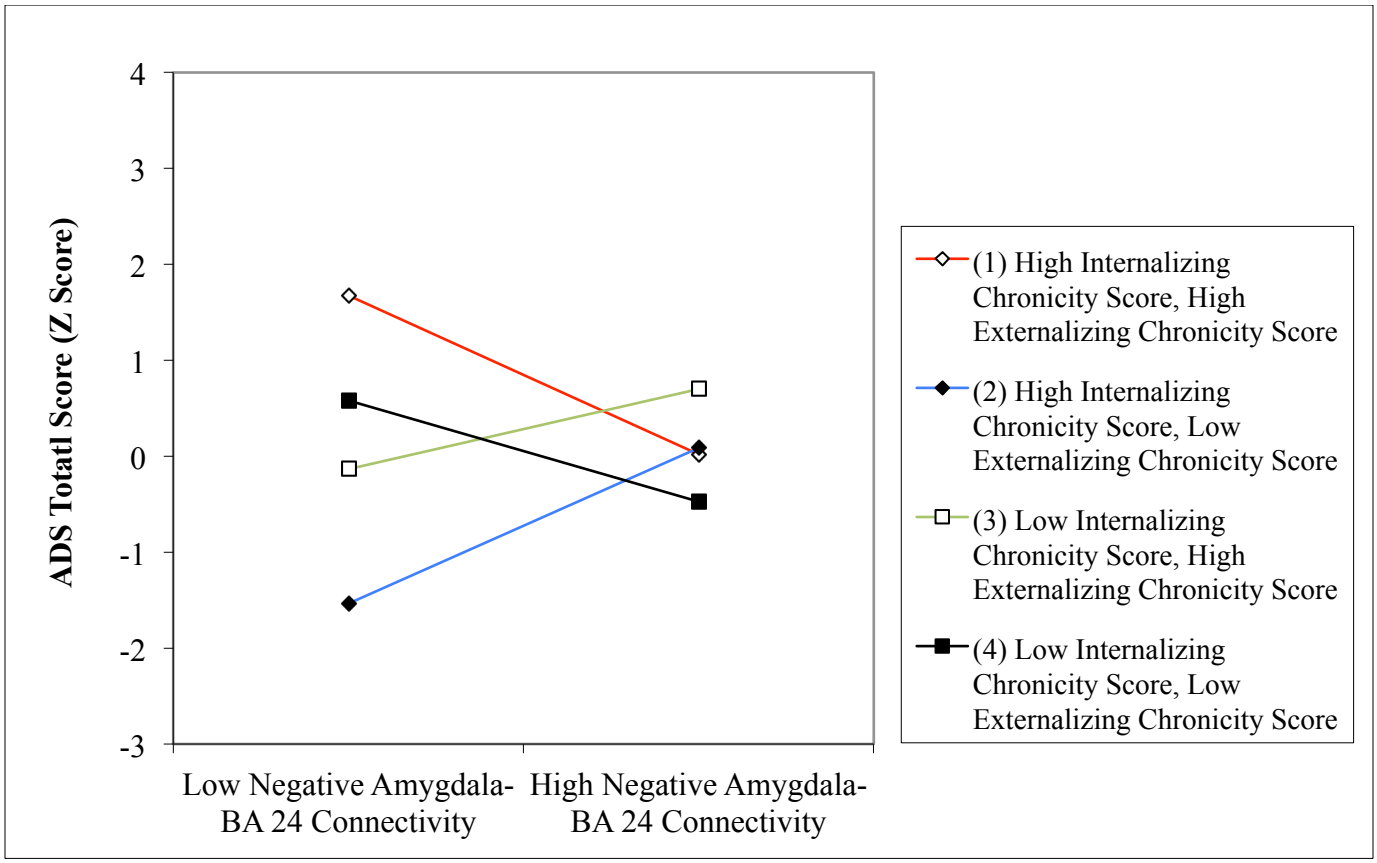

Figure 10. Three-way interaction between internalizing and externalizing chronicity scores and negative amygdala-BA 24 connectivity and ADS total score at age 20 (analysis with age 20 internalizing covariates)

With “average days per month using alcohol” as the outcome measure, the main effects model was not significant. Only the covariate, maternal level of education was significant (previously reported, See Table 13). With “maximum number of days using maximum quantity of alcohol” as the outcome, the full model was significant (R^2_{adj} = 0.20, F(7, 50) = 2.63, p = 0.019). Within this model, the three-way interaction between internalizing and externalizing chronicity scores and amygdala-BA 24 connectivity (Figure 11) was significantly associated with maximum number of days using maximum amount of alcohol (B = -1.05, SE = 0.46, t = -2.28, p = 0.028, $r^2_{partial}$ = -0.33). Within this interaction, there was a significant difference between those individuals high on both internalizing and externalizing chronicity (Line 1, Figure 11) and those high on internalizing and low on externalizing (Line 2, Figure 11), such that there was a negative association between level of negative amygdala-BA 24 connectivity and alcohol use in individuals high on both chronicity measures, and a positive association between connectivity and maximum days using maximum quantity of alcohol in those individuals who were high internalizing/low externalizing (p = 0.045). Similarly, there was a significant difference between those individuals high on both internalizing and externalizing chronicity (Line 1, Figure 8) and those low on internalizing and high on externalizing (Line 3, Figure 8), with those with low internalizing and high externalizing scores demonstrating a positive association (p = 0.036). Finally, there was a significant difference between those low on internalizing and high on externalizing (Line 3, Figure 11) and those low on both measures (Line 4, Figure 11), with those low on both measures demonstrating a negative association between level of negative connectivity and maximum days using maximum quantity of alcohol (p = 0.034). When the age 20 internalizing covariates were entered into the regression, the full model (R^2_{adj} = 0.17, F(9, 50)

= 2.05, p = 0.053), and the three-way interaction remained significant (B = -1.04, SE = 0.48, t = -2.19, p = 0.034, $r^2_{partial}$ = -0.32). The analyses with "average quantity of alcohol used" and "maximum quantity of alcohol" were not significant.

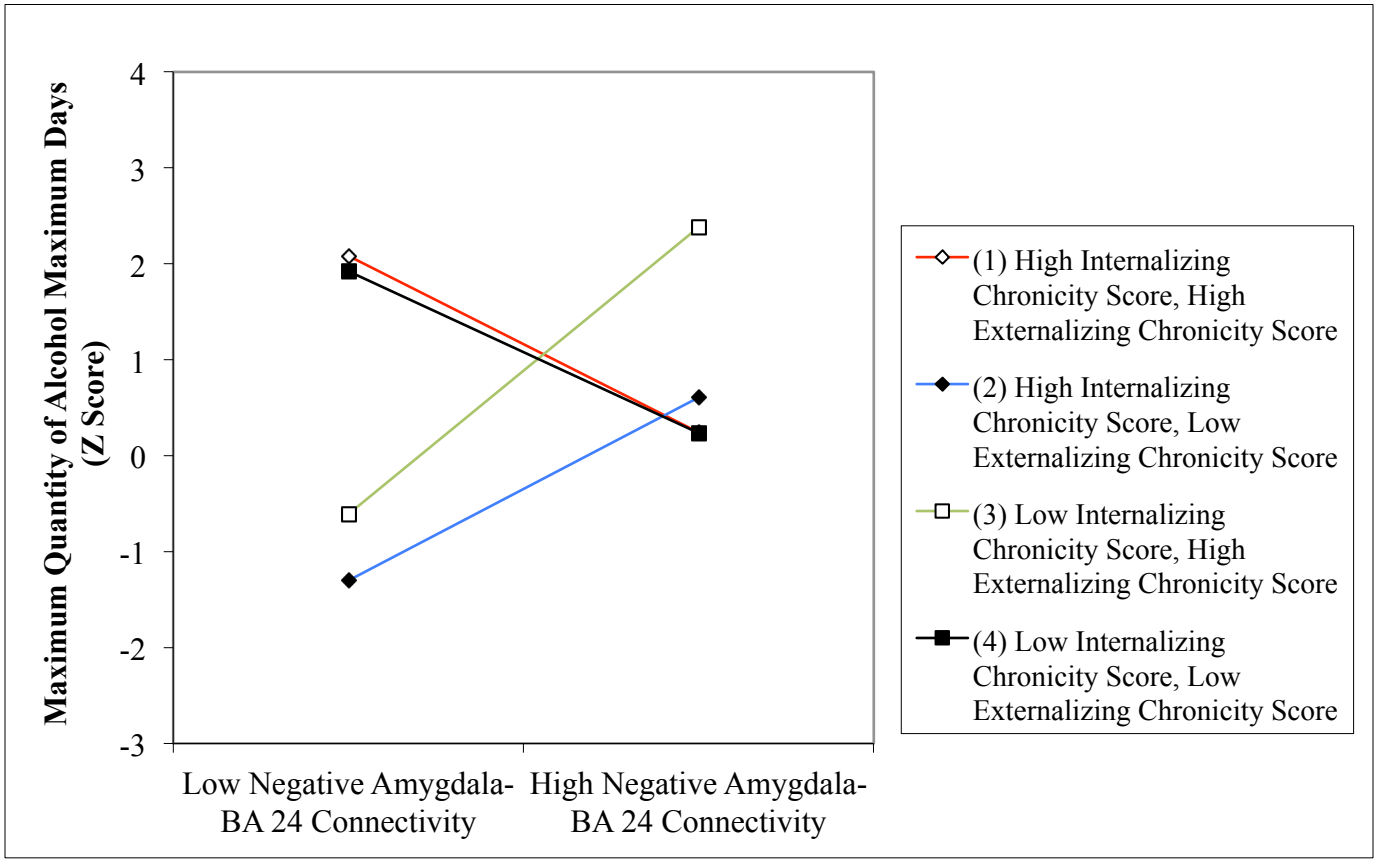

Figure 11. Three-way interaction between internalizing and externalizing chronicity scores and negative amygdala-BA 24 connectivity and maximum days drinking maximum quantity of alcohol at age 20

6.4.6 Severity—Amygdala BA 24 Connectivity (Table 14)

For the ADS analysis, the full model was significant (R^2_{adj} = 0.11, F(7, 103) = 2.08, p = 0.045), with a significant three-way interaction between internalizing and externalizing severity and amygdala-BA 24 connectivity (B = -0.63, SE = 0.20, t = -3.10, p = 0.003, $r^2_{partial}$ = -0.30). Within

this three-way interaction, there was a significant difference between those individuals high on both internalizing and externalizing severity (Line 1, Figure 12) and those high on internalizing and low on externalizing (Line 2, Figure 12), such that there was no difference in ADS score as a function of level of negative amygdala-BA 24 connectivity in individuals high on both severity measures, relative to a positive association between connectivity and ADS in those individuals who were high internalizing/low externalizing (p = 0.010). Additionally, there was a significant difference between those individuals low on internalizing and externalizing severity scores (Line 4, Figure 12) and both those high internalizing/low externalizing (Line 2, Figure 12) and those low internalizing/high externalizing (Line 3, Figure 12), such that there was a negative relation between connectivity and ADS total score in those individuals low on both severity scores (Line 4), relative to a positive association within the other two groups (Lines 2, p = 0.011 and 3, p = 0.009). When the age 20 internalizing covariates were added to the analysis, the full model remained significant (R^2_{adj} = 0.14, F(9, 103) = 2.64, p = 0.007), with a significant interaction between internalizing and externalizing severity scores (B = 0.29, SE = 0.14, t = 2.07, p = 0.041, $r^2_{partial}$ = 0.21), such that there was a positive association between internalizing severity score and ADS in those individuals also high on externalizing severity score and a negative relation in those low in externalizing chronicity (Figure 13). Additionally, the three-way interaction remained significant (B = -0.59, SE = 0.20, t = -2.98, p = 0.004, $r^2_{partial}$ = -0.29). With the early alcohol measures entered as covariates, the full model was also significant (R^2_{adj} = 0.24, F(9, 80) = 3.56, p = 0.001), with age of first intoxication as significant (B = -0.27, SE = 0.10, t = -2.63, p = 0.010, $r^2_{partial}$ = -0.30), such that younger ages of first intoxication were associated with higher ADS scores at age 20. Additionally, there was a two-way interaction between

internalizing and externalizing severity scores (B = 0.32, SE = 0.14, $t = 2.27$, $p = 0.026$, $r^2_{partial} = 0.26$), such that there was no effect of internalizing chronicity on ADS in those individuals high on externalizing, and there was a positive association between internalizing chronicity and ADS in those individuals also high on externalizing chronicity (Figure 14).

Table 14. Regression results for severity and amygdala-BA 24 connectivity

Variable	B	SE B	*B*	t	*p*
ADS					
$R^2_{adj} = 0.11$, $F(7, 103) = 2.65$, $p = 0.011$					
Maternal Education	-0.153	0.205	-0.071	-0.746	0.458
Internalizing (Int)	0.049	0.146	0.035	0.334	0.739
Externalizing (Ext)	0.078	0.161	0.066	0.485	0.629
Amygdala-BA 24 Connectivity	-0.041	0.146	-0.041	-0.283	0.778
Int X Ext	0.261	0.140	0.272	1.869	0.065
Amygdala-BA 24 X Int	0.278	0.198	0.233	1.406	0.163
Amygdala-BA 24 X Ext	0.219	0.146	0.210	1.499	0.137
Amygdala-BA 24 X Int X Ext (Figure 9)	-0.625	0.202	-0.499	-3.098	0.003 **
ADS with age 20 covariates					
$R^2_{adj} = 0.14$, $F(9, 103) = 2.64$, $p = 0.007$					
Maternal Education	-0.106	0.204	-0.050	-0.522	0.603
BAI Age 20	0.142	0.110	0.141	1.290	0.200
BDI Age 20	0.113	0.117	0.112	0.969	0.335
Internalizing (Int)	-0.086	0.159	-0.062	-0.539	0.591
Externalizing (Ext)	0.050	0.160	0.042	0.312	0.755
Amygdala-BA 24 Connectivity	-0.073	0.147	-0.072	-0.497	0.621
Int X Ext (Figure 10)	0.287	0.139	0.299	2.069	0.041 *
Amygdala-BA 24 X Int	0.294	0.196	0.246	1.500	0.137
Amygdala-BA 24 X Ext	0.186	0.146	0.179	1.277	0.205
Amygdala-BA 24 X Int X Ext	-0.594	0.200	-0.474	-2.977	0.004 **
ADS with early alcohol covariates					
$R^2_{adj} = 0.24$, $F(9, 80) = 3.56$, $p = 0.001$					
Maternal Education	-0.103	0.212	-0.049	-0.485	0.629
Age of first intoxication	-0.265	0.101	-0.499	-2.629	0.010 **
Age of first significant alcohol use	0.147	0.121	0.228	1.218	0.227

Internalizing (Int)	0.215	0.159	0.155	1.354	0.180
Externalizing (Ext)	-0.094	0.165	-0.085	-0.567	0.572
Amygdala-BA 24 Connectivity	0.183	0.220	0.155	0.832	0.408
Int X Ext	0.317	0.140	0.354	2.273	0.026 *
Amygdala-BA 24 X Int	0.084	0.231	0.066	0.365	0.716
Amygdala-BA 24 X Ext	-0.013	0.176	-0.013	-0.072	0.943
Amygdala-BA 24 X Int X Ext	-0.252	0.213	-0.210	-1.181	0.242
Average days per month drinking					
R^2_{adj} = 0.054, $F(1, 51) = 3.97$, $p = 0.052$					
Maternal Education	-0.549	0.276	-0.269	-1.991	0.052 *
Average days per month drinking with age 20 covariates (regressions not significant)					
Maximum quantity maximum days					
R^2_{adj} = 0.075, $F(1, 50) = 5.13$, $p = 0.052$					
Maternal Education	-0.621	0.274	-0.305	-2.264	0.028 *
Maximum quantity maximum days with age 20 covariates (regressions not significant)					

* $p < .05$, ** $p < .01$, *** $p < .001$

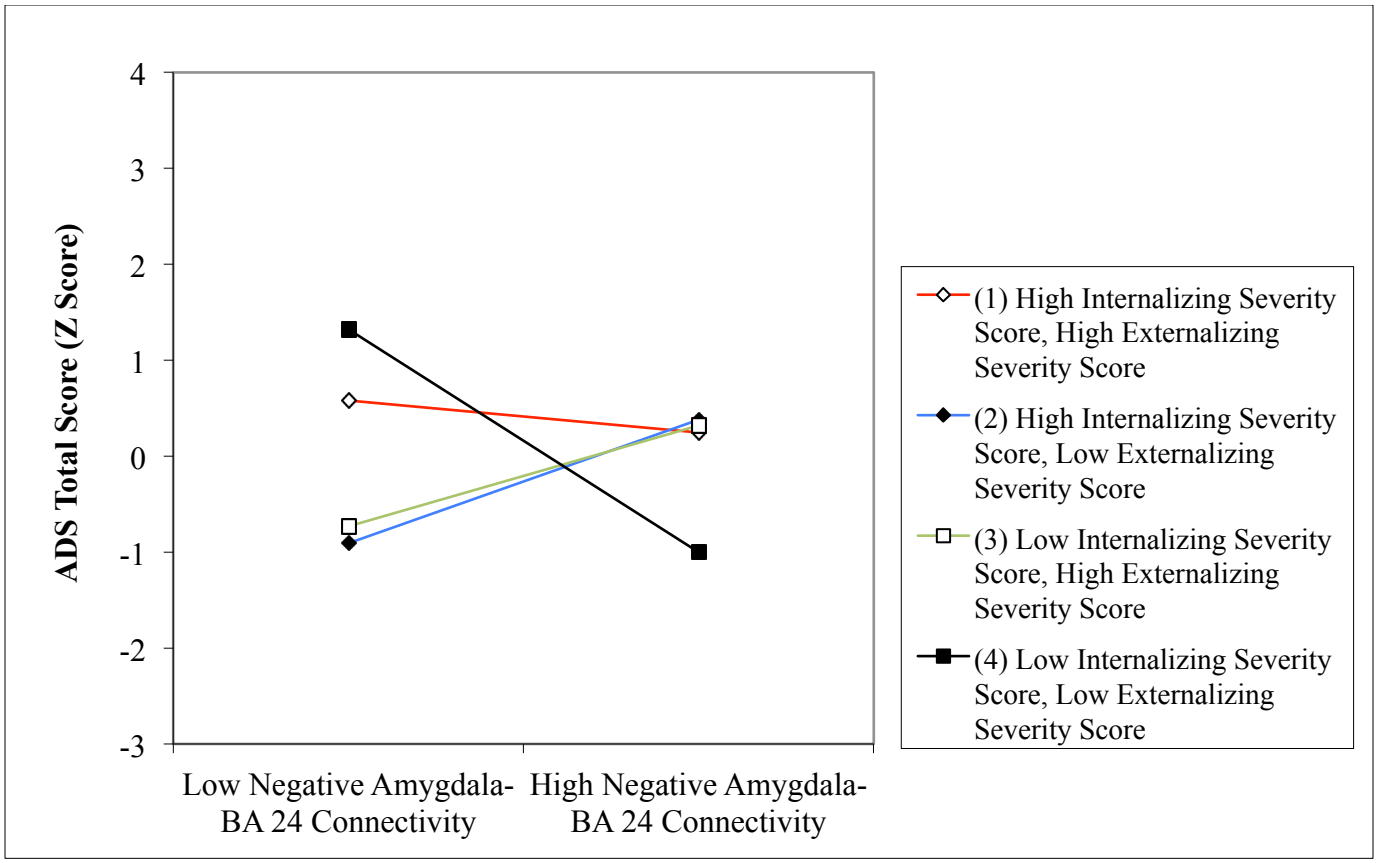

Figure 12. Three-way interaction between internalizing and externalizing severity scores and negative amygdala-BA 24 connectivity and ADS total score at age 20

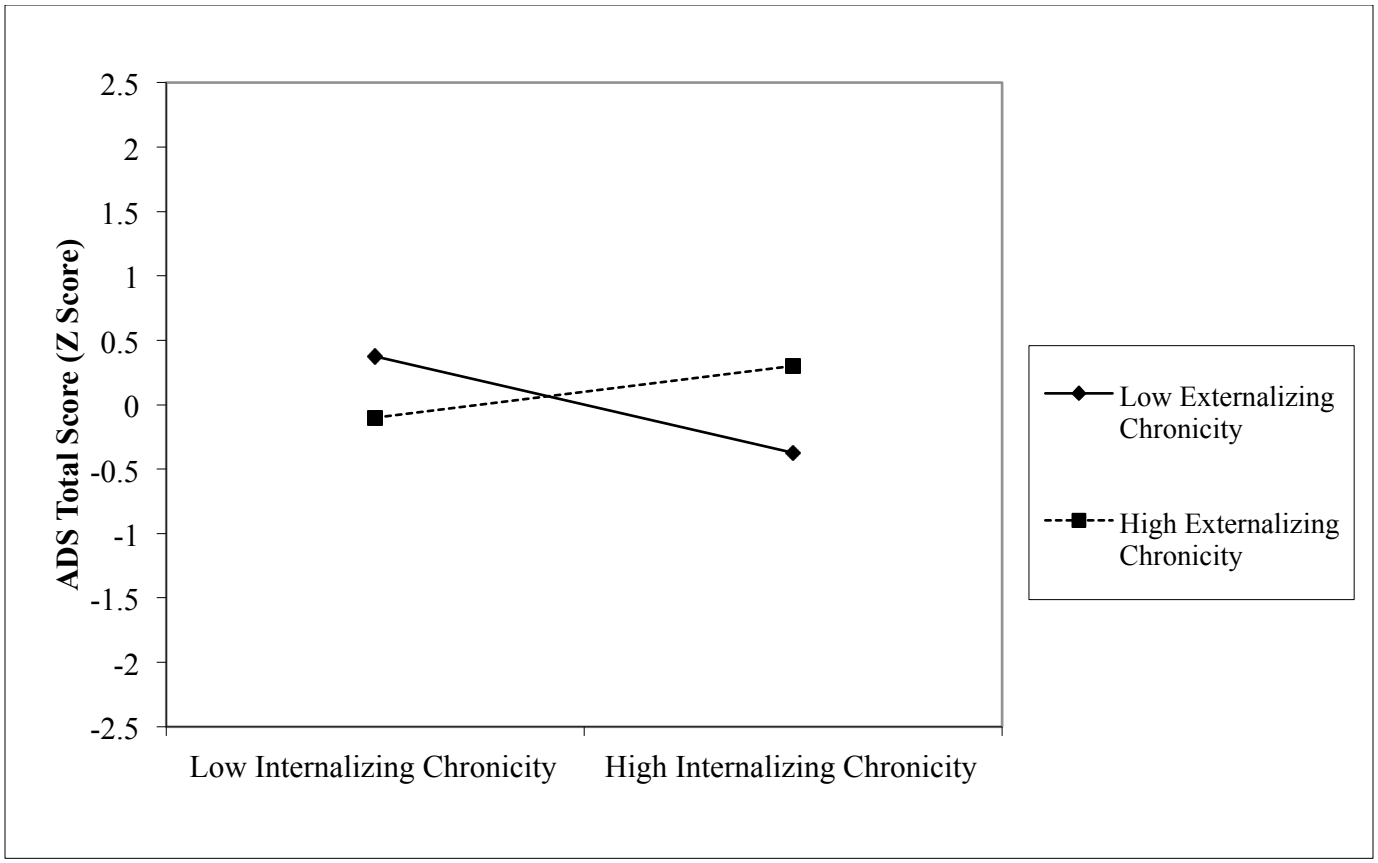

Figure 13. Interaction between internalizing and externalizing severity scores predicts to ADS total score at Age 20 (severity and amygdala-BA 24 connectivity analysis with age 20 internalizing covariates)

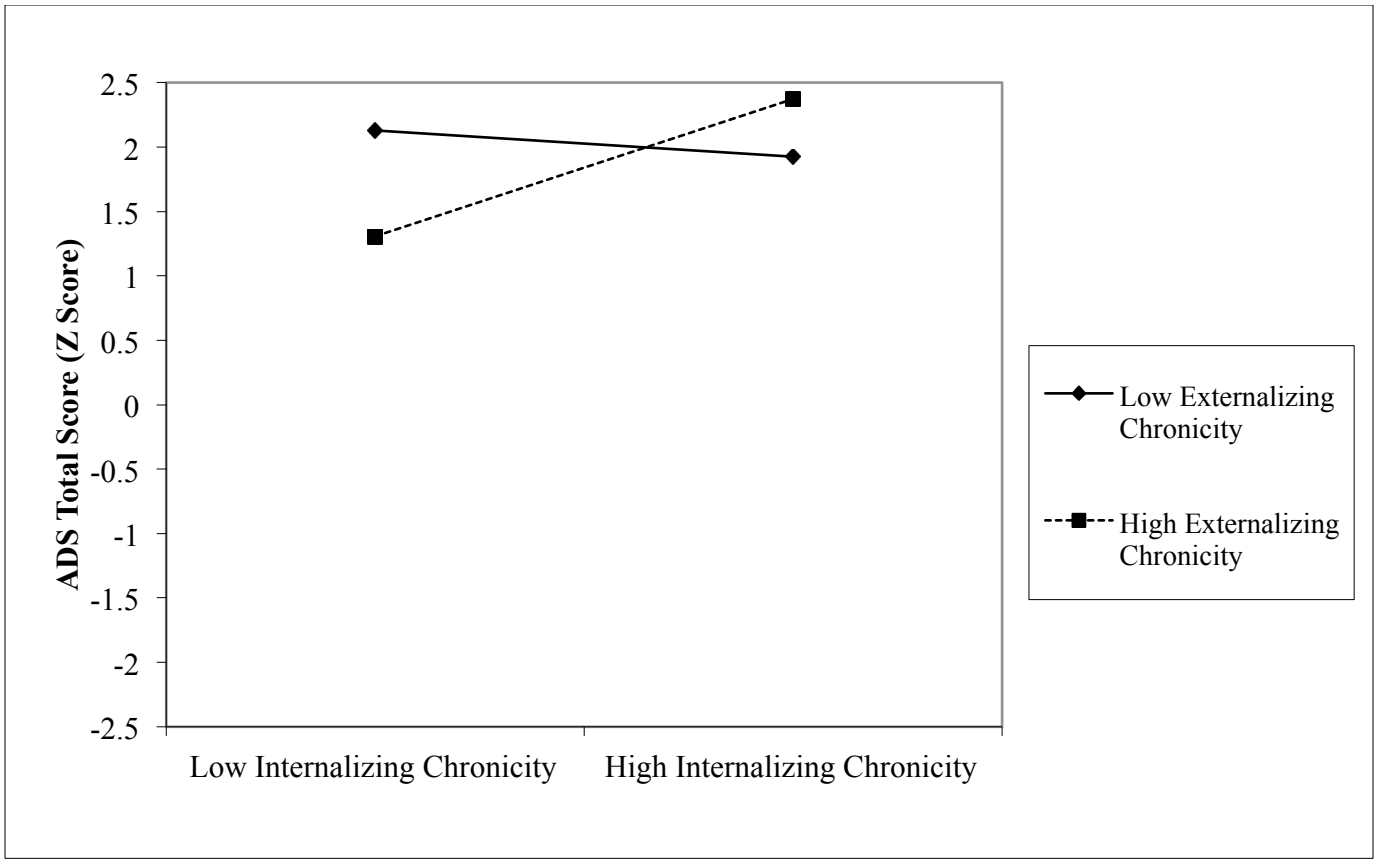

Figure 14. Interaction between internalizing and externalizing severity scores predicts to ADS total score at Age 20 (severity and amygdala-BA 24 connectivity analysis with early alcohol covariates)

With both "average days per month using alcohol" and "maximum number of days using maximum quantity of alcohol" as the outcome measures, the main effects models were not significant. Only the covariate, maternal level of education was significant (previously reported, See Table 14). The analyses with "average quantity of alcohol used" and "maximum quantity of alcohol" were not significant. Additionally, none of the analyses with the age 20 internalizing covariates entered into the regression were significant.

Table 15. Summary table for significant overall results for Question 2

Variable	Diagnostic Group Amygdala	Diagnostic Group Amyg-BA 32	Diagnostic Group Amyg-BA 24	Chronicity Group Amygdala	Chronicity Group Amyg-BA 32	Chronicity Group Amyg-BA 24
ADS with early alcohol	1st intoxication	1st intoxication	1st intoxication Combined Group	1st intoxication	1st intoxication	1st intoxication
Avg days	Maternal Ed	Maternal Ed	Maternal Ed	Maternal Ed	Maternal Ed	Maternal Ed
Maximum quantity maximum days	Maternal Ed	Maternal Ed	Maternal Ed	Maternal Ed	Maternal Ed	Maternal Ed

Note: Analyses with the following outcomes were not significant and are therefore not reported here: ADS with and without age 20 covariates, Average quantity with and without age 20 covariates, Average days per month consuming alcohol with age 20 covariates, maximum quantity of alcohol consumed with and without age 20 covariates, and maximum quantity maximum days with age 20 covariates. ADS: Alcohol Dependence Scale, Maternal Ed: Level of maternal education, 1st intoxication: Age at first intoxication

6.5 Question 2 Analyses (See Table 15 for overall significant findings)

6.5.1 Severity/early history of disorders analysis — Amygdala reactivity

To test the relation between the presence of early internalizing disorders and/or externalizing disorders and age 20 patterns of drinking, the four groups (Internalizing Only, Externalizing Only, Combined, and Comparison) were calculated. However, due to the small cell sizes within the internalizing group, this group was collapsed within the combined group, resulting in 3 groups, which were then compared within a regression model using dummy coding. As with the analyses in question 1, a hierarchical multiple regression analysis was conducted with highest level of maternal education (covariate) entered first, followed by externalizing and combined dummy codes and amygdala composite scores (main effects model), and finally the interaction terms Externalizing Status X Amygdala Reactivity (Connectivity) and Combined Status X Amygdala Reactivity (Connectivity) (full model). Five regression analyses were conducted, varying the alcohol outcome: one with ADS total score and four with the LHD measure. These data were analyzed with and without the age 20 internalizing covariates (BAI and BDI). Additionally, for the analysis using ADS as the outcome measure, the regression was conducted with and without early alcohol use measures as the covariates (age at first intoxication, age at first significant use).

With ADS as the outcome, only the regression with the early alcohol covariates was significant (R^2_{adj} = 0.12, $F(3, 80) = 4.63$, $p = 0.005$). Only the covariate, age at first intoxication, was significant, with younger age predicting to greater ADS scores (B = -0.33, SE = 0.11, t = -3.10,

$p = 0.003$, $r^2_{partial} = -0.33$). For the analyses using the LHD alcohol outcome measures, the main effects models were not significant. Only the covariate, highest level of maternal education, was associated with drinking levels at age 20. These associations are previously reported above (See Table 16). The analyses with "average quantity of alcohol used" and "maximum quantity of alcohol used" were not significant. None of the analyses with the age 20 internalizing covariates entered into the regression were significant.

Table 16. Regression results for diagnostic group status and amygdala reactivity

Variable	B	SE B	*B*	t	*p*
ADS					
(regressions not significant)					
ADS with age 20 covariates					
(regressions not significant)					
ADS with early alcohol covariates					
$R^2_{adj} = 0.12$, $F(3, 80) = 4.63$, $p = 0.005$					
Maternal Education	-0.076	0.220	-0.036	-0.347	0.730
Age at first intoxication	-0.325	0.105	-0.610	-3.102	0.003 **
Age at first significant alcohol use	0.194	0.127	0.300	1.529	0.130
Average days per month drinking					
$R^2_{adj} = 0.054$, $F(1, 51) = 3.97$, $p = 0.052$					
Maternal Education	-0.549	0.276	-0.269	-1.991	0.052 *
Average quantity with age 20 covariates					
(regressions not significant)					
Maximum quantity maximum days					
$R^2_{adj} = 0.075$, $F(1, 50) = 5.125$, $p = 0.028$					
Maternal Education	-0.621	0.274	-0.305	-2.264	0.028 *
Maximum quantity maximum days with age 20 covariates					
(regressions not significant)					

* $p < .05$, ** $p < .01$, *** $p < .001$

6.5.2 Severity/early history of disorders analysis — Amygdala -BA 32 and BA 24 connectivity

The pattern of these results was identical to the amygdala reactivity analyses, with only the covariates predicting to measures of alcohol use and dependence at age 20 (Tables 17, 18). With BA 24 connectivity and ADS, only the model with the early alcohol covariates was significant. The full model was significant (R^2_{adj} = 0.12, $F(7, 80) = 2.33$, $p = 0.028$); however, there were no significant effects of the interaction terms. Within this model, age at first intoxication was significant (B = -0.37, SE = 0.11, t = -3.41, p = 0.001, $r^2_{partial}$ = -0.37), such that younger age of first intoxication predicted to higher ADS scores at age 20. Further, there was a significant effect of the Combined Group Status, such that individuals who had either an internalizing or externalizing DSM-IV diagnosis between ages 8 and 17 demonstrated higher ADS scores relative to those who had never met criteria for a disorder between ages 8 and 17, (B = 0.73, SE = 0.36, t = 2.04, p = 0.045, $r^2_{partial}$ = 0.23).

To determine whether there were group differences in either ADS or amygdala reactivity or connectivity, ANOVA analyses were conducted with group status. There were no significant differences in ADS scores by group status ($F(2, 103) = 0.36$, $p = 0.78$). Additionally, there were no significant differences in either reactivity ($F(2, 109) = 1.96$, $p = 0.13$) or amygdala-BA 24 connectivity ($F(2, 109) = 1.89$, $p = 0.14$) by group status. There was a difference in amygdala-BA 32 connectivity between the internalizing only and externalizing only group, significant at trend ($F(2, 109) = 2.24$, $p = 0.088$), with the internalizing group demonstrating lower negative connectivity relative to the externalizing group. However, it should be noted that the internalizing group comprised only 8 individuals (See Appendix B for sample sizes of the groups).

Table 17. Regression results for diagnostic group status and negative amygdala-BA 24 connectivity

Variable	B	SE B	*B*	t	*p*
ADS					
(regressions not significant)					
ADS with age 20 covariates					
(regressions not significant)					
ADS with early alcohol covariates					
R^2_{adj} = 0.12, $F(7, 80) = 2.33$, $p = 0.028$					
Maternal Education	-0.105	0.233	-0.050	-0.450	0.654
Age at first intoxication	-0.368	0.108	-0.691	-3.406	0.001 ***
Age at first significant alcohol use	0.233	0.130	0.360	1.797	0.076
Combined Group (Combined)	0.734	0.360	0.246	2.038	0.045 *
Externalizing Group (Ext)	-0.155	0.227	-0.079	-0.683	0.497
Amygdala-BA 24 Connectivity	0.040	0.215	0.034	0.185	0.854
Amygdala-BA 24 X Combined	0.073	1.425	0.007	0.051	0.959
Amygdala-BA 24 X Ext	0.186	0.283	0.127	0.658	0.512
Average days per month drinking					
R^2_{adj} = 0.054, $F(1, 51) = 3.97$, $p = 0.052$					
Maternal Education	-0.549	0.276	-0.269	-1.991	0.052 *
Average quantity with age 20 covariates					
(regressions not significant)					
Maximum quantity maximum days					
R^2_{adj} = 0.075, $F(1, 50) = 5.125$, $p = 0.028$					
Maternal Education	-0.621	0.274	-0.305	-2.264	0.028 *
Maximum quantity maximum days with age 20 covariates					
(regressions not significant)					

* $p < .05$, ** $p < .01$, *** $p < .001$

Table 18. Regression Results for Diagnostic Group Status and Negative Amygdala-BA 32 Connectivity

Variable	B	SE B	*B*	t	*p*
ADS					
(regressions not significant)					
ADS with age 20 covariates					
(regressions not significant)					
ADS with early alcohol covariates					
R^2_{adj} = 0.12, *F*(3, 80) = 4.63, *p* = 0.005					
Maternal Education	-0.076	0.220	-0.036	-0.347	0.730
Age at first intoxication	-0.325	0.105	-0.610	-3.102	0.003 **
Age at first significant alcohol use	0.194	0.127	0.300	1.529	0.130
Average days per month drinking					
R^2_{adj} = 0.054, *F*(1, 51) = 3.97, *p* = 0.052					
Maternal Education	-0.549	0.276	-0.269	-1.991	0.052 *
Average quantity with age 20 covariates					
(regressions not significant)					
Maximum quantity maximum days					
R^2_{adj} = 0.075, *F*(1, 50) = 5.125, *p* = 0.028					
Maternal Education	-0.621	0.274	-0.305	-2.264	0.028 *
Maximum quantity maximum days with age 20 covariates					
(regressions not significant)					

* *p* < .05, ** *p* < .01, *** *p* < .001

6.5.3 Chronicity analysis— Amygdala reactivity and connectivity

As noted in the data analytic plan, due to the small cell sizes within each group, the Non-Chronic groups (both internalizing and externalizing) were combined to form a Non-Chronic group, and the Chronic groups (both internalizing and externalizing) were combined to form a

Chronic group. As with the analyses involving diagnostic group status, a hierarchical multiple regression analysis was conducted with highest level of maternal education (covariate) entered first, followed by chronic and non-chronic dummy codes and amygdala reactivity composite (connectivity) scores (main effects model), and finally the interaction terms Chronic Status X Amygdala Reactivity (Connectivity) and Non-Chronic Status X Amygdala Reactivity (Connectivity) (full model). Overall, there were no significant main effects of either amygdala reactivity or connectivity. For the amygdala reactivity analyses with ADS as the outcome measure, the main effects models were not significant. Only the early alcohol covariates model was significant (R^2_{adj} = 0.12, $F(2, 80) = 4.63$, p = 0.005), with age at first intoxication negatively associated with alcohol dependence at age 20 (B = -0.33, SE = 0.11, t = -3.10, p = 0.003, $r^2_{partial}$ = -0.33). Similarly, only highest level of maternal education was significant for analyses with the LHD drinking outcomes at age 20. These associations are previously reported above (See Table 19). The analyses with "average quantity of alcohol used" and "average quantity of alcohol used" were not significant. None of the analyses with the age 20 internalizing covariates entered into the regression were significant.

Table 19. Regression results for chronicity group status and amygdala reactivity

Variable	B	SE B	*B*	t	*p*
ADS (regressions not significant)					
ADS with age 20 covariates (regressions not significant)					
ADS with early alcohol covariates R^2_{adj} = 0.12, $F(2, 80) = 4.63$, p = 0.005					
Maternal Education	-0.076	0.220	-0.036	-0.347	0.730

Age at first intoxication	-0.325	0.105	-0.610	-3.102	0.003 **
Age at first significant alcohol use	0.194	0.127	0.300	1.529	0.130
Average days per month drinking					
R^2_{adj} = 0.054, F(1, 51) = 3.97, p = 0.052					
Maternal Education	-0.549	0.276	-0.269	-1.991	0.052 *
Average quantity with age 20 covariates (regressions not significant)					
Maximum quantity maximum days					
R^2_{adj} = 0.075, F(1, 50) = 5.125, p = 0.028					
Maternal Education	-0.621	0.274	-0.305	-2.264	0.028 *
Maximum quantity maximum days with age 20 covariates (regressions not significant)					

* $p < .05$, ** $p < .01$, *** $p < .001$

In the analyses with amygdala-BA 32 connectivity and ADS as the outcome measure (see Table 20), the full model with early alcohol covariates was significant (R^2_{adj} = 0.094, F(7, 80) = 2.05, p = 0.052); however, within this model only the covariate, age at first intoxication, was associated with alcohol dependence at age 20 (B = -0.30, SE = 0.11, t = -2.78, p = 0.007, $r^2_{partial}$ = -0.31). Similarly, only highest level of maternal education was significant for the analysis with "average days per month" using alcohol as the outcome measure (previously reported, see Table 20). With "maximum number of days using maximum quantity of alcohol" as the outcome (R^2_{adj} = 0.11, F(3, 50) = 2.49, p = 0.056), again only highest level of maternal education was significant (B = -0.60, SE = 0.27, t = -2.24, p = 0.030, $r^2_{partial}$ = -0.31). The analyses with "average quantity of alcohol used" and "average quantity of alcohol used" were not significant. None of the analyses with the age 20 internalizing covariates entered into the regression were significant.

Table 20. Regression results for chronicity group status and negative amygdala-BA 32 connectivity

Variable	B	SE B	*B*	t	*p*
ADS					
(regressions not significant)					
ADS with age 20 covariates					
(regressions not significant)					
ADS with early alcohol covariates					
R^2_{adj} = 0.094, $F(7, 80) = 2.05$, $p = 0.052$					
Maternal Education	-0.060	0.228	-0.029	-0.263	0.793
Age at first intoxication	-0.304	0.109	-0.571	-2.784	0.007 **
Age at first significant alcohol use	0.172	0.132	0.266	1.305	0.196
Chronic	0.162	0.282	0.067	0.575	0.567
Non Chronic	-0.053	0.241	-0.026	-0.221	0.825
Amygdala-BA 32 Connectivity	-0.254	0.200	-0.237	-1.270	0.208
Amygdala-BA 32 X Chronic	0.090	0.304	0.042	0.297	0.767
Amygdala-BA 32 X Non Chronic	0.324	0.267	0.198	1.214	0.229
Average days per month drinking					
R^2_{adj} = 0.054, $F(1, 51) = 3.97$, $p = 0.052$					
Maternal Education	-0.549	0.276	-0.269	-1.991	0.052 *
Average quantity with age 20 covariates					
(regressions not significant)					
Maximum quantity maximum days					
R^2_{adj} = 0.105, $F(3, 50) = 2.49$, $p = 0.056$					
Maternal Education	-0.604	0.270	-0.296	-2.236	0.030 *
Chronic	0.416	0.352	0.166	1.182	0.243
Non Chronic	-0.024	0.306	-0.011	-0.079	0.937
Amygdala-BA 32 Connectivity	0.228	0.127	0.243	1.796	0.079
Maximum quantity maximum days with age 20 covariates					
(regressions not significant)					

* $p < .05$, ** $p < .01$, *** $p < .001$

Similarly, in the analyses with amygdala-BA 24 connectivity and ADS as the outcome measure (see Table 21), the full model with early alcohol covariates was significant (R^2_{adj} =

0.098, *F(*5, 80) = 2.46, *p* = 0.032); however, again within this model only age at first intoxication was associated with alcohol dependence at age 20 (B = -0.32, SE = 0.11, *t* = -2.91, *p* = 0.005, $r^2_{partial}$ = -0.32). Similarly, only highest level of maternal education was significant for analyses with the LHD drinking outcomes at age 20. These associations are previously reported above (See Table 21). The analysis with "average quantity of alcohol used" was not significant. None of the analyses with age 20 internalizing covariates entered into the regression were significant.

Table 21. Regression results for chronicity group status and negative amygdala-BA 24 connectivity

Variable	B	SE B	*B*	t	*p*
ADS					
(regressions not significant)					
ADS with age 20 covariates					
(regressions not significant)					
ADS with early alcohol covariates					
R^2_{adj} = 0.098, *F(*5, 80) = 2.46, *p* = 0.032					
Maternal Education	-0.075	0.226	-0.036	-0.331	0.742
Age at first intoxication	-0.318	0.109	-0.597	-2.907	0.005 *
Age at first significant alcohol use	0.180	0.131	0.278	1.375	0.173
Chronic	0.224	0.282	0.093	0.792	0.431
Non Chronic	0.021	0.238	0.010	0.086	0.932
Amygdala-BA 24 Connectivity	0.099	0.128	0.084	0.776	0.440
Average days per month drinking					
R^2_{adj} = 0.054, *F(*1, 51) = 3.97, *p* = 0.052					
Maternal Education	-0.549	0.276	-0.269	-1.991	0.052 *
Average quantity with age 20 covariates					
(regressions not significant)					
Maximum quantity maximum days					
R^2_{adj} = 0.075, *F(*1, 50) = 5.125, *p* = 0.028					
Maternal Education	-0.621	0.274	-0.305	-2.264	0.028 *

Maximum quantity maximum days with age 20 covariates
(regressions not significant)

* $p < .05$, ** $p < .01$, *** $p < .001$

Additionally, an ANOVA was conducted to determine whether there were differences in ADS total score, amygdala reactivity, and amygdala-BA 32 and 24 connectivity as a function of onset or chronicity of any disorder (early onset before age 15, later onset at age 15 or 17, diagnosis at both early and late periods) relative to individuals never meeting criteria for any disorder. Results were not significant. However, when the ANOVA was rerun to assess potential effects of having met criteria for a disorder in late adolescence (late onset and combined group versus early onset), there was a difference in ADS total score, such that individuals most recently meeting criteria for a disorder (age 15 or 17) had higher levels of alcohol dependence relative to those with early diagnoses (only met criteria before age 15), significant at trend ($F(1, 103) = 2.76$, $p = 0.068$).

7.0 DISCUSSION

Broadly, the purpose of this study was to explore the effects of childhood/adolescence internalizing problems—chronicity, severity, or as DSM disorders—on alcohol use and dependence at age 20. The pattern of findings indicated that, generally, internalizing problems were only related to alcohol use and dependence when they co-occurred with externalizing problems, including early age of first intoxication. Overall, the analyses involving Question 1—with continuous measures of internalizing severity and chronicity, tested in combination with externalizing problems, amygdala reactivity, and ACC-amygdala functional connectivity—yielded the most significant results, although not all of the results were in the hypothesized directions (See Table 8 for summary of significant findings). Within the Question 2 analyses—which included similar variables as Question 1 but which defined internalizing and externalizing categorically, based on DSM diagnostic criteria or clinically-significant symptomatology—only the covariates, age of first intoxication in the ADS analyses and maternal education in the LHD analyses, demonstrated a significant effect on alcohol use and dependence at age 20, with the exception of a significant difference in ADS total score observed between individuals in the Combined Internalizing/Externalizing group relative to the comparison group, with the Combined group demonstrating higher ADS scores relative to the group of individuals never having met criteria for a DSM-IV internalizing or externalizing disorder (see Table 15 for summary of significant findings). The only analysis in which this result was significant was when BA 24 was included in the model, although this variable was not significantly associated with

the alcohol outcome. As discussed in the results section, the analyses of categorical chronicity did not yield any statistically significant findings aside from the covariates.

Across the regression analyses, the significance of internalizing versus externalizing symptomatology depended on both the outcome measures used and the covariates entered, suggesting that different factors contribute to alcohol dependence relative to frequency and level of alcohol consumption. These differences will be discussed in more detail below. Additionally, although the five alcohol outcome measures were significantly correlated, only ADS scores were related to externalizing chronicity and severity scores (positive correlation). Three of the LHD outcomes, which were used as a less severe outcome of problem alcohol use (relative to the ADS), were negatively associated with internalizing chronicity and severity, but only trended toward statistical significance. This difference, however, suggests that internalizing and externalizing symptomatology in childhood and adolescence predicts to different categorizations of problematic alcohol use at age 20, with higher externalizing symptoms potentially indicative of future alcohol dependence and higher internalizing symptoms potentially indicative of decreased quantities of alcohol consumed at age 20. Although the self-medication hypothesis supports the role of internalizing symptomatology predicting to increased drinking behaviors (Kuntsche et al., 2006), this result may not be wholly inconsistent with the extant literature, as there is also evidence suggesting a protective role of internalizing behaviors to alcohol use, potentially through depression/anxiety leading to social withdrawal therefore limiting access to deviant peers and alcohol, and/or through anxiety leading to worry about the consequences of drinking, which might lead to avoidance of alcohol

and situations in which alcohol may be present (Fite, Colder, & O'Connor, 2006; Siewert, Stallings, & Hewitt, 2004).

Additionally, it is worth noting again and further exploring the correlations between the covariates and the target study variables. Unexpectedly, highest level of maternal education was positively associated with externalizing scores, such that individuals whose mothers obtained a high school diploma or higher had greater externalizing scores relative to those individuals whose mothers had less than a high school education. As this finding is not easily explainable, it is possible that this association may be due to a different factor that, while associated with level of maternal education, was not explicitly examined in this study. Maternal education was also negatively associated with two of the LHD measures assessing frequency of alcohol consumption (average days per month using alcohol and maximum days using maximum quantity of alcohol). The age 20 internalizing measures were positively correlated with ADS scores but not with the LHD measures. Interestingly, the most significant predictor of alcohol dependence at age 20 was age at first intoxication, which was also not surprisingly correlated with externalizing symptoms, given the positive correlation between externalizing symptoms and ADS. The younger the age of onset, the greater the alcohol dependence scores observed. Although not explicitly stated as a hypothesis in the current study, this finding is consistent with previously reported results indicating that adolescents who begin drinking before age 15 are four times more likely to develop alcohol dependence later in life (Grant & Dawson, 1997).

7.1 MAIN EFFECTS OF NEUROIMAGING TASK

As expected, the present study yielded robust main effect of task clusters within the amygdala, which were then extracted for use in the regressions. This approach was taken in the present study to eliminate the risk of double correlations resulting when data are extracted from a cluster identified by the effects of a variable of interest and then used in regression and structural models investigating that variable (Vul, Harris, Winkielman, & Pashler, 2009). It should be noted that this is a more conservative approach relative to the majority of findings reported in the introduction, which interrogated neurobiological effects within neuroimaging software (usually SPM) as a function of disorder group. Additionally, these studies sometimes employed a lower statistical threshold (e.g., small-volume correction), which has the potential to inflate the significance of reported results. When amygdala reactivity was tested as moderator in the regression analyses, there were no significant main effects or interactions in any of the analyses. However, as predicted amygdala reactivity was positively correlated with internalizing symptoms (e.g., Fales et al., 2007; Fu et al., 2007; Grimm et al., 2007; Harvey et al., 2005; Sheline et al., 2001), although, there was no relation with externalizing scores.

The two significant ACC regions that emerged from the connectivity analyses, dorsal/supragenual ACC (BA 32) and ventral ACC (BA 24) and direction of the effect (negative connectivity), were consistent with expected results based on the literature reviewed in the introduction. These regions have known negative connections with the amygdala (Roy, Shehzad, Margulies, Kelly, Uddin, Gotimer, Biswal, Castellanos, & Milham, 2009; Stein, Wiedholz, Bassett, Weinberger, Zink, Mattay & Meyer-Lindenberg, 2007;) and have been observed to show dysfunction in individuals with depression (Johnstone, van Reekum, Urry,

Kalin, & Davidson, 2007; Matthews, Strigo, Simmons, Yang, & Paulus, 2008). These specific functional pathways have not been explicitly explored within the context of externalizing disorders; however, similar fronto-amygdala connectivity has been shown to be disrupted in ADHD and CD (for review, see Rubia, 2010).

7.2 ALCOHOL OUTCOME DIFFERENCES

Results of the analyses differed as a function of which alcohol outcome was entered into the regression. Unfortunately, due to the ongoing data coding and cleaning that was not yet completed at the time of analysis, data for the LHD outcomes were only available for approximately half of the total sample. Those results with alcohol dependence (ADS) as the outcome measure will be discussed first. For the ADS outcome measures, overall, the analyses assessing chronicity and severity yielded similar results. However, contrary to the stated hypothesis, there were no main effects of amygdala reactivity on any of the alcohol outcome measures. Nor were there any significant interactions. When assessing the effects on alcohol dependence at age 20 (ADS), externalizing symptomatology emerged as the primary predictor to alcohol dependence when covarying for the effects of both maternal education and age 20 internalizing measures (i.e., BAI, BDI). However, the best fit of the data predicting to ADS was with the early alcohol covariates (age of first intoxication and age of first significant alcohol use). This model accounted for 22% of the variance, with age of first intoxication and internalizing disorders significantly predicting to ADS at age 20. These data suggest that whereas externalizing symptomatology does predict significantly to alcohol dependence at age

20, internalizing severity in conjunction with age at first intoxication may be a stronger predictor of alcohol dependence.

The same pattern was observed when the negative connectivity scores between the amygdala and BA 32 and BA 24 were tested as moderators (separately), with the model including the early alcohol predictors accounting for between 21 and 25% of the variance, again with no significant effects of connectivity. Additionally, when examining the highest internalizing and externalizing mean scores across ages 10 to 17 (severity scores), age at first intoxication was significant in conjunction with the interaction between internalizing and externalizing severity scores, predicting 24-25% of the variance. Unexpectedly, and for reasons that remain unclear, however, within this interaction, it was those individuals with low externalizing scores, regardless of internalizing scores, who demonstrated consistently high levels of alcohol dependence. Nonetheless, the highest scores were observed in the individuals high on both measures.

The three-way interaction between internalizing and externalizing scores and connectivity was only significant in the analyses that tested negative amygdala-BA 24 connectivity both with and without age 20 internalizing covariates. Within this analysis, the group that demonstrated the highest level of alcohol dependence was the group high on both internalizing and externalizing symptoms that was also low in negative amygdala-BA 24 connectivity. This finding is consistent with what was hypothesized about this group. Unexpectedly, however, the group demonstrating the lowest alcohol dependence scores comprised those individuals who were high internalizing/low externalizing who were also in the low connectivity group. This finding, although inconsistent with the predicted results, is

consistent with the idea that internalizing symptoms may be a protective factor against alcohol dependence. This same association was observed within the significant three-way interaction when examining the highest internalizing and externalizing mean scores across ages 10 to 17. Additionally, within this analysis, the group comprising individuals low on both internalizing and externalizing symptomatology demonstrated both the highest and lowest level of alcohol dependence as a function of connectivity. This difference was in the predicted direction, with those in the low connectivity group demonstrating the highest levels of alcohol dependence at age 20.

When the less severe alcohol outcome measures were entered as the covariates, again there were no significant main effects or interactions involving amygdala reactivity. Within these analyses, only two outcomes were significant, average days per month using alcohol and maximum number of days using maximum quantity of alcohol. These were the two measures observed to be correlated with maternal level of education. Given that significant correlation, it is not surprising that maternal education was a significant predictor of frequency of alcohol use. Because of this small sample size, the early alcohol covariates were not examined with the LHD outcomes; therefore, the effects of age of first intoxication in conjunction with internalizing symptomatology seen with the ADS outcome could not be investigated within these analyses. The significant positive association between externalizing chronicity score and alcohol outcome observed with ADS scores was also present with maximum days consuming maximum amount of alcohol; however, this was no longer significant when covarying for age 20 internalizing covariates.

Significant effects were observed as a function of negative amygdala-BA 32 and 24 connectivity, but only when the average internalizing and externalizing scores (chronicity) were entered into the analysis. As with the ADS analyses, the significant findings depended on which covariates were entered into the analysis as well as which alcohol outcome was being investigated as the outcome. With average quantity of alcohol consumed as the outcome, only the analysis with BA 32 connectivity was significant. Within this regression, the analysis with the age 20 covariates was the best fit, explaining 24% of the variance. Similarly to the ADS analyses, there was a positive association between externalizing scores and average quantity of alcohol consumed. The interaction between internalizing and externalizing scores was also significant with the greatest quantities observed in those individuals high on externalizing score regardless of internalizing scores. Interestingly, the lowest scores were those individuals who were high on internalizing but low on externalizing, again offering support for the protective role of internalizing symptomatology against problematic alcohol use. Further, the three-way interaction was also significant. Again, the highest scores were observed among individuals with both high internalizing/externalizing scores and the lowest with those low on both measures. Unexpectedly, the influence of BA 32 connectivity on these associations was in opposite directions, with the highest alcohol outcome scores (average quantity consumed) observed in the group high on both internalizing and externalizing scores with high connectivity and the lowest in the group low on both internalizing and externalizing scores with high connectivity. The pattern of results with maximum quantity of alcohol consumed as the outcome were similar to these, except the three-way interaction was not significant. It should be noted that

these interactions should be interpreted with caution, given the small sample sizes within each group, and replication is necessary to confirm these findings.

Finally, with self-reported maximum days using maximum quantity of alcohol as the outcome measure, there was a significant three-way interaction, but only when negative amygdala-BA 24 connectivity was entered into the analysis. Similar to the other three-way interactions presented, the highest alcohol scores were in the individuals high on externalizing scores, including those who were also high on internalizing scores. Consistent with the other significant three-way interactions, the lowest scores were observed in those individuals who were low on externalizing scores but also high on internalizing symptomatology. Unexpectedly, within this interaction, BA 24 connectivity demonstrated the opposite pattern than BA 32, with the highest scores in the high internalizing/high externalizing group with low connectivity and the lowest in the low internalizing/low externalizing group with low connectivity, although it has been BA 32 that has been previously reported to have decreased functional connectivity in depressed samples (Cullen et al., 2009).

To attempt to disentangle these diverging and unexpected variations as a function of region of connectivity, a consideration of the neuroanatomical connections associated with BA regions 24 and 32 may prove useful. One difficulty is that studies frequently group BA 24 and 32 together and do not discuss them separately (e.g., Johansen-Berg et al., 2007, Morecraft & Tanji, 2009). In addition, the majority of the neuroanatomical work examining the connectivity between these regions has been conducted in animal models, which while informative, has limitations. Nonetheless, studies examining the projections between regions of the PFC and the amygdala in rhesus monkeys suggests some divergent findings with respect to cingulate areas

24 and 32 depending on the region explored. One study indicated that the cingulate area 24 had among the densest projections investigated, and further, that it sent more projections to the amygdala than it received (Ghashghaei, Hilgetag, & Barbas, 2007). The cingulate area 32 had a slightly greater number of output projections relative to inputs; however, the density of connections between area 32 and the amygdala was considerably weaker. Additionally, prior work has indicated that direct communication between the cingulate cortex and other limbic regions occurs primarily through areas 24 and 25 (Paus, 2001). Given these data suggesting a greater number of outgoing projections to the amygdala, the reduced coupling of the connectivity involving BA 24 observed in this study may correspond to less regulation of the amygdala by the PFC, and prior studies reviewed earlier have suggested that reduced coupling is associated with difficulty regulating emotions. Moreover, the alcohol outcome measure that was significant in the analyses involving BA 24 was ADS, which is a more severe measure of alcohol use relative to the alcohol outcome measure that was significant in the analyses involving BA 32 (average quantity of alcohol consumed). Therefore, it may be that the predicted direction of connectivity (low connectivity associated with higher alcohol use) may be specific to the context of alcohol measures assessing dependence. The generalizability of these anatomical findings to humans, however, is limited, as area 32 in the monkey does not directly correspond to BA 32 in humans (Ongur & Price, 2000). Further, it is again noted that the connectivity results should be interpreted with caution, as these differences varied as a function of several variables: alcohol outcome variable, chronicity versus severity scores, and covariates, which stresses the need for replication of these findings.

Overall, results from this study varied as a function of whether alcohol dependence or level/frequency of alcohol consumption was used as the outcome. With alcohol dependence as the outcome, the best predictor was the combination of age of first intoxication and high level of internalizing symptomatology. With LHD scores as the outcome, highest level of maternal education achieved was the best predictor for the measures assessing frequency of alcohol use. Connectivity, in conjunction with level of internalizing and externalizing symptomatology, was the best predictor in the analyses assessing quantity of alcohol consumed, with differing effects of BA 32 and BA 24 connectivity. Overall results support findings from the literature suggesting that internalizing symptomatology (in conjunction with low externalizing scores) may be protective against problematic alcohol use/dependence unless the high internalizing symptomatology is also comorbid with high externalizing or with early onset of alcohol intoxication (Fite, Colder, & O'Connor, 2006; Siewert, Stallings, & Hewitt, 2004). Across the majority of the results, it was this group that demonstrated the highest scores on the alcohol outcome measures. These effects are exacerbated by the strength of the negative connectivity between the amygdala and BA 32 and BA 24, with higher BA 32 connectivity and lower BA 24 connectivity yielding higher rates of alcohol consumption, although these divergent effects were observed in different analyses (average quantity versus maximum days maximum quantity).

7.3 LIMITATIONS

Several limitations of the current study must be noted. First, although this study was primarily interested in investigating the development of AUD, the comorbidity between alcohol use and substance use (predominantly marijuana use) was very high, with over half of the men having used marijuana at least once per month and a quarter using marijuana at least 15 days per month. This comorbidity makes results difficult to interpret in terms of disambiguating the role of alcohol versus drugs. Because of the high overlap between alcohol use and drug use, it was not feasible to exclude for substance use, as this would have eliminated a high number of participants and would have artificially created groups in a population in which "pure" cases are rare. Within this study, the specific effects of drug use were not studied, and therefore it cannot be said with certainty that these results are specific to alcohol use and dependence alone, nor what potential effects the drug use in childhood and adolescence may have had on brain development or how this early drug use may influence the alcohol outcome measures assessed at age 20.

Second, as highlighted in the results section, this sample's diagnostic group frequencies did not lend itself to testing the hypotheses in Question 2 as initially planned. Within this sample, there were very few individuals who met criteria only for an internalizing diagnosis (n = 8); rather the majority of individuals with DSM-defined psychopathology met criteria for either externalizing disorders only (n = 35) or were comorbid for both internalizing and externalizing (n = 19). Whereas the primary goal of this study was to examine the effects of internalizing symptomatology on AUD, the demographic composition of this study lends itself more to understanding the externalizing pathway, at least when investigating the effects of the most

severely impaired individuals (defined by meeting criteria for DSM-IV diagnoses). This limitation also applies to the planned comparisons of internalizing chronicity and severity. Due to the small group sizes, the internalizing and externalizing groups were combined; therefore, the lack of findings within these analyses cannot be attributed to either internalizing or externalizing disorders alone. To address these limitations and investigate further the effects of having a DSM-IV internalizing diagnosis in childhood and adolescence on later alcohol use and dependence, longitudinal high-risk samples may be useful due to the higher incidence of internalizing disorders in children who have family members with a lifetime history of mood disorders (Weissman, Leckman, Merikangas, Gammon, & Prussoff, 1984; Williamson, Birmaher, Axelson, Ryan, & Dahl, 2004).

Third, given that one of the most significant predictors of frequency of alcohol use at age 20 was level of maternal education, the labeling of this study as a working-class sample must be emphasized. Prior work has demonstrated that there is a significant association between growing up in a risky family and/or coming from a low socioeconomic status (SES) background, and engaging in risky behaviors, such as alcohol and drug use; therefore, this finding is not surprising (Maggs, Patrick, & Feinstein, 2008; Repetti, Taylor, Seeman, 2002). However, in studies assessing a broader range of SES in much larger samples, the association between SES and alcohol use is not always present, and additionally, some studies suggest that alcohol use is more common in higher-income households (e.g., Melotti, Heron, Hickman, Macleod, Araya, & Lewis, 2011). Replication of this study in different samples comprising a broader range of SES is necessary.

An additional limitation of this study is the high number of tests conducted, which increases the likelihood of having observed several results by chance. To address questions 1 and 2 of this study, 30 tests each were conducted to investigate the moderating effects of 3 variables (amygdala reactivity, amygdala-BA 32 connectivity, amygdala-BA 24 connectivity) on the relation between the 4 independent variables (internalizing and externalizing severity and chronicity) and the 5 outcome variables (4 LHD measures and 1 ADS measure). Additionally, as the analyses involving the ADS outcome were run with and without covariates, a total of 42 tests per question were conducted. For question 1, 21 of these tests demonstrated a significant effect of either the independent variables or the interaction between independent variables or moderators. For question 2, only one of the regressions was significant. Thus, for question 2, which interrogated the data using DSM diagnostic criteria or clinically-significant symptomatology, there is weak support for the hypotheses tested, especially given the very small number of significant findings. For question 1, half of the analyses conducted resulting in significant findings; however, as previously discussed some of these results were not in the predicted direction, particularly with respect to the connectivity findings. Therefore, further exploration of these results is warranted.

Further, there are several important differences between this study and those highlighted in the introduction that may account, at least in part, to the lack of findings consistent with the predicted hypotheses. First, the majority of studies previously conducted focused on differences as a function of DSM diagnoses rather than based on continuous measures (e.g., Birmaher et al., 1998; Fales, et al., 2007, Fu et al., 2007; Gentili et al., 2008). Notably, prior studies exploring a link between internalizing symptoms and amygdala reactivity,

similar to this study, reported non-significant results (Killgore, Gruber, & Yurgelun-Todd, 2007; Killgore & Yurgelun-Todd, 2005, 2006; Telzer et al., 2008). The analyses planned for Question 2 would have allowed for a more direct comparison with prior findings; however, Question 2 did not yield significant differences as a function of diagnostic group potentially due to the fact that the "internalizing disorder only" was too small to allow for comparison as a separate group. Additionally, although the retention rates in this study are high for a 20-year longitudinal study (~75% response rate at age 20), there were individuals for whom there were missing data across assessments; therefore, the continuous variables approach was taken to maximize the sample size. Additionally, several questionnaires were aggregated, combining measures of depressive symptoms with measures assessing anxiety symptoms. There was sufficient compelling evidence to support combining the two categories of internalizing assessments (Beesdo et al., 2010; Krueger, 1999; Lahey et al., 2008; Wittchen, Stein, & Kessler, 1999). Nonetheless, differences in neurobiological correlates between depressive disorders and anxiety disorders have been observed, and it is worth exploring these potential differences in a similar study in the future. Because this was not investigated in this sample, it is not possible to determine if the lack of significant findings may be due to the combination of the two groups, nor if the significant findings may be driven by one group more than the other.

Finally, the current study was conducted in only males. Given the known differences in rates of both internalizing disorders and externalizing disorders in adult males and females, with females tending toward depression/anxiety and men tending toward externalizing behaviors such as antisocial behavior and substance abuse (Brady & Randall, 1999; Galambos, Leadbeater, & Barker, 2004; Nolen-Hoeksema, 2001; Kessler, Berglund, Demler, et al., 2003), it is not readily

apparent whether some of the findings of this study may be due to the male only composition of the sample. This underscores the need to replicate these results and in a mixed-sex sample in order to determine generalizability of these results. Whereas rates of affective disorders differ in adulthood, adolescent rates of these disorders do not begin to differentiate until late in adolescence (Johnston, O'Malley, Bachman, & Schulenberg, 2008; Young et al., 2002). Of particular relevance to this sample is the potential role of sex differences between males and females with respect to the socialization process that occurs during adolescence, specifically exposure to deviant peer influences, which may contribute to the differing rates of affective disorders that begin to develop at this age. In this sample of males, the presence of high internalizing symptoms in the absence of high externalizing symptoms in adolescence appeared to be protective against problematic alcohol abuse at age 20. One potential explanation for this finding may relate to the possible lack of affiliation with a deviant peer group that may result from social isolation due to high levels of internalizing symptoms. Peer relationships, and in particular deviant peer relationships, have been previously found to be a significant contributor to the development of problematic alcohol use in adolescence (Barber, Bolitho, & Bertrand, 1998; Bates & Labouvie, 1995; Curran, Stice, & Chassin, 1997). Sex differences emerge at this age in both availability and acceptance of deviance, with males experiencing more freedom to experiment with alcohol (Byrnes, Miller, & Shafer, 1999), putting males at a higher risk of engaging in and maintaining problematic alcohol use through continued reinforcement by peers (Suls & Green, 2003). These sex differences in peer relationships place males at high risk for initiation and continuation of alcohol consumption; therefore, it is not surprising that the potential absence of a male peer group resulting from isolation due to depression/anxiety may

result in lower rates of problematic alcohol use due to both potentially limited access to alcohol and lack of negative peer influences. Further exploration of peer influences in conjunction with internalizing/externalizing symptoms in future samples may help elucidate this potential mechanism.

7.4 CONCLUSIONS

It is certainly not surprising that age of first intoxication was a significant predictor of alcohol dependence at age 20. As with early onset of other mental illnesses, the earlier the onset, the poorer prognosis into adulthood. It is interesting that high levels of internalizing symptomatology were only significant within the context of early intoxication. It is possible that those adolescents who may be exhibiting stress as a consequence of high internalizing may find relief from the effects of intoxication. As previously noted, this positive reinforcement is difficult to break from a physiological standpoint, and further, once an adolescent establishes alcohol intoxication as an effective coping strategy, the motivation to develop more adaptive ways to handle stress may diminish (Kushner, Abrams, & Borchardt, 2000). This behavior may be further exacerbated by the neurotoxic effects of alcohol on the developing brain (Monti et al., 2005). Therefore, this early pattern may easily continue into alcohol dependence as an adult. This tendency to drink and make poor decisions is certainly exacerbated or potentially caused by the weaker negative connectivity between the subcortical regions of the brain and the prefrontal regions entrusted to regulate negative emotion. To ascertain the developmental sequence of these neurobiological events, a study similar to this one would need to be

conducted that began scanning the adolescents at an early time point, prior to the initiation of problematic alcohol/internalizing/externalizing behaviors. Likely however, it is not a simple association, and there is probably a neurobiological predisposition that becomes maintained through consequences resulting from a chronic illness. On the other end are the adolescents who are high on internalizing measures who do not begin drinking early. This group does not show high levels of alcohol dependence at age 20; however, it remains to be seen whether this group may go on to develop higher rates of problematic alcohol use as they fully transition into adulthood, and alcohol use becomes a socially-acceptable companion to social interactions.

Based on the findings of this study, it is also those individuals high on both externalizing and internalizing that demonstrate high scores on alcohol outcome measures. In these individuals, it may be the case that internalizing symptoms leads to the initiation of alcohol use (self-medication hypothesis), which may in turn contribute to externalizing behaviors in the context of alcohol intoxication. It may be that the need to dampen negative affect (potentially seen in high internalizing) is a stronger motivator than getting drunk for the sake of fun, which might be the motivating factor behind adolescents who are high on externalizing symptoms only (low internalizing). Those adolescents who are higher on the externalizing spectrum are also likely exposed to more delinquent peers and may have more ready access to alcohol than their non-externalizing counterparts, which highlights a potential interaction between environmental factors and a predisposition to high negative affect. This unfortunate union of a high internalizing/high externalizing presentation also maintains maladaptive coping strategies. Finally, it appears that internalizing in the context of low externalizing may be protective from problematic alcohol use in the future. It is not clear whether this group may be low

externalizing because they do not drink or if it may be that at the extreme, this is a group who is too anxious or socially isolated to interact much with peers, which then protects them from alcohol-exposure. Again, as these young adults mature, it would be interesting to see in what ways these individuals cope with their internalizing symptoms as they may begin to experiment more with alcohol. Additionally, as previously mentioned, marijuana use is very high in this sample; therefore, it would be interesting to track marijuana exposure through childhood into early adulthood as a consequence of internalizing symptomatology.

7.5 CLINCIAL IMPLICATIONS AND FUTURE DIRECTIONS

Although there was a lack of significant findings supporting the hypotheses in this study (e.g., the proposed moderating role of neurobiological correlates of negative affect (amygdala reactivity)), and there were some results that were contrary to the predicted hypotheses (e.g., direction of functional connectivity effects), this study makes important contributions to understanding the role of internalizing symptomatology in the development of AUD in early adulthood. Specifically, this study highlights the need to assess not only internalizing symptomatology, but also externalizing symptomatology in determining future risk for potential problematic alcohol use and dependence. This conclusion is supported by the pattern of findings indicating that the level of symptomatology in *both* of these domains was predictive of problematic alcohol use in early adulthood. Additionally, as age of first intoxication was demonstrated to be an important predictor of future alcohol problems, interventions aimed at delaying the initiation of alcohol use in adolescence would appear warranted. This study also

addressed some of the limitations of the previously reviewed literature in that it 1) investigated both internalizing and externalizing symptomatology and 2) examined these questions within the same longitudinal study. Further, this study used fMRI scans obtained all at the same age, thereby allowing for the exploration of the role of neurobiological contributors to alcohol use and dependence, while eliminating potential effects due to normal brain development.

In this study, there were also notable results related to the neurobiological correlates of emotion processing that merit further investigation. First, amygdala reactivity to emotional stimuli did not make a significant contribution to predicting alcohol use/dependence outcomes. However, as noted previously, this study did not have a substantial internalizing only group, which may have affected the lack of significant findings. Replication in a broader sample of individuals with more varying degrees of internalizing and externalizing symptomatology is therefore needed to confirm this apparent null and unexpected finding. It may be the case that a potential link between amygdala connectivity and alcohol abuse/dependence at age 20 was not observed because amygdala reactivity was not correlated with externalizing symptoms, and externalizing symptoms were a strong predictor of alcohol dependence.

Second, the observed effects of functional connectivity in this study must also be explored further as only one of the two ACC regions (BA 24) was in the predicted direction. The fact that it was amygdala-ACC connectivity and not reactivity that emerged as a significant predictor of alcohol use/dependence cannot be ignored, and indeed future work should probe the role of connectivity—as well as reactivity—in order to provide a more complete neurobiological picture of AUD risk. Finally, the potential protective role of internalizing symptoms in the development of problematic alcohol use/dependence requires further

disentangling to assess in what specific contexts internalizing symptomatology may confer protection against risk and in what contexts it is detrimental. In particular, it will be important to test if this protective finding is still observed in populations with higher rates of internalizing disorders or if this is only observed in samples that are biased toward externalizing disorders.

In sum, additional studies are needed to replicate these novel findings, particularly because of the potential inconsistencies reported between this and previous studies. Continued longitudinal studies of at-risk populations are key to understanding the developmental time course of internalizing and externalizing disorders and AUD in children, adolescents, and young adults. Therefore, continued follow-up with these young adults may uncover trajectories of risk and resilience, as certain subgroups may go on to develop more problematic alcohol abuse/dependence. Results from such studies may in the future aid the development and implementation of effective prevention and treatment studies.

APPENDIX

OPERALIZATION OF VARIABLES OF INTEREST FOR QUESTION 1 ADDRESSING DIMENSIONAL INTERNALIZING VERSUS EXTERNALIZING PATHWAY TO ALCOHOL USE AT AGE 20

Question 1: Are associations between levels of childhood/adolescence internalizing and externalizing symptomatology and alcohol use in early adulthood moderated by differential amygdala reactivity patterns at age 20?

Independent Variables:
Severity:
Internalizing Severity: For each person, CDI total score and MASC total score was standardized and combined to obtain a Z score for each time point. Highest Z score was used in the analysis.
Externalizing Severity: For each person, CBCL Externalizing Factor score and SRD total score was standardized and combined to obtain a Z score for each time point. Highest Z score was used in the analysis.

Chronicity:
Internalizing Chronicity: Mean of all Z scores of internalizing measures across all of the time points attended
Externalizing Chronicity: Mean of all Z scores of externalizing measures across all of the time points attended

Outcome Measure:
Alcohol Use:

Age 20 drinking level as measured by LHD

1. Average days per month
2. Average quantity used
3. Maximum quantity used
4. Maximum number of days using max amount of alcohol

Age 20 level of alcohol dependence as measured by ADS

1. Total Score

Moderator: Amygdala Reactivity

OPERALIZATION OF VARIABLES OF INTEREST FOR QUESTION 2 ADDRESSING CATEGORICAL/DIAGNOSTIC GROUPS OF INTERNALIZING AND EXTERNALIZING PATHWAYS TO ALCOHOL USE AT AGE 20

Question 2: Do individuals with early history of or high-chronicity of internalizing and externalizing disorders demonstrate more severe patterns of drinking at age 20, and is this relation moderated by amygdala reactivity/connectivity?

Independent Variables:
Severity/Early History of Disorders Group Status: Internalizing Only, Externalizing Only, Combined, Comparison—Internalizing and Combined groups collapsed, forming 3 groups.

1. Internalizing Only: The person has met criteria for an internalizing disorder (and no externalizing disorder) between ages 8 and 17 (K-SADS) (N = 8)
   Combined: The person has met criteria for at least one of each internalizing and externalizing disorders between ages 8 and 17 (K-SADS) (N = 19)
2. Externalizing Only: The person has met criteria for an externalizing disorder (and no internalizing disorder) between ages 8 and 17 (K-SADS) (N = 35)
3. Comparison: The person has never met criteria for any disorder (N = 49)

Chronicity Group Status: Chronic Internalizing and Chronic Externalizing collapsed, Non-chronic Internalizing and Non-chronic Externalizing collapsed, Comparison

1. Chronic Internalizing: If he met one of the following criteria in both childhood (8 to 12) and adolescence (15, 17) (N = 4):
   a. Diagnosis of an internalizing disorder (K-SADS)
   b. T score in the borderline or greater range on the CBCL Internalizing Total Factor score (> 63)
   c. Greater than 1 SD above the mean (obtained from a normative group—nonclinical) for the CDI or MASC.

   Chronic Externalizing: If he met one of the following criteria in both childhood and adolescence (N = 14):
   a. Diagnosis of an externalizing disorder (K-SADS)
   b. T score in the borderline or greater range on the Externalizing Total Factor score (> 63)
2. Non-chronic Internalizing: If he met these criteria only in childhood (ages 8 to 12) or only in adolescence (ages 15, 17) (N = 4).
   Non-chronic Externalizing: If he met criteria at only one time point (N = 21)
3. Comparison Group: The person has never met criteria for any disorder (N = 49).

Outcome Measure:
Alcohol Use:

Age 20 drinking level as measured by LHD
1. Average days per month
2. Average quantity used
3. Maximum quantity used
4. Maximum number of days using max amount of alcohol

Age 20 level of alcohol dependence as measured by ADS
1. Total Score

Moderator: Amygdala Reactivity

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