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Neurogenetics of the Externalizing Spectrum
by
Caitlin E Carey

A dissertation presented to
The Graduate School
of Washington University in St. Louis
in partial fulfillment of the
requirements for the degree
of Doctor of Philosophy

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List of Abbreviations

ADHD = attention-deficit/hyperactivity disorder
AFD = age-at-first-drink
ASD = autism spectrum disorder
ASPD = antisocial personality disorder
AUD = alcohol use disorder
BOLD = blood oxygen level-dependent
BP = basepair
CATS = Comorbidity and Trauma Study
CD = conduct disorder
Chr = chromosome
CU = callous-unemotional
DBD = disruptive behavior disorder
DNS = Duke Neurogenetics Study
ESD = externalizing spectrum disorder
fMRI = functional magnetic resonance imaging
GWAS = genome-wide association study
HWE = Hardy-Weinberg equilibrium
LD = linkage disequilibrium
MAF = minor allele frequency
MDS = multidimensional scaling
ODD = oppositional defiant disorder
PC = principal component
PGC = Psychiatric Genomics Consortium
PRS = polygenic risk score(s)
ROI = region of interest
SNP = single nucleotide polymorphism
SUD = substance use disorder
TAOS = Teen Alcohol Outcomes Study
VS = ventral striatum

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ABSTRACT OF THE DISSERTATION

Neurogenetics of the Externalizing Spectrum

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Externalizing spectrum disorders, which include attention-deficit/hyperactivity disorder, oppositional defiant disorder, conduct disorder, alcohol and substance use disorders, and antisocial personality disorder, are characterized by behavioral disinhibition and are thought to be manifestations of a common heritable liability factor throughout the lifespan. However, relatively little is known about their underlying etiology. Here, I probe genetic and neural risk mechanisms for externalizing psychopathology in three complementary studies. First, I report an indirect association between genetic risk for childhood attention-deficit/hyperactivity disorder (ADHD) and problem drinking in young adulthood, mediated by heightened reward-related neural activity within the ventral striatum, among 404 college students. I then provide additional support that such neural activity is a pre-existing risk factor for drinking behaviors by demonstrating that it prospectively predicts early versus late age-at-first-drink among 65 adolescents. Taken together, the results of these studies suggest that heightened reward-related ventral striatum activity is a genetically influenced neural risk marker for the initial stages of drinking behavior and is also related to ADHD genetic risk. Finally, I performed a genome-wide association study (GWAS) of retrospectively reported conduct disorder among 1675 Australian adults enriched for externalizing psychopathology, yielding novel genetic associations with rs12536973 at the single-variant level

and *GOLM1* at the gene level. In the sample of college students used in the first study, both rs12536973 genotype and genome-wide genetic risk calculated based on the results of the GWAS were associated with self-reported psychopathy constructs, and genome-wide genetic risk was additionally associated with blunted anterior insula activity during an emotional face-matching task. Overall, these findings identify potential neurogenetic mechanisms and risk markers for externalizing psychopathology and provide support for etiological relationships across disorders (e.g., ADHD and AUD/drinking behaviors), as well as between pathological and non-pathological externalizing variation (e.g., CD and individual differences in psychopathology among healthy college students).

Chapter 1:

General Introduction

1.1 The Externalizing Spectrum

Externalizing spectrum disorders (ESDs) are characterized by extreme behavioral disinhibition and include attention-deficit/hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), conduct disorder (CD), alcohol and substance use disorders (AUD and SUD), and antisocial personality disorder (ASPD; see Beauchaine & McNulty, 2013, and Beauchaine, Zisner, & Sauder, 2017, for reviews). Lifetime prevalence estimates for ESDs range from 4% for ASPD (Goldstein et al., 2017) to 29% for AUD (Grant et al., 2015), making them relatively common in the population. Moreover, given the nature of ESDs, in which, as their name implies, psychopathology is *externalized*, their societal consequences reach far beyond the individual level. For example, the United States spends an estimated 3.3% of its annual gross domestic product on the consequences of violent crime (Waters et al., 2004), for which ESDs such as CD (Erskine et al., 2016), AUD/SUD (Arseneault, Moffitt, Caspi, Taylor, & Silva, 2000), and ASPD (Yu, Geddes, & Fazel, 2012) are robust independent predictors.

Despite the substantial personal and societal costs of these disorders, few widely utilized, cost-effective, empirically supported treatments exist (Cohen, Feinn, Arias, & Kranzler, 2007; Farmer, Compton, Burns, & Robertson, 2002; Wilson, 2014). With the exception of ADHD and ODD—notably, both early-childhood-onset disorders—the majority of individuals meeting criteria for ESDs forego treatment altogether (Cohen et al., 2007; Goldstein et al., 2017; Merikangas et al., 2011; Wang et al., 2005). Furthermore, in spite of the high degree of homotypic comorbidity (i.e., co-occurrence of disorders in the same individual) and heterotypic continuity

(i.e., sequential diagnosis of different disorders throughout the lifespan) across the externalizing spectrum, most treatments remain disorder-specific (e.g., psychostimulants for ADHD but behavioral interventions for CD; Farmer et al., 2002) and thus fail to adequately address the common underlying externalizing liability (see **Section 1.1.1**; Beauchaine & McNulty, 2013; Beauchaine & Hinshaw, 2015). A better understanding of risk factors for continued impairment, as well as of the biological mechanisms underlying such risk, is thus paramount to developing and disseminating effective treatment and prevention strategies and reducing the personal and societal costs associated with externalizing psychopathology (Beauchaine, Neuhaus, Brenner, & Gatzke-Kopp, 2008).

1.1.1 Structure

As mentioned above, ESDs exhibit a high degree of comorbidity (e.g., 30-50% of children with ADHD also meet criteria for CD; Biederman, Newcorn, & Sprich, 1991) as well as continuity (e.g., children diagnosed with ADHD are greater than 2.5-times as likely as nondiagnosed peers to meet criteria for an SUD in adulthood; Lee, Humphreys, Flory, Liu, & Glass, 2011) and are therefore thought to be driven by a common underlying liability with different manifestations across the lifespan (**Figure 1.1**; Beauchaine & McNulty, 2013; Forbes, Tackett, Markon, & Krueger, 2016). An adult with ASPD, for example, will have likely transitioned from ADHD in childhood, to ODD in grade school, to CD in middle school, to AUD and/or SUD in high school, and finally to ASPD in adulthood (Beauchaine & McNulty, 2013; Moffitt, 1993; Storebo & Simonsen, 2016).

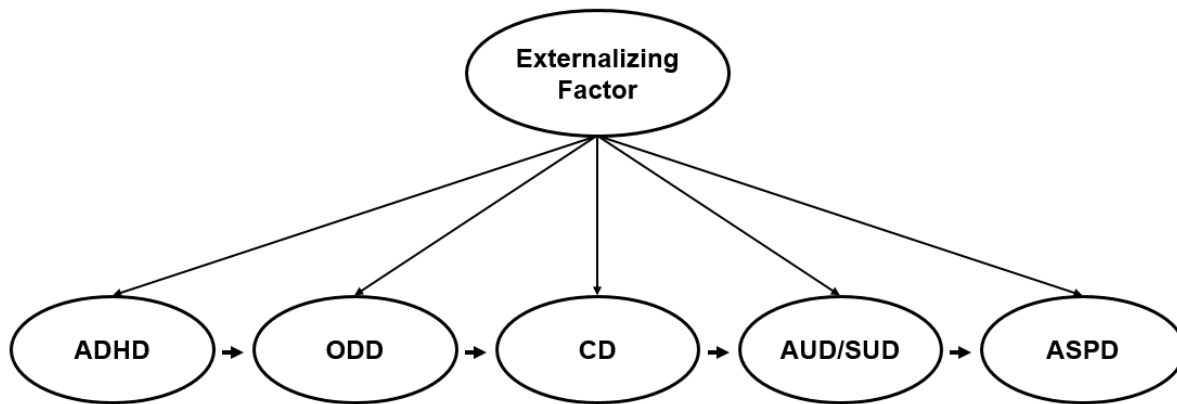


Figure 1.1 Structure and Developmental Trajectory of Externalizing Spectrum Disorders. Latent structure of the externalizing spectrum, with individual psychiatric disorders loading on a single continuous factor. Arrow indicate the developmental trajectory of the externalizing spectrum disorders, beginning with ADHD in childhood and culminating with ASPD in adulthood. Figure adapted from (Beauchaine & McNulty, 2013).

Notably, only a small minority of children with ADHD transition to ASPD in adulthood (Storebø & Simonsen, 2016), and independent risk factors are thought to play a role in disorder-specific outcomes (i.e., callous-unemotional traits in CD and ASPD, Frick & White, 2008; availability of certain drugs in substance-specific SUDs, Kendler et al., 2012, Kendler, Myers, & Prescott, 2007, and Kendler, Prescott, Myers, & Neale, 2003). Nonetheless, family studies (Hicks, Krueger, Iacono, McGue, & Patrick, 2004; Krueger et al., 2002; Young, Stallings, Corley, Krauter, & Hewitt, 2000) and latent factor analyses (Krueger, Markon, Patrick, Benning, & Kramer, 2007; Krueger, Markon, Patrick, & Iacono, 2005; Markon & Krueger, 2005) of these disorders, their symptoms, and related traits (i.e., impulsivity, aggression, and [lack of] empathy) have revealed such behavior to load on one higher-order heritable, continuous “externalizing” construct (Krueger & South, 2009). A conceptualization of ESDs as completely independent thus obscures the common traits and mechanisms underlying lifelong risk.

1.1.2 Etiology

Genetics

Despite their high degree of symptomatic similarity and comorbidity/continuity, little is known about the etiological underpinnings of ESDs. The latent externalizing factor has been shown to be highly heritable ($h^2 \sim 0.80$; Krueger et al., 2002), though few replicable individual genetic risk variants have been identified to date (see Gizer, Otto, & Ellingson, 2016, for review). Earlier studies focused primarily on candidate genes in known biological systems (e.g., *DRD4*, which codes for a dopamine receptor, in ADHD, Faraone, Doyle, Mick, & Biederman, 2001; *ADH1B*, which codes for an enzyme that metabolizes alcohol, in AUD, Li, Zhao, & Gelernter, 2011; and *MAOA*, which codes for enzymes involved in the breakdown of key neurotransmitters, in CD, ASPD, and general aggression, Ficks & Waldman, 2014), with some meta-analytic evidence of association. More recently, genome-wide association studies (GWAS), in which each available variant in the genome is tested for association with the outcome phenotype, have dominated the psychiatric and behavioral genetics literatures, with significant loci having been identified for multiple ESDs (e.g., **Table 1.1**) and broader transdiagnostic constructs (e.g., behavioral disinhibition, Derringer et al., 2015; aggressive behavior in childhood, Pappa et al., 2016). Thus far, GWAS of externalizing psychopathology have both confirmed prior candidate gene associations (e.g., *ADH1B* with AUD; Gelernter et al., 2014) and identified promising novel loci (**Table 1.1**).

Table 1.1 Largest Genome-wide Association Study to Date for Each ESD

Disorder	N_{cases}	$N_{controls}$	Significant SNPs	Citation
ADHD	20,183	35,191	rs11420276, rs1222063, rs9677504, rs4858241, rs28411770, rs4916723, rs5886709, rs74760947, rs11591402, rs1427829, rs281324, rs212178	Demontis et al., 2017
ODD ^a	-	-	-	-
CD	872	3091 ^b	rs16891867, rs7950811, rs11838918, rs1861046	Dick et al., 2011
AUD/SUD ^c	7677	7102	rs1437396, rs1229984 ^d	Gelernter et al., 2014
ASPD	543	9616	rs4714329	Rautiainen et al., 2016

^aNo case-control GWAS of ODD have been performed to date, but see (Aebi et al., 2016) for a multivariate GWAS of ODD subtypes.

^bPrimary analyses were performed using *DSM-IV* CD symptom counts.

^cSelected study is of *DSM-IV* alcohol dependence.

^dSNPs significant in the transethnic meta-analysis. See paper for additional results in ethnic subsamples.

Due to the dimensional nature of the externalizing spectrum, spanning both normal variation in behavior and personality as well as extreme deviations, the underlying genetic architecture is posited to be highly polygenic in nature, composed of thousands of individual variants each of small effect (Gizer et al., 2016; Plomin, Haworth, & Davis, 2009). In the largest GWAS meta-analysis of ADHD to date, for example, the risk allele at the top locus was associated with only 1.11 increased odds of developing ADHD (Demontis et al., 2017). As such, single common variants are unlikely to be clinically useful at the individual level for predicting future diagnoses, though some notable exceptions exist (e.g., *ALDH2* genotype and risk for AUD in East Asian populations; Luczak, Glatt, & Wall, 2006). Nonetheless, GWAS, when combined with methodological advances in bioinformatics and statistical genetics, can provide valuable insights into disorder etiology through functional annotation of top hits (e.g., Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), estimation of genome-wide genetic correlations between putatively related disorders/phenotypes (e.g., Anttila et al., 2016), and prediction of potential intermediate phenotypes in independent samples based on genome-wide genetic risk calculated from summary statistics (e.g., Holmes et al., 2012).

Neural Structure and Function

At the neural level, externalizing psychopathology is likely the result of dysfunction across multiple systems (**Table 1.1**). Studies of ESDs have typically categorized brain regions and paradigms into those influencing “hot” and “cool” executive functions and social-emotional processing (Alegria, Radua, & Rubia, 2016). “Hot” executive functions are goal-directed processes with motivational and affective significance, such as reward- and punishment-based decision-making and reinforcement learning, which are subserved by paralimbic regions of the brain including the orbitofrontal cortex, anterior cingulate, amygdala, parahippocampal gyrus, insula, and ventral striatum (Alegria et al., 2016; Kiehl, 2006; Rubia, 2011). “Cool” executive functions, in contrast, lack an affective component and include processes such as attention, inhibition, and working memory, which are controlled primarily by circuits linking the frontal, parietal, and temporal cortices to the dorsal striatum and cerebellum (Rubia, 2011). Social-emotional processing, sometimes considered in tandem with “hot” executive functions due to regional and paradigmatic overlap (Rubia, 2011), includes functions such as empathy, theory-of-mind, and emotion processing, which are associated with paralimbic regions of the brain such as the amygdala, insula, anterior cingulate, orbitofrontal cortex, and temporal lobe (Lindquist, Wager, Kober, Bliss-Moreau, & Barrett, 2012; Ochsner, 2008; Raschle, Menks, Fehlbauer, Tshomba, & Stadler, 2015).

Table 1.2 Prior Reviews/Meta-analyses of Externalizing Spectrum Neuroimaging Studies

Citation	Phenotype(s)	Modality	Paradigms	<i>N_{studies}</i>	Main Findings ^a
Alegria et al., 2016	DBDs (CD and ODD) and conduct problems	functional	“hot” and “cool” EF, emotion processing, and empathic pain	24	↓ activity in the rostral and dorsal anterior cingulate, medial prefrontal cortex, and ventral striatum
Cortese et al., 2012	ADHD	functional	all available	55	↓ activity across the frontal, parietal, and temporal lobes, as well as in the putamen; ↑ activity in the angular gyrus, middle occipital gyrus, posterior cingulate, middle cingulate, Heschl’s gyrus, and inferior frontal gyrus
McCarthy et al., 2014	ADHD	functional	“cool” EF	20	↓ frontal lobe activity relative to controls
Noordermeer et al., 2016	ODD and CD, w/ and w/o comorbid ADHD	structural & functional	“hot” and “cool” EF	structural: 12 functional: 17	↓ grey matter volume and activity (driven primarily by that of “hot” executive functioning tasks) in the amygdala, striatum, insula, frontal gyrus, and precuneus
Plichta & Scheres, 2014	ADHD, trait impulsivity	functional ^b	reward processing	ADHD: 10 healthy: 12	↓ ventral striatum activation, particularly during reward anticipation, among ADHD vs. controls; ↑ ventral striatum activation among healthy individuals scoring higher on trait impulsivity
Raschle et al., 2015	aggressive behavior	structural & functional	emotion processing and empathy	structural: 8 functional: 9	↓ grey matter volume and activity in the dorsomedial prefrontal cortex and insula
Rogers et al., 2016	conduct problems	structural	-	13	↓ grey matter volume in the insula, amygdala, medial superior frontal gyrus, and fusiform gyrus
Yang et al., 2016	AUD	structural	-	12	↓ grey matter volume in the insula, superior temporal gyrus, striatum, dorsolateral prefrontal cortex, precentral gyrus, anterior cingulate, thalamus, and hippocampus

^aFindings are reported from the broadest analysis in each study (e.g., analyses across structural and functional data, or all task types).

^bStudy considered activation within a ventral striatum region of interest only.

Note. Highlighted studies should be considered representative but not exhaustive. DBD = disruptive behavior disorder. EF = executive functions.

When comparing neural activity across disorders and constructs comprising the externalizing spectrum, as would be expected when studying syndromes that load on both first-order disorder-specific factors and a higher-order common externalizing factor (Beauchaine & McNulty, 2013), both similarities (e.g., Alegria et al., 2016; Frodl, 2010) and disorder-specific differences (e.g., Plichta & Scheres, 2014; Rubia, 2011) have been noted. For example, differences in reward-related activity within the ventral striatum, a region critical to generating and orchestrating motivated behavior including reward sensitivity and reinforcement learning (Berridge & Kringelbach, 2015), have been independently associated with ADHD (Plichta & Scheres, 2014), disruptive behavior disorders (DBDs; i.e., CD and ODD; Alegria et al., 2016; Noordermeer et al., 2016), AUD/SUD (Balodis & Potenza, 2015; Hommer, Bjork, & Gilman, 2011), drinking patterns (Weiland, Zucker, Zubieta, & Heitzeg, 2016), and trait impulsivity (Kennis, Rademaker, & Geuze, 2013), though directionality of association has been mixed and is the subject of some controversy (e.g., see Balodis & Potenza, 2015; Hommer et al., 2011; Plichta & Scheres, 2014, 2015). In contrast, highlighting potential disorder-specific associations, though ADHD and DBDs frequently co-occur, comparisons have revealed differences in “cool” executive function regions to be somewhat specific to ADHD, while dysfunctions of “hot” executive function and emotion processing regions are unique to DBDs (Alegria et al., 2016; Rubia, 2011). This finding would suggest that youths with comorbid ADHD and DBD have an “extra” neurobiological impairment relative to those with ADHD alone, placing them at increased risk for long-term negative outcomes such as substance (mis)use (Brook, Brook, Zhang, & Koppel, 2010; Wilens et al., 2011).

Notably, one major limitation of prior cross-sectional case-control imaging studies is that it has not been possible to disentangle premorbid risk factors from downstream effects of disorder

expression and related confounds (e.g., medication). This is a particularly salient issue in studies of AUD and SUD, wherein long-term use of substances is thought to alter brain structure and function (e.g., Filbey et al., 2014; Squeglia, Jacobus, & Tapert, 2014). Two complementary approaches—genetic/familial high-risk and longitudinal studies—may be useful in distinguishing [putative] cause from effect. For example, using a discordant twins/siblings design, Pagliaccio and colleagues (2015) demonstrated that decreased amygdala volume among cannabis users is likely a genetically influenced preexisting risk factor for cannabis use, rather than a consequence of use. Longitudinal studies have likewise shown that heightened reward-related activity in the striatum prospectively predicts the onset of substance use (Heitzeg et al., 2014; Stice, Yokum, & Burger, 2013). Using such techniques, the transdiagnostic and specific biological risk factors for ESDs may be elucidated, providing neurobiological targets for treatment and prevention strategies to halt the progression of externalizing sequelae across the lifespan.

1.2 Current Aims

The current collection of studies aims to identify neurogenetic mechanisms underlying various forms of externalizing behavior (i.e., ADHD in **Chapter 2**, drinking behavior in **Chapters 2 and 3**, and CD and psychopathy in **Chapter 4**) using archival data from three complementary, independent datasets covering different developmental periods (i.e., early-to-late adolescence, young adulthood, and middle adulthood). Building on prior literature, these studies focus specifically on identifying premorbid genetically influenced neurobiological risk factors, rather than downstream consequences of disorder expression.

In **Chapter 2**, based on epidemiological observations of enhanced risk for future alcohol and substance use disorders among children diagnosed with ADHD (Charach, Yeung, Climans, &

Lillie, 2011; S. S. Lee et al., 2011), and family studies demonstrating such externalizing continuity to be due primarily to heritable genetic factors (Derks, Vink, Willemsen, van den Brink, & Boomsma, 2014; Quinn et al., 2015), I tested whether a putative transdiagnostic neural phenotype, reward-related blood oxygen level-dependent functional magnetic resonance imaging (BOLD fMRI) activity within the ventral striatum, links genetic risk for childhood ADHD to problematic alcohol use in young adulthood among 404 college students. Such an association would be consistent with developmental models of the externalizing spectrum, which posit that shared genetic and neurobiological risk manifest as different ESDs across the lifespan (Beauchaine & McNulty, 2013; M. K. Forbes et al., 2016).

In **Chapter 3**, using the same fMRI reward paradigm as in **Chapter 2**, I investigated whether reward-related neural activity within the ventral striatum prospectively predicts alcohol use initiation, indexed by relative age-of-first-drink, in a longitudinal neuroimaging study of 65 adolescents, consistent with (Heitzeg et al., 2014; Stice et al., 2013). Given the substantial continuity between ADHD and problematic alcohol use (Charach et al., 2011; S. S. Lee et al., 2011), convergence in the results of **Chapters 2** and **3** would suggest a common genetically influenced neural mechanism underlying both disorders.

Finally, in **Chapter 4**, I performed a GWAS of retrospectively reported CD among 1675 adult participants enriched for externalizing psychopathology. Based on the results of the GWAS, I tested whether genetic risk for CD (i.e., significant GWAS loci and genome-wide genetic risk scores based on summary statistics) was also associated with self-reported psychopathy and neural activity to a social-emotional processing task in the college sample from **Chapter 2**, consistent with literature suggesting emotion processing to be uniquely impaired in DBDs such as CD and adult antisocial behavior and psychopathy (Alegria et al., 2016; Rubia, 2011). Identification of

genetic and genetically influenced neural risk factors related to both CD diagnosis and normal variability in trait psychopathy within a healthy young adult population would provide evidence for a spectrum of externalizing risk, particularly as it relates to antisocial behavior and psychopathy, with common genetic and neurobiological influences.

Chapter 2: Neurogenetic Underpinnings of Childhood ADHD and Problematic Alcohol Use Continuity

2.1 Introduction

Attention-deficit/hyperactivity disorder (ADHD) is characterized by persistent hyperactivity-impulsivity and/or inattention, and is among the most common childhood psychiatric disorders, affecting approximately 5% of children worldwide (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007). In addition to deficits in cognitive (Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005), academic (Barry, Lyman, & Klinger, 2002), and socioemotional (Strine et al., 2006) domains during childhood, evidence suggests a continued pattern of impairment extending to adolescence and adulthood (Shaw et al., 2012). Problematic alcohol use, typically conceptualized as levels of use associated with significant socioemotional, academic, or workplace impairment, is especially prevalent among these individuals, with prospective studies demonstrating that children with ADHD are at substantially increased (e.g., 1.35-1.72) odds of developing alcohol use disorder (AUD) as adults (Charach et al., 2011; Lee et al., 2011). Supportive of a common etiology (Carragher et al., 2014), twin studies have shown that shared additive genetic factors primarily account for this association (e.g., 91-99.8% of phenotypic covariance; Derks et al., 2014; Quinn et al., 2015). However, what neural mechanisms, if any, may mediate the effects of this shared genetic liability remain largely unexplored. Identifying such mechanisms is important not only for better understanding the etiology of ADHD but also for informing the development of

novel strategies for preventing comorbid dysfunction by providing target intermediate neural phenotypes that can serve as risk biomarkers.

Differences in neural response to reward may be one way through which shared genetic risk for ADHD and problematic alcohol use manifests. For example, emotional and motivational facets of impulsivity (i.e., positive and negative urgency) prospectively mediate the association between childhood ADHD and adult problematic alcohol use (Pedersen et al., 2016). At the neural level, ADHD (Plichta & Scheres, 2014; von Rhein, Cools, Zwiers, et al., 2015), trait impulsivity (Hariri et al., 2006; Plichta & Scheres, 2014), and problematic alcohol use (Balodis & Potenza, 2015; Hommer et al., 2011) have each been associated with variability in reward-related brain function, particularly within the ventral striatum (VS; Berridge & Kringelbach, 2015). Moreover, a recent study identified transcripts associated with impulsive behavior within a mouse model and then showed that variation in the sequence of the genes coding for these transcripts in humans was associated with impulsivity, VS reactivity to reward, and problematic alcohol use during adolescence (Peña-Oliver et al., 2016). Collectively, this evidence suggests that genetically influenced individual differences in reward-related brain function may contribute to shared risk between childhood ADHD and later problematic alcohol use.

Though prior studies of comorbidity and continuity have relied upon twin and family designs enriched for clinical cases (Derks et al., 2014; Knopik et al., 2006; Quinn et al., 2015), recent advances in statistical genetics allow for quantitation of genetic risk in healthy individuals without knowledge of family history. Building on the idea that psychiatric disorders represent extremes of continuous phenotypes normally distributed throughout the population (Plomin et al., 2009), it follows that those with a greater number of common risk variants for a particular disorder will be at heightened risk for diagnosis. Indices of genome-wide genetic risk, commonly referred

to as *polygenic risk scores* (PRS; Purcell et al., 2009), can be calculated based on the number of disorder risk alleles an individual possesses. The increasing availability of genome-wide association study (GWAS) summary statistics, made possible through collaborative efforts such as the Psychiatric Genomics Consortium (PGC; <http://www.med.unc.edu/pgc/>), has facilitated the exploration of mechanisms of genetic risk for psychiatric disorders in independent non-clinical samples (Belsky et al., 2016; Hagenaars et al., 2016; Krapohl et al., 2015), which typically benefit from larger sample sizes and are generally without the confounds of clinical symptom expression and medication.

In the current study, I investigated whether alterations in reward-related VS activity mediated links between polygenic risk for childhood ADHD and problematic alcohol use among 404 non-Hispanic Caucasian young adults. VS activity to reward was measured using blood oxygen level-dependent functional magnetic resonance imaging (BOLD fMRI), and defined as relative activity to positive versus negative feedback associated with monetary reward (Corral-Frias et al., 2015). ADHD PRS were calculated based on a PGC meta-analysis of childhood ADHD (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). Based on existing literature (Balodis & Potenza, 2015; Hariri et al., 2006; Hommer et al., 2011; Peña-Oliver et al., 2016; Plichta & Scheres, 2014; von Rhein, Cools, Zwiers, et al., 2015), I hypothesized that both polygenic risk for childhood ADHD and self-reported problematic alcohol use would be associated with individual differences in reward-related VS activity, and that variability in VS activity would mediate the association between polygenic risk and problematic alcohol use during young adulthood.

2.2 Methods

2.2.1 Participants

Self-report, neuroimaging, and genomic data were available from 438 non-Hispanic Caucasian 18- to 22-year-old undergraduate students who completed the ongoing Duke Neurogenetics Study (DNS; Corral-Frias et al., 2015) by April 4, 2015. Ancestry was determined by self-report and confirmed genetically (see **Section 2.2.3**). Participants provided informed written consent prior to the DNS protocol, which was approved by the Duke University Medical Center Institutional Review Board. Study exclusion criteria were as follows: 1) medical diagnosis of cancer, stroke, diabetes requiring insulin treatment, chronic kidney or liver disease, or, importantly, lifetime psychotic symptoms; 2) use of psychotropic, glucocorticoid, or hypolipidemic medication; and 3) conditions affecting cerebral blood flow and metabolism (e.g., hypertension). DSM-IV Axis I and select Axis II (i.e., borderline and antisocial personality) disorders were assessed with the electronic Mini International Neuropsychiatric Interview (Sheehan et al., 1998) and Structured Clinical Interview for the DSM-IV Axis II (First, Gibbon, Spitzer, Williams, & Benjamin, 1997).

Participants were excluded for the following quality control issues: a large number of movement or signal intensity outliers in fMRI data ($n=24$), an inadequate behavioral feedback schedule ($n=2$), scanner/equipment malfunction ($n=3$), subjects falling asleep ($n=1$), incidental structural findings ($n=1$), and relatedness ($n=2$, removed randomly from related pairs of participants). Following these exclusions, a final sample of 404 participants ($M_{age}=19.79\pm1.25$; 213 females) remained.

2.2.2 Measures

Problematic alcohol use was assessed using the 10-item Alcohol Use Disorders Identification Test (AUDIT; Saunders, Aasland, Babor, de la Fuente, & Grant, 1993), which was designed to assess past-year hazardous (e.g., “How often do you have six or more drinks on one occasion?”), dependent (e.g., “How often during the last year have you found that you were not able to stop drinking once you had started?”), and harmful (e.g., “Have you or someone else been injured because of your drinking?”) patterns of alcohol consumption in primary-care settings. Given that the AUDIT is useful only for assessing problem use among drinkers, nondrinkers (i.e., those who reported not having drunk alcohol in the past 12 months, $n=50$) were excluded from analyses involving this measure, leaving 354 drinkers for analysis. Consistent with prior studies in college students (e.g., Kokotailo et al., 2004), the AUDIT demonstrated acceptable psychometric properties in in this sample ($\alpha=0.818$; $M_{AUDIT}=6.59\pm4.20$; **Figure 2.1**) but were log-transformed prior to analyses to reduce positive skew.

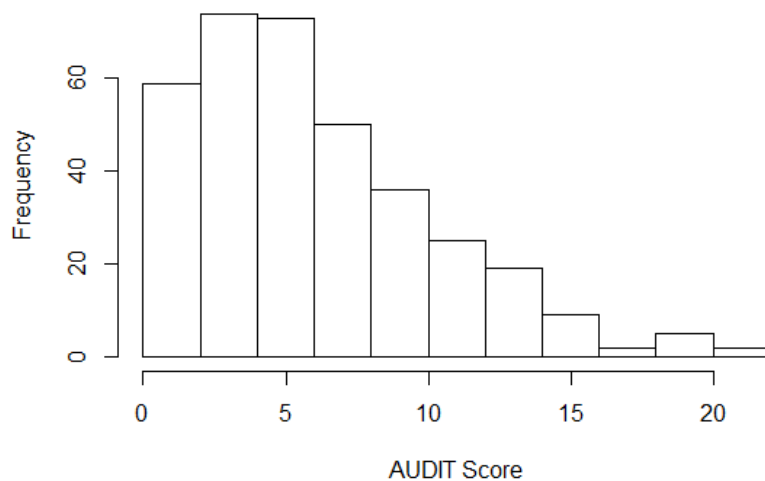


Figure 2.1 Raw Distribution of AUDIT Scores Among Past-year Drinkers. Scores of 8 and above, out of a maximum possible 40, are considered indicative of problem drinking (Saunders et al., 1993).

2.2.3 Genetics

Genotyping

DNA was isolated from saliva derived from Oragene DNA self-collection kits (DNA Genotek) customized for 23andMe (www.23andme.com). DNA extraction and genotyping were performed by the National Genetics Institute (NGI), a CLIA-certified clinical laboratory and subsidiary of Laboratory Corporation of America. One of two different Illumina arrays with additional custom content, the HumanOmniExpress or HumanOmniExpress-24 (Hu et al., 2016), was used to provide genome-wide single nucleotide polymorphism (SNP) data. Genotype imputation was run separately for participants typed on each array using the pre-phasing/imputation stepwise approach implemented in SHAPEIT (Delaneau, Marchini, & Zagury, 2012) and IMPUTE2 (Howie, Fuchsberger, Stephens, Marchini, & Abecasis, 2012) using only biallelic SNPs and the default value for effective size of the population (20,000), and chunk sizes of 3Mb and 5Mb for the respective arrays. Within each array batch, genotyped SNPs used for imputation were required to have missingness <0.02 , Hardy-Weinberg equilibrium (HWE) p -values $>10^{-6}$, and minor allele frequency (MAF) >0.01 . The imputation reference set consisted of 2,504 phased haplotypes from the full 1000 Genomes Project Phase 3 dataset (May 2013, over 70 million variants, release “v5a”). Imputed SNPs were retained if they had high imputation quality (INFO >0.9), low missingness ($<5\%$), and MAF >0.01 .

Relatedness was assessed using pairwise identity by descent estimation in PLINK (v.1.07; Purcell et al., 2007). Population stratification reflecting genetic heterogeneity associated with ancestry was assessed using identity by state analysis of whole-genome SNPs. The top 10 multidimensional scaling (MDS) components of this analysis were used to confirm self-reported ancestry (i.e., that no individuals were outliers of greater than $\pm 6SD$ on any component). The top

2 components were then selected based on visual inspection of the scree plot for use as covariates in all analyses to account for occult population stratification.

Polygenic Risk Scores

Polygenic risk scores were derived from GWAS summary statistics for the ADHD subset of the PGC's cross-disorder meta-analysis, which included 1947 trio cases, 1947 trio pseudocontrols, 840 cases, and 688 controls (<http://www.med.unc.edu/pgc/results-and-downloads>; Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). Because the original meta-analysis did not assess predictive utility of PRS in additional case-control samples and thus did not identify an ideal threshold for the inclusion of variants, PRS were constructed for p-value thresholds 0.0001, 0.001, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and 1.0, to be consistent with the PRS analyses conducted in the PGC cross-disorder paper. SNPs were required to have MAF >0.02, genotyping rates >0.98, and HWE p-values >10⁻⁶. SNPs within the major histocompatibility complex (MHC) region (chr6:25000000:35000000) were excluded due to their complex patterns of linkage disequilibrium. All remaining SNPs were pruned using p-value-informed clumping (i.e., grouping linked SNPs; $R^2=0.10$, 500kb window). For each P-value threshold, using the --score method in PLINK (v.1.07; Purcell et al., 2007), the log odds-ratio for ADHD for each component SNP (i.e., those in the original meta-analysis with p-values below the cutoff threshold) was multiplied by the number of reference alleles for that SNP before being summed and divided by the total number of contributing SNPs to produce a single metric for each participant representing cumulative genome-wide risk for childhood ADHD. To assess the specificity of ADHD polygenic risk relative to risk for neurodevelopmental disorders more generally, PRS were generated for autism spectrum disorders (ASD) in the same manner based on summary statistics from a meta-analysis conducted by the

PGC Autism Spectrum Disorders Workgroup (5305 cases, 5305 pseudocontrols; <http://www.med.unc.edu/pgc/results-and-downloads>; Robinson et al., 2016).

2.2.4 Neuroimaging

Corticostriatal Reactivity Paradigm

A number-guessing paradigm (**Figure 2.2**; Corral-Frias et al., 2015; Delgado, Nystrom, Fissell, Noll, & Fiez, 2000), consisting of a pseudorandom presentation of three blocks each of predominantly positive (80% correct guess) or negative (20% correct guess) feedback, interleaved with three control blocks, was used to probe VS activity associated with positive and negative feedback linked to monetary gains and losses.

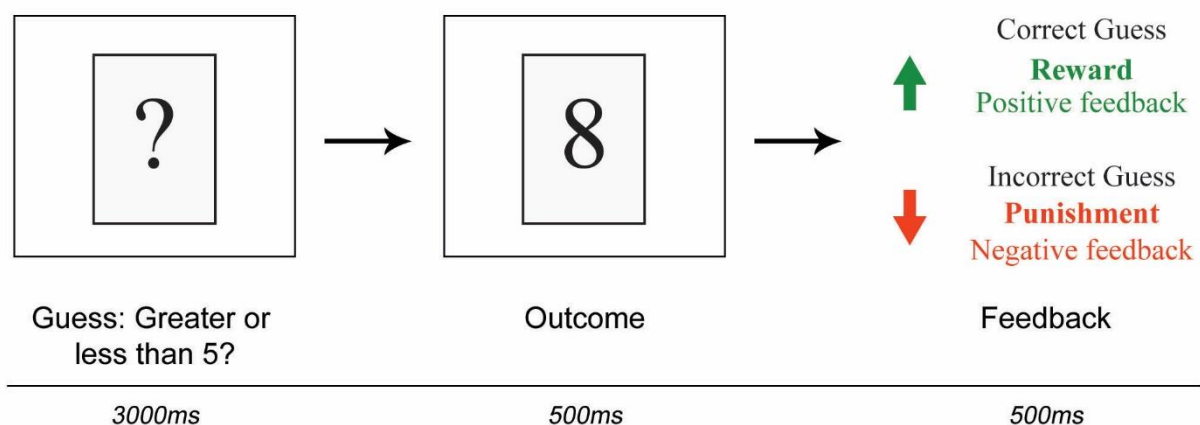


Figure 2.2 BOLD fMRI Corticostriatal Reactivity Paradigm. Paradigm consisted of a pseudorandom presentation of 3 blocks each of predominantly positive (80% correct guess) or negative (20% correct guess) fixed feedback, interleaved with 3 control blocks. During each trial, depicted here, participants saw the back of a card and were given 3000ms to guess whether the card was greater or less than 5 (face cards excluded). After a choice was made, the value of the card was displayed for 500ms, followed by appropriate feedback for 500ms and then a crosshair for 3000ms.

During each trial, participants saw the back of a card and were given 3000ms to guess whether the card was greater or less than five, face cards excluded. After a choice was made, the value of the card was displayed for 500ms, followed by appropriate feedback for 500ms and then a crosshair for 3000ms. Correct versus incorrect feedback was indicated by a green upward-facing arrow or a

red downward-facing arrow, respectively. In the control condition, participants saw an X for 3000ms, during which they were instructed to push a button, which was followed by an asterisk for 500ms and then a yellow circle for 500ms. Participants were unaware of the fixed outcome probabilities associated with each block and led to believe that their performance would determine their monetary gain at the end of the scanning sessions. However, all participants received \$10 in winnings regardless of performance.

Acquisition

Participants were scanned using one of two identical research-dedicated GE MR750 3T scanners equipped with high-power high-duty-cycle 50-mT/m gradients at 200 T/m/s slew rate, and an eight-channel head coil for parallel imaging at high bandwidth up to 1MHz at the Duke-UNC Brain Imaging and Analysis Center. A semi-automated high-order shimming program was used to ensure global field homogeneity. A series of 34 interleaved axial functional slices aligned with the anterior commissure-posterior commissure (AC-PC) plane were acquired for full-brain coverage using an inverse-spiral pulse sequence to reduce susceptibility artifact (TR/TE/flip angle=2000 ms/30 ms/60; FOV=240 mm; 3.75×3.75×4 mm voxels [selected to provide whole brain coverage while maintaining adequate signal-to-noise and optimizing acquisition times]; interslice skip=0). Four initial RF excitations were performed (and discarded) to achieve steady-state equilibrium. To allow for spatial registration of each participant's data to a standard coordinate system, high-resolution three-dimensional structural images were acquired in 34 axial slices co-planar with the functional scans (TR/TE/flip angle=7.7 s/3.0 ms/12; voxel size=0.9×0.9×4 mm; FOV=240 mm, interslice skip=0).

Preprocessing

The general linear model of Statistical Parametric Mapping 8 (SPM8; <http://www.fil.ion.ucl.ac.uk/spm>) was used for whole-brain image analysis. Individual subject data were first realigned to the first volume in the time series to correct for head motion before being spatially normalized into the standard stereotactic space of the Montreal Neurological Institute (MNI) template using a 12-parameter affine model. Next, data were smoothed to minimize noise and residual differences in individual anatomy with a 6mm full width at half maximum (FWHM) Gaussian filter. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean. Then the ARTifact Detection Tool (ART; http://www.nitrc.org/projects/artifact_detect/) was used to generate regressors accounting for image outliers due to large motion (i.e., >0.6mm relative to the previous time frame) or spikes (i.e., global mean intensity 2.5 standard deviations from the entire time series). Participants for whom more than 5% of acquisition volumes were flagged by ART were removed from analyses. A 5mm sphere based on the maximum voxels from a prior study (Hariri et al., 2006) was used to ensure adequate ventral striatum coverage; no participant had <90% coverage of the region.

Analysis

Following preprocessing steps, the general linear model in SPM8, employing canonical hemodynamic response functions, was used to estimate condition-specific (i.e., positive feedback, negative feedback, and control block) BOLD responses for each individual. Consistent with prior studies (Corral-Frias et al., 2015; Hariri et al., 2006; Nikolova, Knodt, Radtke, & Hariri, 2016), I focused on activity resulting from the contrast of all positive feedback blocks relative to negative feedback blocks (Positive Feedback > Negative Feedback) as an index of neural activity associated with monetary gains versus losses. As outlined in SPM8's random-effects analysis via summary

statistics approach (Holmes & Friston, 1998; Penny, Holmes, & Friston, 2003), Positive Feedback > Negative Feedback contrast images (i.e., weighted sums of single-condition beta images) for each individual were used in a second-level random effects model to determine mean contrast-specific responses using a one-sample t-test.

Left and right VS regions of interest (ROIs) were defined using a 10mm sphere based on the maximum voxels from a prior study (Hariri et al., 2006). A voxel-level statistical threshold of $p < 0.05$, family-wise error (FWE) corrected for multiple comparisons across the ROIs, and a cluster-level extent threshold of 10 contiguous voxels, were applied to these analyses. In-line with prior work (Corral-Frias et al., 2015) and recent recommendations (Tong et al., 2016), parameter estimates from functional clusters within these ROIs were extracted, which were then used for all statistical analyses. To maintain variability but constrain the influence of extreme outliers, prior to analyses all imaging variables were winsorized (i.e., following data quality control procedures, outliers more than $M \pm 3SDs$ [$n=5$ for left VS, $n=3$ for right VS] were set at $\pm 3SDs$ from the mean).

2.2.5 Statistical Analyses

Sex and the top two ancestry-informative multidimensional scaling components (Purcell et al., 2007) were entered as covariates for all analyses to control for possible confounding effects of sex and occult population stratification. For analyses involving alcohol phenotypes, being of legal drinking age in the US (i.e., at least 21) was also included as a covariate of no interest ($n_{legal}=132$, $n_{underage}=222$).

Ordinary least squares regression was used to test the association between each thresholded ADHD PRS and left and right VS activity, as well as direct associations with AUDIT scores. To address multiple testing, I implemented a label-swapping permutation procedure to assess whether the overall pattern of association across thresholds differed from that expected by chance. In each

of 10,000 permutations, an individual's genetic data (i.e., PRS at all thresholds and ancestry-informative MDS components) were swapped but dependent variables (i.e., left and right VS activity to reward, AUDIT scores) and non-genetic covariates (i.e., sex and, when appropriate, being of legal drinking age) were left intact in order to generate a null distribution. For each permutation, the number of nominally significant (i.e., $p < 0.05$) associations were summed. Empirical significance of the overall association pattern, P_{PRS} , was calculated as the number of permutations for which the number of nominally significant associations equaled or exceeded that reported in our analyses, divided by the number of permutations performed (i.e., 10,000). I adopted this approach due to the nested structure of PRS scores across p-value thresholds (i.e., each threshold contains all SNPs in more stringent p-value thresholds).

To examine links from genes to brain to behavior, a mediational model was tested in MPlus (v.7.11; Muthén & Muthén, 2012) with ADHD PRS as the predictor, VS activity as the mediating variable, and AUDIT scores as the dependent variable. Because such a model is dependent upon associations between ADHD PRS and VS activity, I conducted these analyses at the p-value threshold most strongly associated with VS activity (i.e., $P < 0.30$). Full information maximum likelihood estimation was used, and unstandardized indirect effects were computed for each of 5000 bootstrapped samples, consistent with the recommendations of (Hayes, 2009).

2.3 Results

2.3.1 Main Effects

Consistent with prior work, the contrast of interest (i.e., Positive Feedback > Negative Feedback) yielded robust bilateral VS activity (**Figure 2.3A**). ADHD PRS were significantly associated with bilateral VS activity (Left VS: significant at 6 of 10 P-value thresholds; largest effect at $P < 0.30$:

$\beta=0.132$, $\Delta R^2=0.017$, $p=0.008$; Right VS: significant at 6 of 10 P-value thresholds; largest effect at $P<0.30$: $\beta=0.124$, $\Delta R^2=0.015$, $p=0.013$; **Figure 2.3B**).

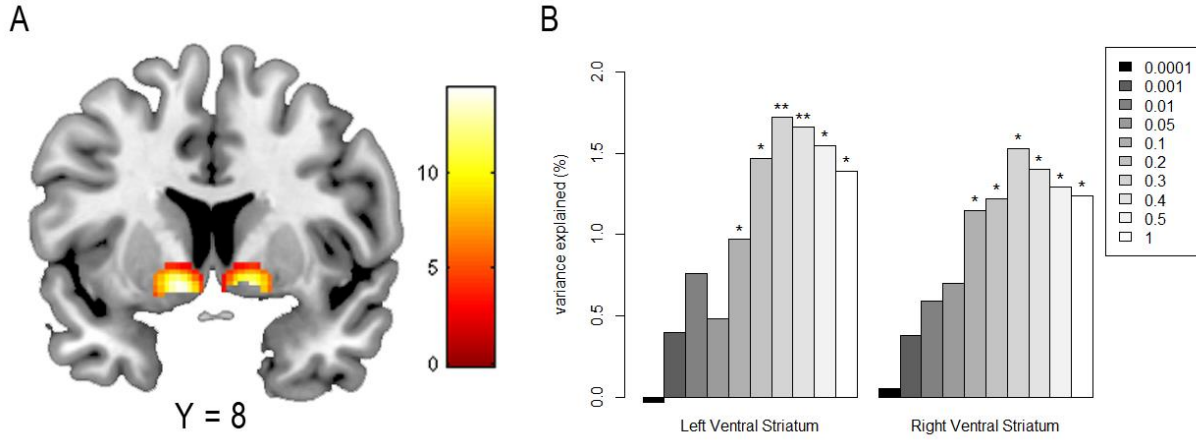


Figure 2.3 Main Effects of fMRI Task and ADHD Polygenic Risk on Reward-Related Activity in the Ventral Striatum. **A)** Bilateral ventral striatal (VS) reactivity to reward (Positive Feedback > Negative Feedback) across all participants. Right hemisphere: Montreal Neurological Institute (MNI) coordinates = 12, 10, and -8 ($t=13.287$, $p<0.05$ FWE), cluster size = 239 voxels. Left hemisphere: MNI coordinates = -12, 8, and -8 ($t=14.394$, $p<0.05$ FWE), cluster size = 293 voxels. **B)** ADHD polygenic risk scores (PRS) and bilateral ventral striatal reactivity to reward. Y-axis is the percent of variation in VS reactivity explained by ADHD PRS. Positive values indicate a positive association between ADHD PRS and VS reactivity. Negative values are for display purposes only and indicate a negative association between ADHD PRS and VS reactivity. Shades of gray in legend indicate the P-value threshold at which the risk score was calculated.

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

Permutation analyses revealed that the overall bilateral pattern of associations across thresholds significantly deviated from that expected by chance ($P_{PRS}=0.013$). The negative control, ASD PRS, was not associated with VS activity at any threshold (all $ps>0.15$). ADHD PRS failed to predict problematic alcohol use across thresholds (significant only at $P<0.01$: $\beta=0.103$, $\Delta R^2=0.010$, $p=0.050$; $P_{PRS}=0.223$). However, bilateral VS activity was significantly associated with problematic alcohol use (Left VS: $\beta=0.172$, $\Delta R^2=0.029$, $p<0.001$; Right VS: $\beta=0.149$, $\Delta R^2=0.022$, $p=0.004$). Repetition of analyses with raw (i.e., non-winsorized, untransformed) data did not alter results.

2.3.2 Structural Equation Model

Due to the consistency in results across hemispheres, a combined bilateral ROI was used for structural equation modelling. Within the full model (**Figure 2.4A**), polygenic risk scores positively predicted VS activity ($\beta_{PRS}=0.132$, 95% CI [0.025, 0.244], $p=0.019$; **Figure 2.4B**), which, in turn, positively predicted problematic alcohol use ($\beta_{VS}=0.162$, 95% CI [0.068, 0.259], $p<0.001$; **Figure 2.4C**). The indirect pathway from ADHD PRS to problematic alcohol use through VS activity was significant ($\beta_{IND}=0.021$, 95% CI [0.005, 0.051], $p<0.01$).

2.3.3 Supplemental Follow-up Alcohol Use Initiation Analysis

To provide preliminary evidence that heightened VS activity to reward likely *preceded* the emergence of problematic drinking, as implied by the setup of my structural equation model, I analyzed whether bilateral VS activity predicted the future initiation of alcohol use among baseline nondrinkers ($n=50$). After successful completion of the baseline protocol including fMRI, participants were subsequently contacted by e-mail every 3 months and invited to complete a short online assessment which included the AUDIT. Follow-up analyses were performed using data from the most recent timepoint ($n=29$; M days since baseline completion= 609.71 ± 390.80 , range: 91-1615). Of these individuals, 15 reported drinking at follow-up. Logistic regression analyses with sex, two ancestry-informative MDS components, whether one was of legal drinking age (i.e., 21+) at follow-up, and days since baseline assessment as covariates of no interest demonstrated that heightened bilateral VS activity to reward at baseline was predictive of drinking initiation at follow-up ($\beta=3.735$, Nagelkerke $\Delta R^2=0.276$, $p=0.021$).

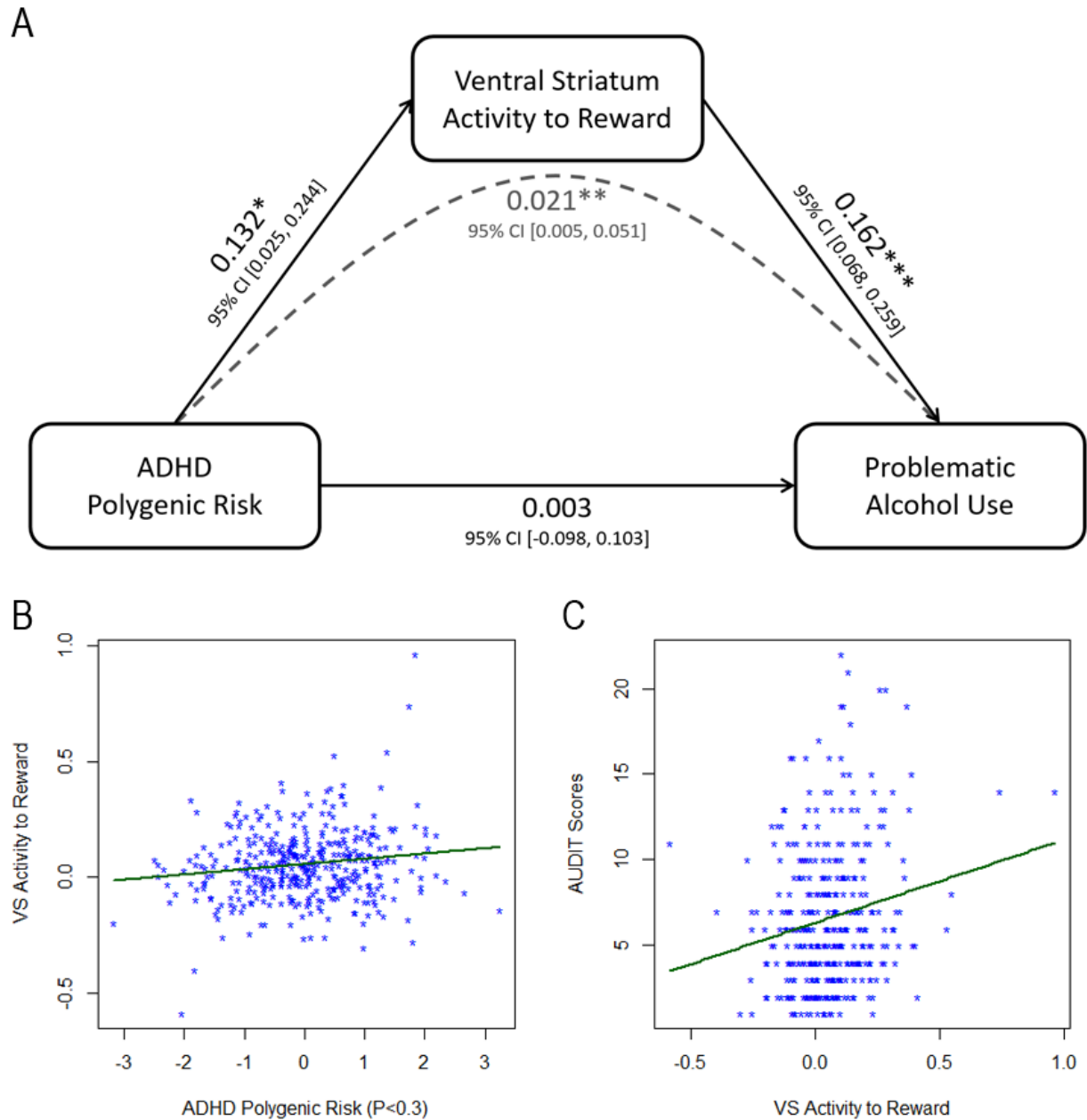


Figure 2.4 Structural Equation Model Demonstrating the Effect of ADHD Polygenic Risk on Problematic Alcohol Use through Bilateral Ventral Striatum Activity to Reward. The overall model is depicted in **A**, with raw data plots of the significant *a* and *b* pathways shown in **B** and **C**, respectively. The dashed line in **A** represents the indirect effect of ADHD PRS on problematic alcohol use through bilateral ventral striatum activity to reward. Sex and the top two ancestrally-informative MDS components were included as covariates of no interest for all paths in the full model. Being of legal drinking age in the US (i.e., age 21+) was included as a covariate in paths involving alcohol use outcomes (i.e., *b* and *c*).

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

2.4 Discussion

Twin and family studies have shown that shared genetic factors account for the majority of phenotypic variance between childhood ADHD and problematic alcohol use during adolescence and adulthood (Derks et al., 2014; Knopik et al., 2006; Quinn et al., 2015). Here, I have extended this work by providing initial evidence that polygenic risk for childhood ADHD predicts heightened reward-related ventral striatum activity, which, in turn, is associated with problematic alcohol use in young adulthood (**Figure 2.4**). Alongside evidence that elevated neural responsivity to reward is found among adolescents with ADHD and their unaffected siblings (von Rhein, Cools, Zwiers, et al., 2015), and that such reactivity is associated with alcohol use initiation and escalation (Heitzeg et al., 2014; Weiland et al., 2016), these results suggest that elevated ventral striatum activity to reward may be a genetically-influenced neural mechanism mediating the link between polygenic risk for childhood ADHD and later problematic alcohol use.

2.4.1 Childhood ADHD and Later Problematic Alcohol Use

Despite compelling prior epidemiological evidence of associations between childhood ADHD diagnosis and later problematic alcohol use (Charach et al., 2011; Lee et al., 2011), as well as previous twin and family studies demonstrating substantial genetic overlap between the two (Derks et al., 2014; Knopik et al., 2006; Quinn et al., 2015), I observed no direct association between polygenic risk for childhood ADHD and problematic alcohol use. Of note, though not significant across p-value thresholds, the directionality of associations was consistent with the epidemiological literature (i.e., higher childhood ADHD polygenic risk leading to greater reported problem drinking). While it is possible that an absence of a statistically significant direct association is an artifact of my likely underpowered sample (i.e., 80% power to detect an R^2 of

0.023, when variance explained by PRS in population-based studies is generally 1% or less; Belsky et al., 2016; Hagenaars et al., 2016; Krapohl et al., 2015), I have previously reported a similar lack of association in a larger ($N=2573$) sample ascertained for substance use disorders, including alcohol dependence (Carey et al., 2016). It is possible that the association between polygenic risk for ADHD and problematic alcohol use occurs indirectly through altered neural mechanisms of reward sensitivity and reinforcement learning and/or that a direct association would require a larger sample to detect.

2.4.2 ADHD and Associations with Reward-Related VS Activation

Although neural and behavioral research on ADHD has traditionally focused on deficits in executive functioning (Cortese et al., 2012; McCarthy, Skokauskas, & Frodl, 2014; Willcutt et al., 2005), more recent theoretical models of the disorder have proposed a key role for dysfunctional motivation and reward-related processes (Castellanos, Sonuga-Barke, Milham, & Tannock, 2006; Luman, Tripp, & Scheres, 2010). For example, in healthy adults, individual differences in VS activity during both reward anticipation and outcome are positively associated with behavioral and self-reported indices of impulsivity and reward responsiveness (Forbes et al., 2009; Hariri et al., 2006; Plichta & Scheres, 2014). ADHD symptoms and diagnosis, in contrast, have been predominantly characterized by lower VS responsiveness to reward-predicting cues (see Plichta & Scheres, 2014, for meta-analysis and review, but see also von Rhein, Cools, Zwiers, et al., 2015, and subsequent commentary in Plichta & Scheres, 2015, and von Rhein, Cools, Mennes, & Buitelaar, 2015). Neural activation to reward outcome has been less systematically studied in ADHD, with some evidence of relative hyper-responsiveness (Furukawa et al., 2014; von Rhein, Cools, Zwiers, et al., 2015; Paloyelis, Mehta, Faraone, Asherson, & Kuntsi, 2012; but see also Scheres, Milham, Knutson, & Castellanos, 2007, and Wilbertz et al., 2012). This discrepancy in

relative VS activation to reward anticipation versus outcome may reflect impaired reward learning (Furukawa et al., 2014), consistent with evidence of impaired behavioral modification based on prior reinforcement history in ADHD (Tripp & Alsop, 1999). Given the design of the corticostriatal reactivity paradigm in the DNS, in which mean VS activation was compared across positive versus negative feedback blocks (with non-valence-specific cues), my results may more appropriately reflect emergent data linking ADHD to increased VS response to reward outcomes (Furukawa et al., 2014; Paloyelis et al., 2012; von Rhein, Cools, Zwiers, et al., 2015). Notably, the current findings of elevated VS activity to positive versus negative feedback among young adults at higher polygenic risk for childhood ADHD complement those of a prior family study (von Rhein, Cools, Zwiers, et al., 2015) in suggesting that heightened neural responsiveness to reward may be a heritable neural mechanism through which ADHD expression manifests.

2.4.3 Reward-Related VS Activation and Risk for Problematic Alcohol Use

As with ADHD, addiction has been contextualized as a disorder of altered reward sensitivity and abnormal reinforcement learning. Influential theories of addiction emphasize the importance of conceptualizing the disorder within stages, whereby substance use initiation and initial problematic use are related to the positively reinforcing aspects of a substance, while later compulsive use is driven by negative reinforcement and diminished cognitive control resulting from chronic use-induced changes in neural plasticity and a revised homeostasis that includes the presence of the substance (Everitt & Robbins, 2016; Koob, 2013; Koob & Le Moal, 2005; Volkow, Fowler, Wang, Baler, & Telang, 2009; Wise & Koob, 2014). Heightened VS activity to reward may characterize the initial stages (i.e., initiation and escalation) of substance use, causing at-risk individuals to be more likely to engage in initiation and also be more sensitive to the positively reinforcing aspects of the substance (Hommer et al., 2011). Repeated use of substances, in contrast, may result in

overstimulation and consequent downregulation of reinforcement mechanisms (Volkow et al., 2009; Volkow, Fowler, Wang, & Swanson, 2004), thus decreasing VS activity to natural reward and increasing the threshold required to reach prior levels of responsiveness (Hommer et al., 2011). Consistent with this model, increased striatum activity to reward in adolescence prospectively predicts substance use initiation (Stice et al., 2013) and problem drinking (Heitzeg et al., 2014), and is retrospectively related to age-of-first-drunkenness across both individuals with AUD and healthy volunteers (Weiland et al., 2016). In contrast, the vast majority of studies among individuals with AUD have reported decreased VS reactivity to monetary reward cues (Balodis & Potenza, 2015; Hommer et al., 2011) but hyperresponsive to alcohol cues relative to controls (Wrase et al., 2007), indicating a shift in reward responsiveness from more generally salient stimuli (e.g., money) to the conditioned substance of choice as the disorder progresses.

My results, showing that elevated VS activation during reward processing indirectly links polygenic risk for ADHD to problematic alcohol use among young adult college students, are therefore consistent with a heightened reward sensitivity model of substance use initiation/escalation conferred by childhood ADHD polygenic risk. Due to my relatively healthy sample, however, these findings cannot exclude the possibility that childhood ADHD polygenic risk impacts later stages of substance use disorders (e.g., transition to and maintenance of dependence) either through the VS or alternative neural pathways (e.g., the cognitive control circuit: Holmes, Hollinshead, Roffman, Smoller, & Buckner, 2016; Rooney, Chronis-Tuscano, & Huggins, 2015). Additional studies of genetic risk, in concert with longitudinal studies, are needed to better elucidate the temporal associations between reward-related VS activity and ADHD and AUD.

2.4.4 Limitations

Several limitations are important to consider when interpreting the results of this study. First, despite being large for a neuroimaging study ($N=404$), the sample was small for genetic association analyses, which, as in other imaging genetics studies, may increase the risk of false negative and false positive findings and result in imprecise effect estimates (Bogdan, Pagliaccio, Baranger, & Hariri, 2016). Correspondingly, the childhood ADHD GWAS meta-analysis used to generate PRS in this study is small relative to more recent meta-analytic efforts (e.g., schizophrenia: $N_{case}=36,989$, $N_{control}=113,075$; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), and, perhaps due to its resultant lack of power, did not yield any genome-wide significant loci. As a result, it will be important for this observed association to be replicated and extended once larger ADHD GWAS meta-analyses and additional neurogenetics samples become available.

Second, because ADHD diagnosis and symptomatology were not assessed in the DNS, it is not possible to rule out whether disorder expression is a mediating factor between polygenic risk and reward-related VS activity. One possible explanation for my results is that higher polygenic risk leads to ADHD, and that the disorder itself is then associated with increased VS activity to reward. Correspondingly, I was unable to validate whether childhood ADHD PRS were indeed predictive of retrospective diagnosis in the current sample. However, a prior population-based study demonstrated that ADHD PRS derived from the same meta-analysis used for this study was predictive of childhood, but, intriguingly, not adult, ADHD, thus indicating the utility and, somewhat surprisingly, childhood specificity of these scores (Moffitt et al., 2015).

Third, the DNS is cross-sectional, making it impossible to establish temporal order for the association between VS activity and problematic alcohol use. As a result, I cannot rule out that

alcohol misuse may have preceded increases in VS activity. However, longitudinal studies of the effect of VS activity on future alcohol use are consistent with our model (Heitzeg et al., 2014). Furthermore, within our subsample of baseline nondrinkers who also had follow-up AUDIT data (n=29), VS activity predicted future initiation of drinking, providing some preliminary evidence for temporality.

Fourth, the functional paradigm used in the DNS (Corral-Frias et al., 2015), due to its blocked design, does not allow for the examination of distinct components of reward processing. The current results thus cannot be directly compared to those of prior studies of reward-related VS activity in ADHD and AUD, which have generally examined reward anticipation and outcome separately (Balodis & Potenza, 2015; Plichta & Scheres, 2014).

Lastly, the indirect association between polygenic risk and problematic alcohol use is a small effect that is not currently informative on an individual level. Nonetheless, the effect size is consistent with those reported in prior studies using PRS (Belsky et al., 2016; Hagenaars et al., 2016; Krapohl et al., 2015), and these findings identify a promising neural mechanism that provides etiologic insight into associations between childhood ADHD and problematic alcohol use and is worthy of future study.

2.4.5 Conclusion

Limitations notwithstanding, the current study provides initial evidence that relatively heightened reward-related VS activity mediates associations between polygenic risk for childhood ADHD and problematic alcohol use in young adulthood. Future studies may wish to interrogate dissociations between reward anticipation and receipt as they relate to genetic risk for ADHD and problematic alcohol use, and to extend these findings to additional substance use and externalizing disorders. Furthermore, as imaging and genetics sample sizes increase, pathway enrichment analyses (e.g.,

Lee, O'Dushlaine, Thomas, & Purcell, 2012) and functional annotation (e.g., Finucane et al., 2015) may allow for the discovery of specific molecular pathways that underlie genetic associations between ADHD, neural reward activity, and problematic alcohol use. Overall, the current findings suggest that elevated VS response to reward is a promising neural mechanism through which ADHD polygenic risk and problematic drinking may manifest.

Chapter 3:

Neural Risk Markers for Adolescent Alcohol Use Initiation

3.1 Introduction

Alcohol use is a significant public health issue, with approximately 3.3 million deaths worldwide per year directly attributable to alcohol consumption (World Health Organization, 2014) and roughly 30% of individuals in the United States meeting criteria for Alcohol Use Disorder (AUD) during their lifetimes (Grant et al., 2015). Alcohol misuse has been increasingly contextualized within a developmental framework, with adolescence as a particularly influential period (Chassin, Sher, Hussong, & Curran, 2013; Zucker, 2015). The typical age-of-first-drink in the US is in early adolescence (i.e., median age of 14; Chen, Yi, & Faden, 2013), with patterns such as regular use, binge drinking, and heavy use typically emerging and escalating throughout adolescence, peaking in the early 20s, and then plateauing or tapering off in adulthood (Johnston, 2016; Substance Abuse and Mental Health Services Administration, 2015). Individuals with an earlier onset of alcohol use are at higher risk for problematic drinking patterns, including AUD diagnosis, in later adolescence and adulthood (Chou & Pickering, 1992; Dawson, Goldstein, Chou, Ruan, & Grant, 2008; DeWit, Adlaf, Offord, & Ogborne, 2000; Grant & Dawson, 1997; Hingson, Heeren, & Winter, 2006). As such, it is important to identify biological risk factors associated with early initiation of alcohol use during adolescence to better understand and perhaps prevent developmental transitions to problematic use patterns and eventual dependence.

One potential neurobiological mechanism underlying the initiation of alcohol use in adolescence is the staggered maturation of the brain's subcortical reward (e.g., ventral striatum

[VS]) and cortical cognitive control (e.g., prefrontal cortex) systems (see Casey & Jones, 2010, for review). During adolescence, the subcortical reward system matures first (Galván, 2013), while the cognitive control system lags behind (Gogtay et al., 2004). This creates a developmental period of imbalance during which “bottom-up” reward/motivation signals are relied upon to a greater degree in driving behavior than “top-down” cognitive control signals (Casey & Jones, 2010). Correspondingly, heightened subcortical activity to reward, particularly within the VS, is thought to characterize the initial stages of substance use, causing at-risk individuals to be more likely to initiate use and also be more sensitive to the positively reinforcing aspects of the substance (Homer et al., 2011). Increased striatum activity to reward in adolescence, for example, prospectively predicts substance use initiation (Stice et al., 2013) and problem drinking (Heitzeg et al., 2014), and is retrospectively related to age-of-first-drunkenness across individuals with AUD and healthy volunteers (Weiland et al., 2016). Given its implication in alcohol and substance use and its maturational overlap with a time of increase in both, reward-related activity within the VS, and the reward system in general, is a strong candidate neural risk mechanism underlying the timing of alcohol use initiation.

In this study, using archival longitudinal data from 65 baseline alcohol-naïve participants in the Teen Alcohol Outcomes Study (TAOS; Bogdan, Williamson, & Hariri, 2012), I tested whether reward-related ventral striatum activity prospectively predicted relatively early (i.e., age 15 or less) vs. late (i.e., age 18 or greater) age-at-first-drink. TAOS participants were scanned in early adolescence (age range: 12 to 15) while performing a monetarily incentivized number-guessing task, during which blood oxygen level-dependent functional magnetic resonance imaging (BOLD fMRI) data were acquired (Corral-Frias et al., 2015; Hariri et al., 2006). Self-reported alcohol use was assessed at a maximum of six timepoints annually, with the initial assessment

coinciding with the fMRI scan. Based on prior literature (Heitzeg et al., 2014; Stice et al., 2013; Weiland et al., 2016), as well as the results of **Chapter 2**, I hypothesized that increased reward-related VS activity would be associated with earlier initiation of alcohol use.

3.2 Methods

3.2.1 Participants

Teen Alcohol Outcomes Study (TAOS) participants ($N=331$), ages 11 to 15 at baseline, were recruited via phone from the San Antonio, Texas, metro area within a 30-mile radius of University of Texas Health Science Center at San Antonio (UTHSCA; Bogdan et al., 2012). As TAOS seeks to investigate factors contributing to risk for psychopathology, participants with a family history of major depressive disorder (MDD), which is associated with increased risk for MDD, anxiety, and substance use disorders (Weissman et al., 2006), were over-sampled. Participants provided assent, with parents providing written informed consent, following procedures approved by the UTHSCA Institutional Review Board. To be eligible for the study, participants were required to be medically healthy, free of contraindications to MRI (e.g., braces), and not have a psychiatric or substance use disorder, aside from anxiety, at baseline, as assessed by the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (K-SADS-PL; Kaufman et al., 1997). Reported binge drinking at baseline, as defined by the NIAAA guidelines (i.e., 4+ drinks on one occasion for females or 5+ drinks on one occasion for males; National Institute on Alcohol Abuse and Alcoholism), was also grounds for exclusion. After assenting/consenting, participants completed in-person interviews, self-report behavioral assessments, and fMRI scanning. Participants were re-contacted annually to complete diagnostic

interviews and questionnaires, for a maximum of six timepoints (including baseline) to date, and also underwent a follow-up scanning session approximately two years post-baseline.

For the current analyses, participants who reported having had at least one alcoholic drink by the time of the baseline assessment ($n=9$), or for whom baseline drinking status could not be determined due to missingness or discrepancies in self-report ($n=25$), were excluded. Additionally, to ensure adequate assessment of age-at-first-drink, participants were required to have either initiated drinking during the course of the study ($n=98$), or to have continued the study to the age of 18 without having initiated drinking ($n=30$). Of the remaining participants, 9 did not complete the corticostriatal reactivity paradigm scan, and an additional 31 were removed for issues of imaging quality control (i.e., a large number of movement or signal intensity outliers, $n=23$, or an inadequate behavioral feedback schedule, $n=8$; see **Section 3.3.2**). Following these exclusions, usable self-report and baseline imaging data were available for 88 participants ($M_{age}=13.59\pm0.96$; 40 females; 61% non-Hispanic Caucasian, 23% Hispanic, 11% Other).

3.2.2 Measures

Age-at-first-drink

At baseline and each annual follow-up session, participants were asked to report their lifetime and past-year use of 16 types of licit and illicit substances, including alcohol, on a substance use checklist. For each substance a participant endorsed, they completed the corresponding section of the Substance Use Questionnaire (SUQ; Molina et al., 2007), which asks questions regarding the quantity, frequency, and experiences of past-year substance use. Participants who reported having initiated alcohol use at any timepoint (i.e., “Did you have your very first drink since your last interview here?”) were subsequently asked at what age they had their first drink (i.e., “How old

were you the first time you had a drink, not just a sip or a taste?”). This was used as an *age-at-first-drink* metric ($M=15.50\pm1.61$; **Figure 3.1**) among alcohol use initiators ($n=66$).

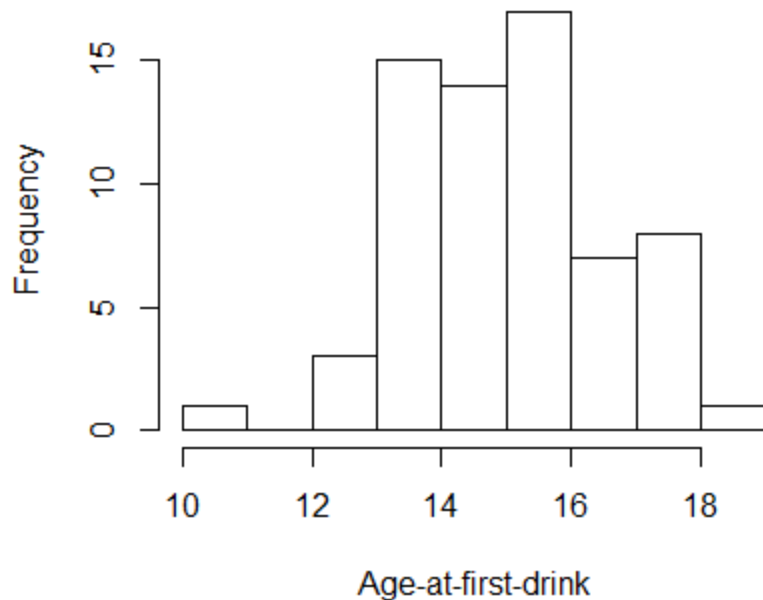


Figure 3.1 Distribution of Age-at-first-drink Among Initiators.

To incorporate continuous nondrinkers (i.e., participants who reached the age of 18 without having initiated alcohol use) into analyses and simultaneously maximize power, I assigned participants to two groups based on early (i.e., age-at-first-drink ≤ 15 ; $n=34$) versus late (i.e., age-at-first-drink ≥ 18 ; $n=31$) initiation of alcohol use (**Table 3.1**). A third intermediate group (i.e., $15 < \text{age-at-first-drink} < 18$, $n=23$) was also created but not used in primary analyses. Age cutpoints were selected based on a desire to maximize group sizes while maintaining at least two years of distance between “early” and “late” initiators.

Table 3.1 Age-at-first-drink Subsample Demographics

Variable	Early	Middle	Late
N	34	23	31
Age at Baseline	13.49(0.89)	14.01(0.86)	14.29(0.70) ^{*, 1}
Tanner Stage >III at Baseline	61.8%	56.5%	83.9%
Female	44.1%	47.8%	45.2%
Non-Hispanic Caucasian	79.4%	47.8%	54.8% ¹
Hispanic	20.6%	26.1%	32.3%

*Indicates a significant difference between early and late groups.

¹Indicates a significant difference between early and middle groups.

²Indicates a significant difference between middle and late groups.

Pubertal Status

Pubertal status was assessed at baseline using the Tanner scales (Marshall & Tanner, 1969, 1970) and binarized, consistent with a prior TAOS study (Dotterer, Hyde, Swartz, Hariri, & Williamson, 2017), into individuals in Stage III or less, reflecting less pubertal development, and those above Stage III, reflecting greater development.

3.2.3 Neuroimaging

Corticostriatal Reactivity Paradigm

A monetarily incentivized number-guessing paradigm (**Figure 2.2**; Corral-Frias et al., 2015; Delgado et al., 2000), described in detail in **Section 2.2.4**, was used to elicit reward-related VS activity.

Acquisition

BOLD fMRI images were acquired on a Siemens 3T Trio scanner using a gradient-echo, echo planar imaging (EPI) sequence (TR=2000 ms, TE=25 ms, FOV=192 mm, matrix=64x64), covering 34 interleaved 3 mm-thick axial slices. To allow for spatial registration of each participant's data to a standard coordinate system, structural MRI images were acquired with a T1-weighted MPRAGE sequence (TR=2200 ms, TE=2.8 ms, slice thickness=0.8 cm, FOV=256 mm).

Preprocessing

The general linear model of Statistical Parametric Mapping 8 (SPM8; <http://www.fil.ion.ucl.ac.uk/spm>) was used for whole-brain image analysis. Individual subject data were first realigned to the first volume in the time series to correct for head motion before being spatially normalized into the standard stereotactic space of the Montreal Neurological Institute (MNI) template using a 12-parameter affine model. Next, data were smoothed to minimize noise and residual differences in individual anatomy with a 6mm FWHM Gaussian filter. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean. Then the ARTifact Detection Tool (ART; http://www.nitrc.org/projects/artifact_detect/) was used to generate regressors accounting for image outliers due to motion (i.e., >2 mm or degrees relative to the previous time frame) or spikes (i.e., global mean intensity >4 standard deviations from the entire time series). Participants for whom more than 5% of acquisition volumes were flagged by ART were removed from analyses ($n=23$). For the remaining participants, ART generated regressors to control for outlier volumes in analyses. A 5mm sphere based on the maximum voxels from a prior study (Hariri et al., 2006) was used to ensure adequate (i.e., 90%) ventral striatum coverage bilaterally; all participants had sufficient coverage of the region.

Analysis

Following preprocessing steps, linear contrasts employing canonical hemodynamic response functions were used to estimate task-specific BOLD responses to different forms of feedback (i.e., positive and negative) for each individual. Consistent with prior studies (Corral-Frias et al., 2015; Hariri et al., 2006; Nikolova et al., 2016), I focused on activity resulting from the contrast of all positive feedback blocks relative to negative feedback blocks (Positive Feedback > Negative Feedback) as an index of neural activity associated with monetary gains versus losses. As outlined

in SPM8's random-effects analysis via summary statistics approach (Holmes & Friston, 1998; Penny et al., 2003), Positive Feedback > Negative Feedback contrast images (i.e., weighted sums of single-condition beta images) for each individual were used in a second-level random effects model to determine mean contrast-specific responses using a one-sample t-test.

Left and right VS regions of interest (ROIs) were defined using a 5mm sphere based on the maximum voxels from a prior study (Hariri et al., 2006). A voxel-level statistical threshold of $p < 0.05$, family-wise error (FWE) corrected for multiple comparisons across the ROIs, and a cluster-level extent threshold of 10 contiguous voxels, were applied to these analyses. In-line with prior work (Corral-Frias et al., 2015) and recent recommendations (Tong et al., 2016), parameter estimates at the maximum voxels of functional clusters within these ROIs were extracted, which were then used for all statistical analyses. To maintain variability but constrain the influence of extreme outliers, prior to analyses all imaging variables were winsorized (i.e., following data quality control procedures, outliers more than $M \pm 3SDs$ [$n=2$ for left VS, $n=1$ for right VS] were set at $\pm 3SDs$ from the mean).

3.2.4 Statistical Analyses

To examine reward-related neural predictors of age-at-first-drink, logistic regressions with membership in the early versus late initiation group as the dependent variable and either left or right VS activation as the predictor were performed. In addition to these analyses, an exploratory whole-brain independent samples t-test was conducted in SPM8, employing cluster-level family-wise error (FWE) correction as implemented by AFNI's 3dClustSim (Cox, 1996). A mask was created by thresholding SPM8's a priori probabilistic grey matter image at >0.20 to restrict analyses to grey matter only. Consistent with recent recommendations (Cox, Chen, Glen, Reynolds, & Taylor, 2017; Cox, Reynolds, & Taylor, 2016), smoothness of the residuals for the

whole-brain independent samples t-test was first assessed using AFNI's 3dFWHMx with the -acf option, which fits the estimated spatial autocorrelation function (ACF) to a Gaussian-plus-mono-exponential mixed model. Estimated ACF parameters were then averaged across residuals and fed into 3dClustSim, which uses 10,000 Monte Carlo simulations to form a null distribution of cluster sizes (i.e., nominally significant positive voxels touching at either faces or edges; NN=2, one-sided thresholding, $\alpha=0.05$) based on a pre-specified cluster-forming threshold. The output of 3dClustSim is the minimum cluster size k at the specified cluster-forming threshold such that α is controlled. Due to evidence that liberal cluster-forming thresholds (i.e., $p<0.05$) do not appropriately control the Type I error rate (Eklund, Nichols, & Knutsson, 2016), a conservative cluster-forming threshold of $p<0.001$ was used. Covariates for all analyses included age and pubertal status at baseline, gender, and race/ethnicity.

3.3 Results

Consistent with prior work, the contrast of interest (i.e., Positive Feedback > Negative Feedback) yielded robust VS activity, though somewhat left-lateralized (**Figure 3.2A**). Left, but not right, VS activity predicted early vs. late initiation of drinking (Left VS: $\beta=0.926$, $p=0.019$; Right VS: $\beta=-0.070$, $p=0.844$), such that for every standard-deviation increase in left VS activity, odds of early vs. late initiation increased by a factor of 2.524 (**Figure 3.2B**). No significant differences emerged between the middle group and either of the extreme initiation groups ($ps>0.20$). Use of a continuous variable, right-censored at the age of 19, rather than binned age groups, and a linear model, yielded a nonsignificant trend ($\beta=-0.142$, $p=0.106$), wherein greater left VS activation at baseline was associated with earlier age-at-first-drink. Exploratory whole-brain analyses of early vs. late initiators did not reveal any regions surviving family-wise error correction (i.e., $k=108$ at

$p < 0.001$ uncorrected); however, it is worth noting that a voxel within a cluster in the left VS was the most differentially active between the two groups (**Figure 3.2C**).

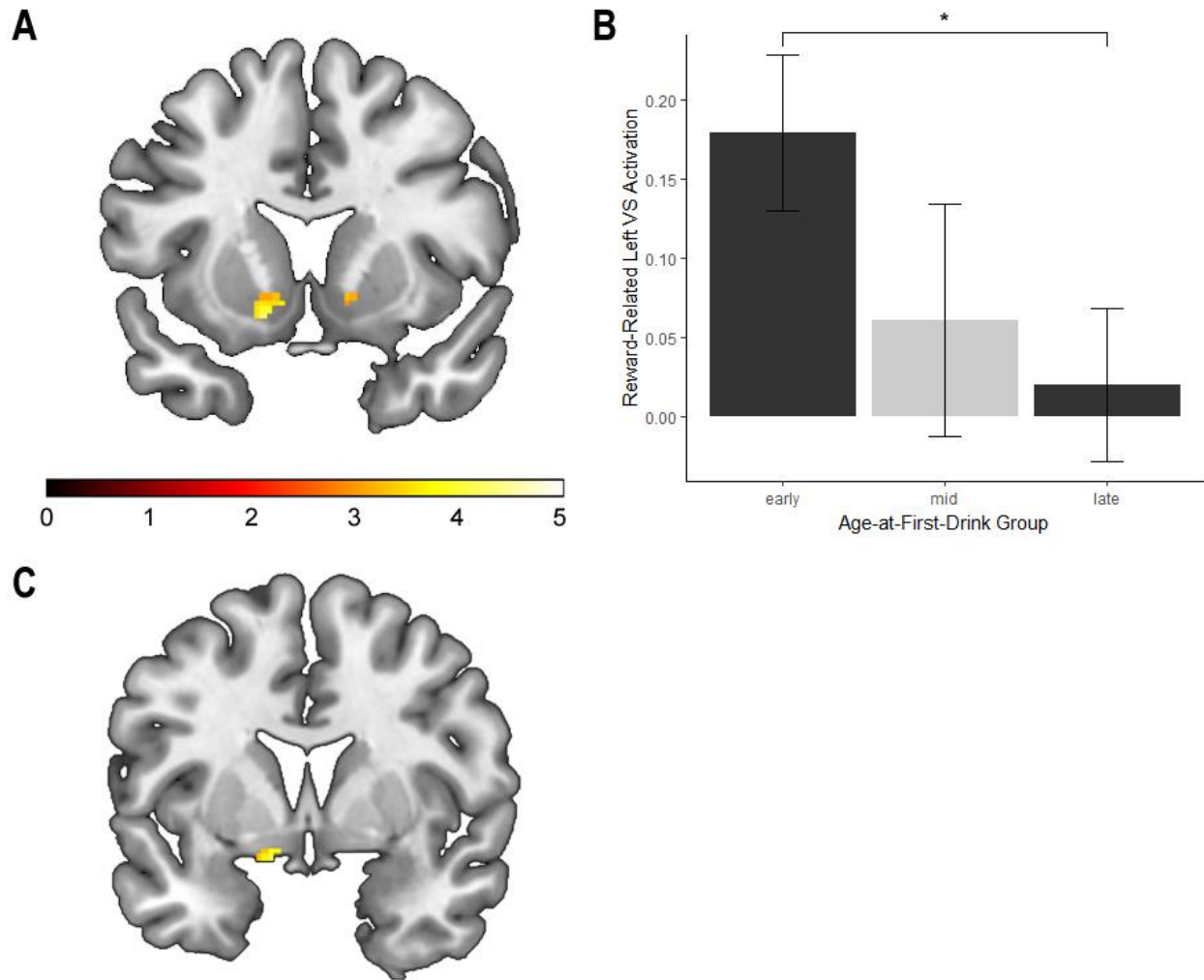


Figure 3.2 Reward-related VS Activation and Relationship to Age-at-first-drink. **A)** Bilateral ventral striatal (VS) activity to reward (Positive Feedback > Negative Feedback) across all participants. Right hemisphere: max voxel MNI coordinates=[12, 10, -6], $t=3.44$, $p < 0.05$ FWE, $k=13$ voxels. Left hemisphere: max voxel=[-14, 14, -12], $t=4.37$, $p < 0.05$ FWE, $k=56$ voxels. **B)** Mean reward-related left VS activation for each initiation group. Values are unadjusted. The middle group (AFD at ages 16-17) is greyed out, as it was not included in initial analyses between early and late initiators. Error bars reflect standard error of the mean. **C)** Maximally activated voxel in the whole-brain contrast of early initiators vs. late initiators. Max voxel=[-12, 4, -14], $t=4.03$, $p < 0.001$ uncorrected, $k=12$ voxels.

* $p < .05$.

3.3 Discussion

Prior studies have repeatedly linked earlier age-at-first-drink (AFD) to problematic alcohol use and AUD diagnosis (e.g., Dawson et al., 2008; DeWit et al., 2000; Grant & Dawson, 1997), though biological risk mechanisms for AFD itself have remained largely unexplored. The results of the current study provide support for the hypothesized prospective relationship between reward-related ventral striatum activation and AFD, wherein greater VS activity to positive versus negative feedback in a monetarily incentivized BOLD fMRI number-guessing task at baseline predicted relatively early (i.e., age 15 or younger) versus late (i.e., age 18 or older) AFD. These findings, considered with those of previous imaging studies of alcohol/substance use initiation (Heitzeg et al., 2014; Stice et al., 2013), escalation (Weiland et al., 2016), and problem drinking (**Chapter 2**), suggest a key role for reward processing in general, and ventral striatum activity in particular, in the early stages of alcohol use.

As discussed in **Chapter 2**, pathways to addiction are thought to occur in stages—including initiation, progression and escalation to regular/heavy use, and finally disordered use (Heath, Martin, Lynskey, Todorov, & Madden, 2002; Hines, Morley, Mackie, & Lynskey, 2015; Neale, Harvey, Maes, Sullivan, & Kendler, 2006)—with age-at-first drink thus being the first point of individual differences in this model. General and specific biological and environmental risk factors are thought to affect each stage (Hines et al., 2015; Kendler, Myers, Dick, & Prescott, 2010; Sartor, Lynskey, Heath, Jacob, & True, 2007). AFD, for example, has been associated with behavioral disinhibition and reward responsiveness, and is therefore thought to be more generally related to externalizing psychopathology risk than to risk for alcohol use disorder in particular (McGue, Iacono, Legrand, Malone, & Elkins, 2001; Pardo, Aguilar, Molinuevo, & Torrubia, 2007; Zernicke, Cantrell, Finn, & Lucas, 2010; but see also Dawson et al., 2008). In contrast, later

progression to dependence may be more so influenced by susceptibility to the physiological and psychological effects of substances (Koob et al., 2004; Schuckit, 1994), thus reflecting SUD-specific risk factors. Correspondingly, rather than acting as an independent causal factor in the pathway to eventual AUD (Prescott & Kendler, 1999; Ystrom, Kendler, & Reichborn-Kjennerud, 2014), earlier age-at-first-drink is thought to be reflective of a heritable propensity towards externalizing behavior (McGue, Iacono, Legrand, & Elkins, 2001; McGue, Iacono, Legrand, Malone, et al., 2001; Pardo et al., 2007; Zernicke et al., 2010), with evidence of common genetic factors influencing both AFD and AUD (Sartor et al., 2009; Ystrom et al., 2014). Within this framework, age-at-first-drink is not only a predictor of future AUD (i.e., 14% decrease in the odds of dependence for each year of delay in AFD; Grant & Dawson, 1997), but also a behavioral expression of heritable biological risk.

The current findings provide evidence that increased VS activity during reward processing is one such biological risk factor influencing AFD and likely the eventual expression of AUD. The few previous prospective imaging studies of substance use initiation have focused primarily on the impulsivity-related construct of response inhibition, reporting less activation in cognitive control-related regions among future initiators relative to continuous nondrinkers (Mahmood et al., 2013; Norman et al., 2011; Wetherill, Squeglia, Yang, & Tapert, 2013). Combined with the results of this study and another which showed increased dorsal striatum (i.e., caudate and putamen) activity in response to monetary reward among adolescents who initiated substance use within one year of the initial scan (Stice et al., 2013), this suggests that the neural underpinnings of multiple facets of impulsivity, including response inhibition and reward sensitivity, may be markers for substance use initiation. Notably, however, my study was the first to investigate AFD, controlling for age at

baseline, rather than whether or not an individual initiated within a certain follow-up period (e.g., 1 year from baseline).

Importantly, these findings have critical implications for the treatment and prevention of alcohol and substance use disorders. If AFD is indeed an expression of heritable risk related to neural reward processing and behavioral disinhibition, rather than a causal environmental factor, then public health efforts to restrict access to alcohol and other substances during adolescence will not address the underlying liability. Instead, interventions targeting reward processing, general impulsiveness, and the early and adequate treatment of other forms of externalizing pathology may be more effective in preventing/delaying initiation and subsequent progression to disordered use. Future studies with both imaging and genetic or familial data should seek to further establish whether these behavioral and functional neural risk markers exhibit co-heritability with AFD, and whether they fully mediate associations between AFD and progression to dependence.

3.3.1 Limitations

The results of the current study should be interpreted in the context of several limitations. First, AFD as a construct has been the subject of recent controversy (see Kuntsche, Rossow, Engels, & Kuntsche, 2016b, and subsequent commentary in Alati, 2016; Diemen & Kessler, 2016; Hingson, Zha, & White, 2016; Kuntsche, Rossow, Engels, & Kuntsche, 2016a; and Windle, 2016), with some researchers arguing that the transition from first drink to problem drinking is a more robust predictor of problematic alcohol use and AUD in adulthood (Kuntsche et al., 2013). Most criticism of AFD has focused on lack of consensus regarding how to operationalize “first drink” (i.e., whether to include sips versus full drinks only) as well as lack of evidence for specific causal influence on later drinking problems (Kuntsche et al., 2016b). Within the current study, first *drink*, relative to first *sip*, was explicitly defined as “a 12-oz. can or a bottle of beer, a 6-oz. glass of wine,

a 12-oz. wine cooler, a shot glass of hard liquor, or a mixed drink with 1 shot (1 1/2 oz.) of hard liquor, like whiskey, scotch, vodka, gin, rum, or tequila,” thus providing a more specificity relative to prior studies in which first sip was included. Additionally, the direct causality of AFD was not relevant to the current study, as I was investigating neural risk markers *for* AFD rather than using it as a predictor for future problems.

Relatedly, AFD was assessed via self-report and thus may not be entirely accurate. For example, two baseline nondrinkers in the current sample subsequently reported ages-of-first-drink preceding their baseline scans. I tried to mitigate this issue by assessing age-at-first-drink at the first timepoint at which a participant ever reported drinking—presumably while his or her first drink would be fresh in his or her mind—and also by binning AFDs into early, middle, and late initiation groups. It is worth noting that it is possible that some individuals in the late initiator group may have continued to abstain; however, given that 86.4% of adults report drinking during their lifetimes (Substance Abuse and Mental Health Services Administration), it is more likely that they simply delayed initiation. Additionally, my definitions of early (i.e., $AFD \leq 15$), middle (i.e., $15 < AFD < 18$), and late (i.e., $AFD \geq 18$) initiation were sample-specific and selected to maximize cell sizes for the extreme groups contrast. As such, they should not be viewed as reflective of population-level initiation patterns, which have generally demonstrated the median age-of-first-drink to be 14 (Chen et al., 2013).

Finally, due to lack of variability in post-initiation drinking behaviors within this sample (see **Appendix A** for a more detailed discussion of this issue), I was unable to test for relationships between reward-related VS activation and later stages of alcohol (mis)use. Future longitudinal studies, perhaps of longer duration or covering a later developmental period, may wish to investigate whether reward-related VS activation is also related to escalation of use and/or

transition to problem drinking—including binge drinking, heavy use, and alcohol use disorder—above-and-beyond the association with AFD. One might expect, for example, that increased VS activation would be related to escalation of use and transition to problem drinking, but not with transition to or maintenance of AUD. Such research would allow for a more thorough interrogation of the role of neural reward processing, specifically within the VS, in the context of stage models of addiction.

3.3.2 Conclusions

Despite these limitations, the current findings provide additional evidence that increased reward-related VS activity is a neural risk marker for earlier initiation of alcohol use, as indexed by age-at-first-drink. Such an association is consistent with increased reward sensitivity being a key factor in the initial stages of alcohol, and possibly other-substance, use (Hommer et al., 2011), particularly during the sensitive developmental period of adolescence (Casey & Jones, 2010). This study confirms and extends prior studies of the role of reward-related activation within the striatum in early stages of alcohol use (Heitzeg et al., 2014; Stice et al., 2013; Weiland et al., 2016), as well as lays the groundwork for future studies of reward-processing in later stages of addiction.

Chapter 4:

Conduct Disorder Genome-Wide Association Study and Extension to Social-Emotional Brain Function

4.1 Introduction

Conduct disorder (CD) is a child and adolescent externalizing disorder characterized by persistent aggressive, deceitful, and rule-breaking behavior (American Psychiatric Association, 2013) that affects 2.1% of individuals under the age of 18 worldwide (Polanczyk, Salum, Sugaya, Caye, & Rohde, 2015). The disorder is associated with a significant global burden of disease (i.e., 5.75 million years lived with disability; Erskine et al., 2014) and substantial public cost (i.e., >\$15,000 per diagnosed child per year in the United States; Beecham, 2014; Foster & Jones, 2005). Further, individuals with CD are at greater risk for other psychiatric disorders (e.g., depression/anxiety: OR=2.10), substance involvement (e.g., illicit drug use: OR=2.11), academic underachievement (e.g., failure to complete high school: OR=2.69), risky sexual behaviors (e.g., early pregnancy: OR=3.03), and criminality (e.g., violence: OR=3.52; Erskine et al., 2016). Despite these negative outcomes, remarkably little is known about the etiology of CD; there is a relative paucity of biological, and, in particular, genetic, research compared to other childhood neuropsychiatric disorders such as attention-deficit/hyperactivity disorder (ADHD) and autism (Moffitt et al., 2008; Salvatore & Dick, 2016). A more thorough understanding of the mechanisms underlying CD is essential to improving treatment and prevention strategies.

Though twin studies suggest that CD is moderately heritable ($h^2 \sim 0.50$; Polderman et al., 2015), few replicable individual risk variants been identified (Salvatore & Dick, 2016). Most

molecular genetic research on CD and related phenotypes has focused on candidate genes in known biological systems (e.g., *MAOA* and *SLC6A4*; Holz et al., 2016; Salvatore & Dick, 2016; Veroude et al., 2016), with some meta-analytic evidence for replicable main (Ficks & Waldman, 2014) and gene-by-environment interaction (Byrd & Manuck, 2014) effects. Genome-wide association studies (GWAS) of conduct disorder (Anney et al., 2008; Dick et al., 2011) and related phenotypes (e.g., behavioral disinhibition: Derringer et al., 2015; aggression: Mick et al., 2011, and Pappa et al., 2016; psychopathic/callous-unemotional [CU] traits: Viding et al., 2010, and Viding et al., 2013; and antisocial personality disorder [ASPD]: Rautiainen et al., 2016, Salvatore et al., 2015, and Tielbeek et al., 2012) have begun to identify suggestive and genome-wide significant (i.e., $p < 5E-08$; **Table 4.1**) loci.

Table 4.1 Prior Genome-wide Association Studies of Conduct-disorder-related Phenotypes

Citation	Phenotype	N	Significant SNPs	Significant Genes
Anney et al., 2008	CD diagnosis	208 cases, 730 controls	none	-
Viding et al., 2010	psychopathic tendencies	300 cases, 300 controls	none	-
Dick et al., 2011	CD symptoms	3963 (872 cases, 3091 controls)	rs16891867, rs7950811, rs11838918, rs1861046	-
Mick et al., 2011	behavior dysregulation	341	none	-
Tielbeek et al., 2012	antisocial behavior	4816 (298 cases, 4518 controls)	none	None
Viding et al., 2013	CU behavior	2930	none	-
Derringer et al., 2015	behavioral disinhibition	1901	none	<i>MAGI2</i> , <i>NAV2</i> , <i>CACNA1C</i> , <i>PCDH9</i> , <i>MYO16</i> , <i>IQCH</i> , <i>DLGAP1</i>
Salvatore et al., 2015	antisocial behavior	1379	none	<i>ABCB1</i>
Pappa et al., 2016	aggressive behavior	18,988	none	<i>AVPR1A</i>

Rautiainen et al., 2016	ASPD	discovery: 370 cases, 5850 controls	rs4714329	-
		replication: 173 cases, 3766 controls		

One way in which genetic risk for conduct disorder may manifest phenotypically is through abnormal neural responsiveness to social-emotional stimuli. Theoretical models (Blair, 2013; Kiehl, 2006; Rubia, 2011) and recent meta-analyses (Alegria et al., 2016; Noordermeer et al., 2016; Raschle et al., 2015) have emphasized a role for regions within the paralimbic system (e.g., orbitofrontal cortex, dorsolateral and medial prefrontal cortex, superior temporal cortex, insula, and amygdala) associated with affect, motivation, and emotion processing. When viewing social-emotional stimuli (e.g., images of facial expressions or individuals in painful situations), for example, individuals with CD, and especially those with comorbid psychopathic/callous-unemotional (CU) traits, tend to show blunted responses in regions such as the amygdala and insula (e.g., (Lockwood et al., 2013; Marsh et al., 2013; Passamonti et al., 2010), but see also (Fairchild et al., 2014)). Despite this evidence, it is unclear whether group differences in these regions are heritable neurobiological risk factors, correlates of environmental risk (e.g., childhood maltreatment), or consequences of disorder expression. As with behavioral genetic studies of CD, prior neurogenetic investigations have focused primarily on candidate genes (e.g., *MAOA*: Buckholtz & Meyer-Lindenberg, 2008), and, to my knowledge, no imaging studies to date have examined unaffected relatives of individuals with conduct disorder. As such, it remains uncertain whether individual differences in activation within these brain regions represent genetically influenced risk markers for CD.

In this study, I performed a GWAS of retrospectively reported *DSM-IV* CD diagnosis in a sample of 1675 Australians of European ancestry from the Comorbidity and Trauma Study (CATS; Nelson et al., 2016). I then tested whether genetic risk factors identified by the GWAS (i.e., genome-wide significant loci and polygenic risk scores) were also associated with self-reported psychopathy constructs in a sample of 406 non-Hispanic Caucasian U.S. college students in the Duke Neurogenetics Study (DNS; Corral-Frias et al., 2015). Finally, based on evidence that CD, psychopathy, and related phenotypes are associated with blunted neural activity to emotional expression in others (see Alegria et al., 2016, Noordermeer et al., 2016, and Rubia, 2011, for meta-analyses), I probed whether genetic risk for CD is associated with individual variability in brain activity to emotional faces in the DNS sample.

4.2 Methods

4.2.1 Genome-wide Association Study

Participants

CATS participants of European ancestry ($N=1675$; $M_{age}=36.25\pm9.13$; 735 females) who had genetic and CD diagnosis data were included in analyses. CATS is a case-control study of opioid-dependent individuals ($n=1232$), aged 18 or older, who were recruited from clinics providing opioid substitution therapy in the greater Sydney, Australia, region (Nelson et al., 2016). Non-opioid-dependent controls ($n=443$) were recruited from socially disadvantaged neighborhoods in geographic proximity to locations where cases had been recruited. Institutional Review Board approval was obtained from University of New South Wales, Washington University School of Medicine, QIMR Berghofer Medical Research Institute, and Sydney area health service ethics committees.

Measures

DSM-IV CD diagnosis ($n_{case}=680$, $n_{control}=995$) was retrospectively assessed using a modified version of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA-OZ; Bucholz et al., 1994; Heath et al., 2011). Consistent with a prior GWAS of CD that also utilized the SSAGA (Dick et al., 2011), the *DSM-IV* criterion requiring temporal symptom clustering (i.e., symptoms must co-occur within a six-month period) was not used, due to the retrospective nature of report. The interview also assessed *DSM-IV* substance abuse and dependence, as well as other forms of psychopathology (**Table 4.2**).

Table 4.2 CATS Sample Demographics

Variable	Overall	Cases	Controls
N	1675	680	995
Age	36.25(9.13)	35.29(8.58)	36.91(9.44)*
Female	43.9%	35.3%	49.6%*
Conduct disorder	40.6%	100%	0%*
Conduct disorder sx	3.28(2.61)	5.49(2.09)	1.77(1.70)*
Opioid dependence	73.6%	86.0%	65.0%*
Alcohol dependence	38.0%	49.3%	30.3%*
Cannabis dependence	49.3%	63.2%	39.8%*
Cocaine dependence	23.8%	31.9%	18.3%*
Nicotine dependence	59.6%	70.2%	52.4%*
Sedative dependence	28.5%	39.0%	21.3%*
Stimulant dependence	43.0%	58.3%	32.6%*
PTSD	33.7%	43.0%	27.4%*
MDD	58.5%	63.5%	55.1%*
ASPD	39.2%	96.1%	0%*

*Indicates a significant difference between cases and controls.

Note. All binary measures are reported in percentages. Continuous measures are presented as $M(SD)$. CATS = Comorbidity and Trauma Study. PTSD = Post-traumatic stress disorder. MDD = Major depressive disorder. ASPD = Antisocial personality disorder.

Notably, suggestive that conduct problems were not secondary to problematic substance use in this sample, onset of substance use disorders largely occurred after age 15, the upper limit for conduct disorder diagnosis in CATS (**Table 4.3**).

Table 4.3 CATS Substance Dependence Average Ages-of-Onset

Substance	Overall	Cases	Controls
Opioid	22.94(6.50)	21.44(6.20)	24.30(6.47)*
Alcohol	20.27(6.48)	19.05(6.11)	21.62(6.61)*
Cannabis	19.83(5.90)	18.73(5.46)	21.02(6.13)*
Cocaine	27.08(7.62)	25.95(7.17)	28.41(7.95)*
Nicotine	21.20(6.37)	20.05(5.86)	22.24(6.63)*
Sedative	25.18(7.34)	23.57(7.12)	27.17(7.13)*
Stimulant	22.78(6.95)	21.65(6.58)	24.14(7.14)*

*Indicates a significant difference between cases and controls.

Note. Measures are presented as $M(SD)$.

Genetics

Genotyping was performed at Johns Hopkins Center for Inherited Disease Research (CIDR) using the Illumina Human660W-Quad BeadChip. Following quality control and data cleaning procedures (duplicate genotyping concordance=99.9%, Hardy-Weinberg equilibrium (HWE) p -values $>10^{-6}$, minor allele frequency (MAF) >0.01 , SNP call and sample genotype rates $>99\%$, GenomeStudio quality score >0.7), 494,990 SNPs were available for analysis.

Ancestry-informative principal components (PCs) were generated using the smartpca program (Patterson, Price, & Reich, 2006) within the EIGENSOFT package (v.5.0.1; <https://www.hsph.harvard.edu/alkes-price/software/>). Consistent with prior genetic analyses of this sample (Carey et al., 2015; Nelson et al., 2016), PCs within the top 10 which showed an association with the outcome phenotype (i.e., CD diagnosis) were used in analyses to control for occult population stratification.

Statistical Analyses

Associations between each autosomal SNP and CD diagnosis were tested using logistic regression and an additive model in PLINK (v.1.07; Purcell et al., 2007). Covariates included sex, age quintile, and four ancestry-informative principal components. Because childhood CD is highly predictive of future substance involvement (Elkins, McGue, & Iacono, 2007), primary analyses

were conducted without opioid dependence status as a covariate. Follow-up analyses with opioid dependence case status as an additional covariate were then conducted.

Versatile Gene-based Association Study 2 (VEGAS2; Mishra & Macgregor, 2015) software was used to conduct gene-based analyses by aggregating SNP-level summary statistics across 24,769 autosomal RefSeq-annotated individual gene definitions, $\pm 50\text{kb}$ in order to capture promoter and flanking variants. Gene-level p-values were calculated using Monte Carlo simulation, accounting for linkage structure based on the 1000 Genomes European reference panel. The adjusted gene-based significance threshold, controlling for the number of genes tested, was $p < 2.02\text{E-}06$ ($\alpha = 0.05 / 24,769$ autosomal genes).

Bioinformatics Investigation of Variant-Associated Gene Expression

Genome-wide significant SNPs were probed for associations with gene expression in brain tissue using data from the CommonMind Consortium (dorsolateral prefrontal cortex from 279 control participants; <http://commonmind.org>; Fromer et al., 2016), UK Brain Expression Consortium's Brain eQTL Almanac (BRAINEAC; 10 brain regions [frontal cortex, occipital cortex, temporal cortex, cerebellar cortex, hippocampus, intralobular white matter, medulla, putamen, thalamus, substantia nigra] obtained from 134 individuals with no evidence of neurodegenerative disorder; <http://www.braineac.org/>; Ramasamy et al., 2014), and Genotype-Tissue Expression project (GTEx; 10 brain regions [anterior cingulate cortex, caudate, cerebellar hemisphere, cerebellum, cortex, frontal cortex, hippocampus, hypothalamus, nucleus accumbens, putamen] available in ≥ 72 individuals; <https://www.gtexportal.org>; Lonsdale et al., 2013) in order to better understand potential mechanisms of action.

4.2.2 Neuroimaging Follow-Up

Participants

Data were available from 438 non-Hispanic Caucasian undergraduate student participants in the DNS, as described in detail in **Section 2.2.1**. Participants were excluded for issues of quality control: a large number of movement or signal intensity outliers in fMRI data ($n=6$), inadequate coverage of the amygdala ($n=10$), poor behavioral accuracy ($n=5$), scanner/equipment malfunction ($n=6$), experimenter error ($n=1$), incidental structural findings ($n=2$) and relatedness ($n=2$, removed randomly from related pairs of participants). Following these exclusions, a final sample of 406 participants ($M_{age}=19.75\pm1.25$; 211 females) remained.

Measures

The 29-item Self-Report of Psychopathy-Short Form (SRP-SF; Paulhus, Neumann, & Hare, 2009) was used to assess psychopathic traits. The SRP-SF has a known four-factor structure (Dotterer et al., 2016), each consisting of seven items scored on a Likert scale from 1 (strongly disagree) to 5 (strongly agree). The *Interpersonal* subscale may be conceptualized as interpersonal manipulation and consists of items such as “I would get a kick out of scamming someone” and “You can get what you want by telling people what they want to hear.” The *Affective* subscale reflects callous-unemotional traits and is composed of items such as “People sometimes say that I’m cold-hearted” and “Most people are wimps.” The *Lifestyle* subscale reflects features such as recklessness and is composed of items such as “I’m a rebellious person” and “I rarely follow the rules.” The *Antisocial* subscale represents criminal tendencies and behavior and is composed of items such as “I have tricked someone into giving me money” and “I have broken into a building or vehicle in order to steal something or vandalize.” Subscale scores showed evidence of acceptable internal consistency ($\alpha s=0.654-0.815$) but were log-transformed to reduce positive skewness (**Figure 4.1**). Finally, to

maintain variability but constrain the influence of extreme outliers, scores were winsorized (i.e., outliers more than $M \pm 3SDs$ [$n_{Antisocial}=5$, all other subscales $n=0$] were set at $\pm 3SDs$ from the mean) prior to analyses.

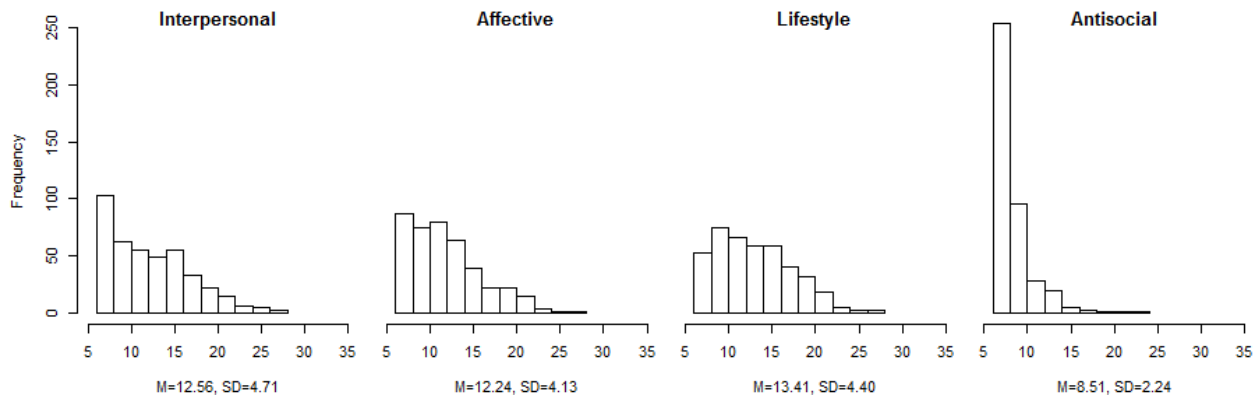


Figure 4.1 Raw Distributions of SRP-SF Subscale Scores. Mean and standard deviation for each subscale are listed below the corresponding histogram. Possible scores range from 5 to 35.

Genetics

DNA was isolated from saliva, genotyped, imputed, and subjected to quality assurance procedures, after which polygenic risk scores (PRS; Purcell et al., 2009) based on the CATS CD GWAS results were generated, and genome-wide significant variants extracted, for analysis. Non-Hispanic Caucasian ancestry was confirmed and proper controls for population stratification were generated using identity by state analysis and multidimensional scaling (MDS) in PLINK (v.1.07; Purcell et al., 2007). Further details of these procedures are available in **Section 2.2.3**.

Neuroimaging

Blood oxygen level-dependent functional magnetic resonance imaging (BOLD fMRI) data were acquired while participants completed an emotional face-matching paradigm (**Figure 4.2**; Carre, Hyde, Neumann, Viding, & Hariri, 2013), consisting of four expression-specific (i.e., Neutral, Angry, Fear, Surprise) face-matching task blocks interleaved with five sensorimotor shape-matching control blocks.

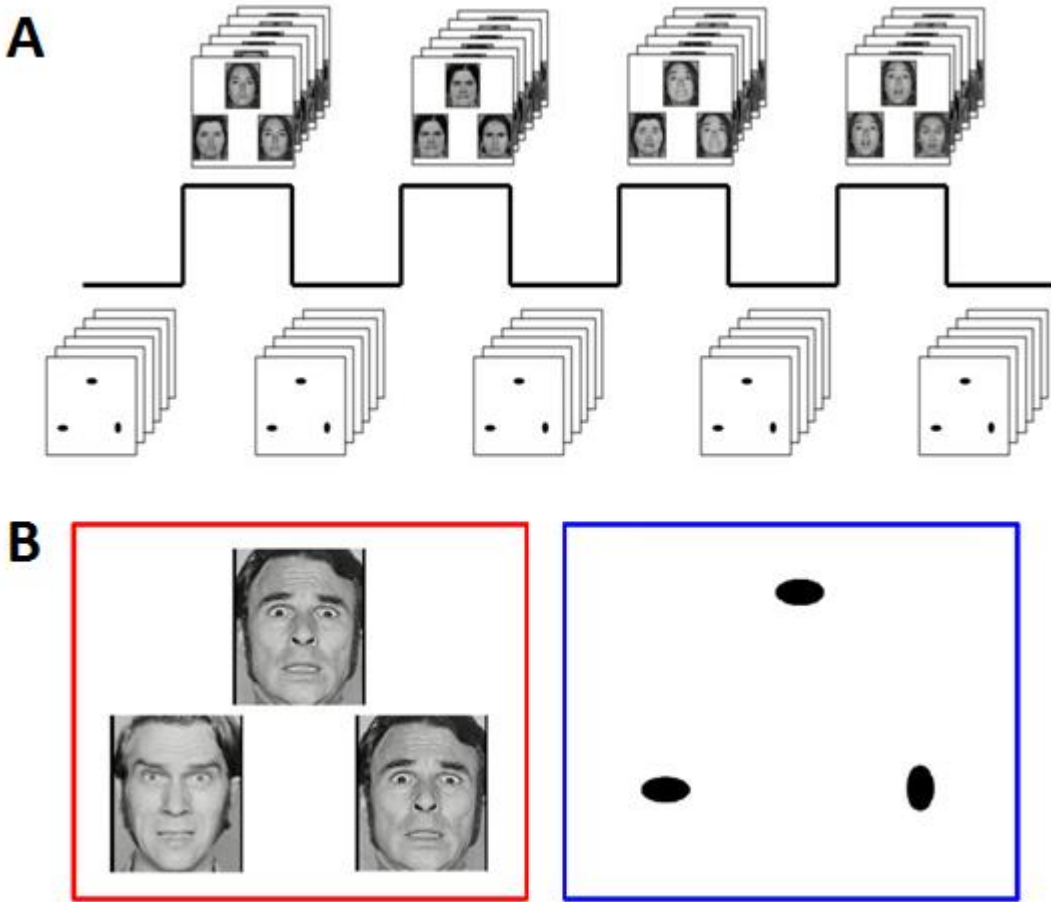


Figure 4.2 BOLD fMRI Corticolimbic Reactivity Paradigm. **A)** Participants completed four expression-specific (Neutral, Angry, Fear, Surprise) face-matching task blocks interleaved with five sensorimotor shape-matching control blocks. Order for task blocks was counterbalanced across participants. **B)** Depiction of one face-matching trial (in red) and one shape-matching trial (in blue).

Order for task blocks was counterbalanced across participants. In each face-matching trial within a block, participants viewed a trio of faces derived from a standard set of facial affect pictures (Ekman & Friesen, 1975) and selected which of two faces presented on the bottom row of the display matched the target stimulus presented on the top row. Each emotion-specific block (e.g., fearful facial expressions only) consisted of six individual trials, balanced for gender of the face. Block order was pseudo-randomized across participants. Each of the six face trios was presented for 4 seconds with a variable inter-stimulus interval of 2-6 seconds; total block length was 48 seconds. In the shape-matching control blocks, participants viewed a trio of geometric shapes (i.e.,

circles, horizontal and vertical ellipses) and selected which of two shapes displayed on the bottom matched the target shape presented on top. Each control block consisted of six different shape trios presented for 4 seconds with a fixed inter-stimulus interval of 2 seconds, comprising a total block length of 36 seconds.

Information regarding BOLD fMRI acquisition and preprocessing procedures is available in **Section 2.2.4**. Following preprocessing, linear contrasts employing canonical hemodynamic response functions were implemented using the general linear model of Statistical Parametric Mapping 8 (SPM8; <http://www.fil.ion.ucl.ac.uk/spm>) to estimate effects of condition (i.e., neutral faces, angry faces, fearful faces, surprised faces, and shapes blocks) for each participant. Contrast images for All Faces Blocks > Shapes Blocks, which reflects broad reactivity to emotionally salient facial stimuli, were then calculated for each participant for use in second-level whole-brain regressions.

Statistical Analyses

A series of linear regressions tested whether genetic risk for CD (i.e., genome-wide significant polymorphisms and PRS from the CATS GWAS), was associated with individual differences in self-reported psychopathy (i.e., SRP-SF *Interpersonal*, *Affective*, *Lifestyle*, and *Antisocial* subscales). I made no a priori predictions regarding which of the four SRP-SF factors would be associated with CD genetic risk and thus treated these analyses as exploratory.

Associations between CD genetic risk and neural reactivity to emotional faces were assessed at the whole-brain level in SPM8. All Faces Blocks > Shapes Blocks contrast images for each participant were entered into a second-level multiple regression model with CD genetic risk as the covariate of interest. PRS were calculated at the P-value threshold of $P < 0.50$ based on the CATS CD GWAS to maximize the number of variants included while requiring some evidence of

association; post hoc tests at other P-value thresholds were also conducted. To correct for multiple comparisons, significant regions of activation were required to meet a voxel-wise $p < 0.05$ FWE threshold and a cluster extent threshold of 10 contiguous voxels.

Sex and two ancestry-informative MDS components were entered as covariates of no interest for all analyses.

4.3 Results

3.3.1 Genome-wide Association Study

One variant, rs12536973 (A/G; call rate=0.998, HWE $p=0.271$, MAF=0.113) in an intergenic region on chromosome 7, attained genome-wide significance ($p=3.74\text{E-}08$), with several other independent signals suggestive of association (i.e., $p < 1\text{E-}05$; **Table 4.4**; **Figure 4.3**). At the top locus, the number of minor G alleles was associated with relatively decreased odds (i.e., 0.505, 95% CI[0.396, 0.644]) of CD diagnosis.

Table 4.4 SNPs Associated with Conduct Disorder Diagnosis in CATS Participants ($p < 1\text{E-}05$)

SNP	Chr	BP	Gene	A1 ^a	A2	A1 Freq	OR [95% CI]	P-value	P-value, OD ^b
rs12536973	7	10062341	Intergenic	G	A	0.11	0.51 [0.40, 0.64]	3.74E-08	5.74E-08
rs1928228	9	88698820	<i>GOLM1</i>	A	C	0.11	0.53 [0.41, 0.67]	3.56E-07	3.95E-07
rs1595146	4	29316636	Intergenic	G	A	0.42	0.71 [0.61, 0.82]	4.74E-06	5.48E-06
rs7893421	10	72991385	<i>UNC5B</i>	A	G	0.44	0.72 [0.62, 0.83]	5.59E-06	1.48E-05
rs10974824	9	4869801	Intergenic	A	C	0.06	0.47 [0.34, 0.65]	6.72E-06	1.12E-05
rs8045276	16	29025978	Intergenic	A	G	0.30	0.70 [0.59, 0.82]	7.23E-06	4.96E-06

^aA1 denotes the minor allele in our sample. ORs were calculated using A1 as the effect allele.

^bP-value when covarying for opioid dependence status.

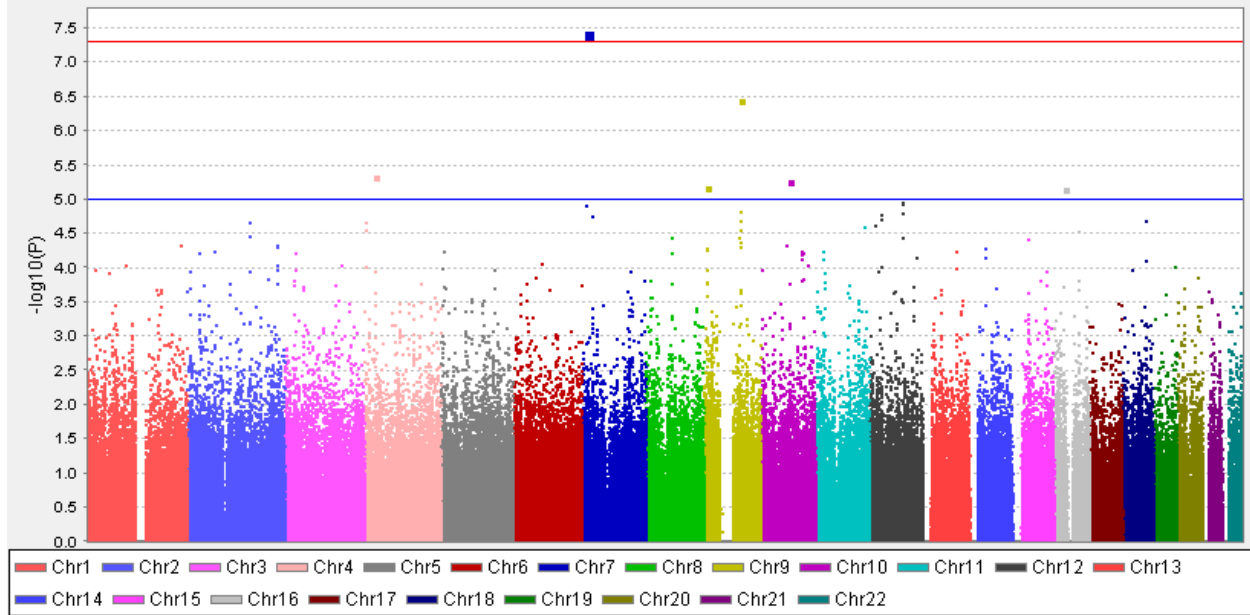


Figure 4.3 Manhattan Plot of Results from the Conduct Disorder GWAS. Suggestive association threshold (i.e., $p < 1E-05$) is indicated by the blue line; GWAS significance threshold (i.e., $p < 5E-08$) is indicated by the red line. Figure was created using HaploView (<https://www.broadinstitute.org/haploview/>; (Barrett, Fry, Maller, & Daly, 2005)).

Annotation analyses revealed no strong evidence that rs12536973 genotype or SNPs in LD with it are associated with differential gene expression. There was a nominally significant association between rs12536973 genotype and *PHF14* expression in prefrontal tissue within the CommonMind Consortium (dorsolateral prefrontal cortex: $\beta=0.020$, $p=0.019$, uncorrected) and BRAINEAC (t2990043; frontal cortex; $p=0.045$, uncorrected; but not other regions, all $ps > 0.20$) databases, wherein the A allele—the “risk” allele in the CD GWAS—was associated with relatively increased *PHF14* expression. However, this relationship did not approach significance when accounting for multiple testing across genes and brain regions and was not even nominally significant within GTEx.

Gene-based tests revealed one gene, *GOLM1*, associated with CD diagnosis ($p=2.00E-06$; see **Table 4.5** for the top 10 gene-based associations). Within *GOLM1*, 13 of 23 SNPs were

nominally associated (i.e., $p < 0.05$) with CD, with the strongest evidence of association at rs1928228 ($p = 3.56 \times 10^{-7}$; **Figure 4.4**).

Table 4.5 Top 10 Gene-level Associations in CATS

Gene	Chr	Starting BP	Ending BP	# SNPs	Gene-level P-value
<i>GOLM1</i>	9	88591057	88765116	23	2.00E-06
<i>NAA35</i>	9	88506056	88687217	19	3.00E-06
<i>MIR101-2</i>	9	4800296	4900375	47	6.00E-05
<i>RCL1</i>	9	4742833	4911077	67	9.20E-05
<i>GGH</i>	8	63877638	64001610	17	1.71E-04
<i>LOC100144595</i>	2	155242364	155363950	48	2.15E-04
<i>LOXL4</i>	10	99957442	100078007	24	3.26E-04
<i>MIR4515</i>	15	83686086	83786167	11	0.000328
<i>BTBD1</i>	15	83635180	83786106	13	0.000337
<i>C5orf49</i>	5	7780490	7901603	42	0.000376

Note. Associations surviving correction for multiple comparisons are bolded. Base pair positions for each gene extend 50kb from the 5' and 3' UTR.

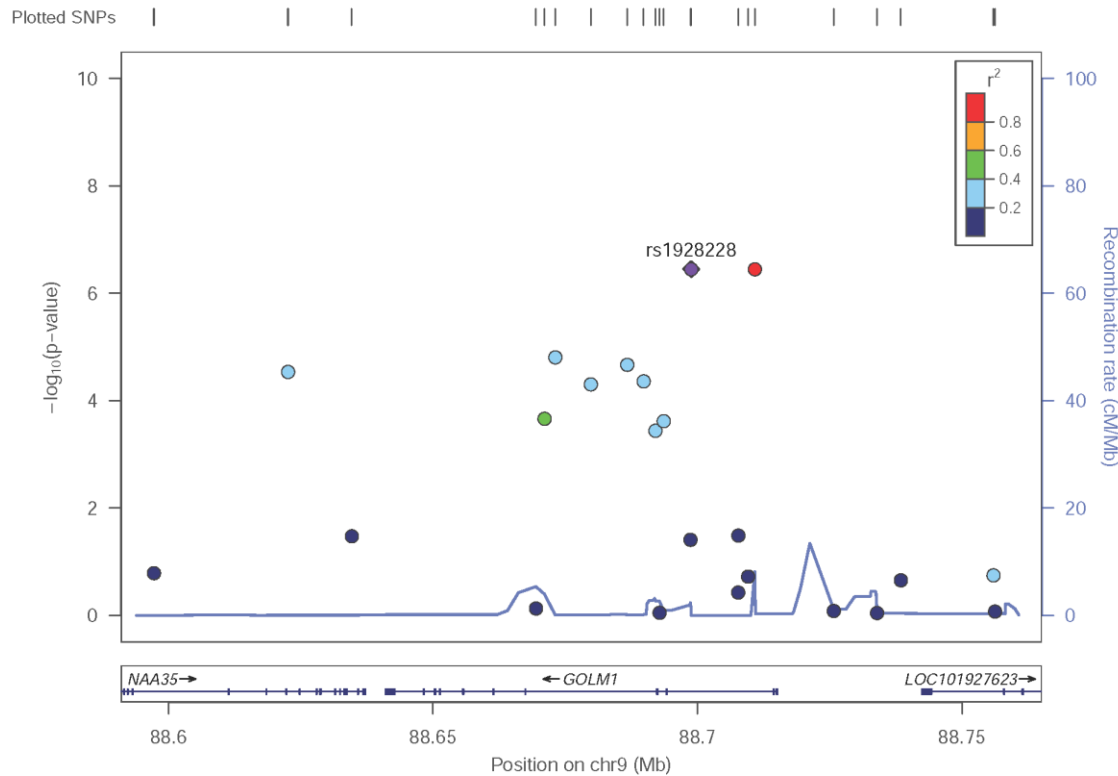


Figure 4.4 Regional Association Plot for All SNPs within *GOLM1*. The x-axis represents the position of each SNP on chromosome 9. In the scatterplot, the y-axis is the negative log of the p-value of each association. Colors indicate linkage patterns with the most significant SNP in the region, rs1928228, whereby warmer colors represent higher r^2 values, while cooler colors represent lower R^2 values. The recombination rate across the region, based on HapMap data, is represented by the solid blue line. Figure was created using LocusZoom (<http://locuszoom.org/>; Pruim et al., 2010).

Replication of Prior Associations

Following these exploratory analyses, prior published SNP- and gene-level associations (**Table 4.1**) were queried for significance in the current sample. Of all previously identified SNPs, only one variant, rs4714329 (A/G; call rate=1.000, HWE $p=0.614$, MAF=0.410) in an intergenic region on chromosome 6, attained nominal significance (i.e., $p=0.022$; **Table 4.6**). As in the original paper (Rautiainen et al., 2016), the number of minor G alleles was associated with relatively increased odds (i.e., 1.184, 95% CI[1.024, 1.369]) of CD diagnosis. No prior gene-level associations were replicated in the current sample (**Table 4.7**).

Table 4.6 Conduct Disorder Associations with Previously-Identified SNPs

Citation	SNP	Chr	BP	A1 ^a	A2	A1 Freq	Original OR	OR [95% CI]	P-value
Dick et al., 2011 ^b	rs1861046	4	15397906	A	G	0.03	1.65	0.77 [0.51, 1.16]	0.21
	rs7950811	11	92651002	A	C	0.04	1.65	0.83 [0.58, 1.19]	0.31
Rautiainen et al., 2016	rs4714329	6	40273457	G	A	0.41	1.59	1.18 [1.02, 1.37]	0.02

^aA1 denotes the minor allele in our sample. ORs were calculated using A1 as the effect allele.

^bDick et al., 2011, meta-analyzed across both European-American and African-American subsamples. In European-American ancestry, rs1861046 and rs16891867 are in perfect LD, and rs11838918 is not represented.

Table 4.7 Conduct Disorder Associations with Previously-Identified Genes

Citation	Gene	Chr	Starting BP	Ending BP	# SNPs	Gene-level p-value
Derringer et al., 2015	<i>MAGI2</i>	7	77596373	79132890	479	0.68
	<i>NAV2</i>	11	19322270	20193147	306	0.39
	<i>CACNA1C</i>	12	2112415	2857115	188	0.24
	<i>PCDH9</i>	13	66826965	67854468	186	0.77
	<i>MYO16</i>	13	109198499	109910355	179	0.17
	<i>IQCH</i>	15	67497137	67844142	21	0.08
	<i>DLGAP1</i>	18	3446029	4505266	279	0.57
Salvatore et al., 2015	<i>ABCB1</i>	7	87083178	87392639	76	0.39
Pappa et al., 2016	<i>AVPR1A</i>	12	63486538	63596590	22	0.87

Note. Base pair positions for each gene extend 50kb from the 5' and 3' UTR.

4.3.2 Neuroimaging Follow-Up

The genome-wide significant SNP in the CD GWAS, rs12536973, was extracted for analysis in the DNS (directly genotyped, call rate=0.995; HWE $p=1.000$, MAF=0.116; $n=404$). Due to low MAF, minor allele homozygotes ($n_{GG}=6$) were combined with heterozygotes ($n_{AG}=82$) to form one

group of “CD-protected” minor allele carriers ($n_{AG/GG}=88$), who were compared to major allele homozygotes ($n_{AA}=316$).

Consistent with the CATS GWAS results, rs12536973 A allele homozygotes had greater SRP-SF *Lifestyle* scores relative to G allele carriers ($\beta=0.123$, $\Delta R^2=0.015$, $p=0.010$). No other subscales (i.e., *Affective*, *Antisocial*, or *Interpersonal*) were associated with rs12536973 genotype (i.e., $ps>0.40$; **Table 4.6**).

Table 4.6 Top GWAS Hit Associations with SRP-SF Scores in DNS

Subscale	β	ΔR^2	P
Interpersonal	0.039	0.001	0.420
Affective	0.017	0.000	0.709
Lifestyle	0.123	0.015	0.010
Antisocial	0.016	0.000	0.740

PRS derived from the CD GWAS were nominally associated with the SRP-SF *Antisocial* subscale (significant at 3 of 10 P-value thresholds; largest effect at $P<0.10$: $\beta=0.116$, $\Delta R^2=0.013$, $p=0.019$; **Figure 4.5**), but not with *Lifestyle* or other subscales (i.e., $ps>0.08$).

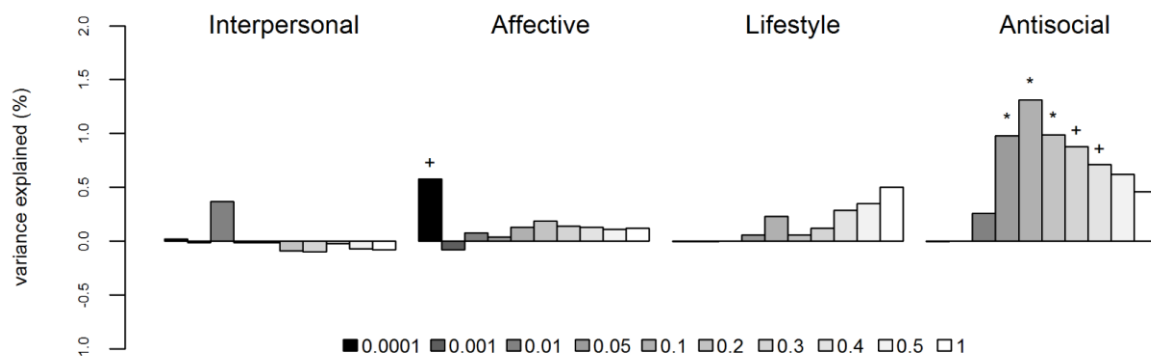


Figure 4.5 Associations between CD PRS and SRP-SF Subscales in the Duke Neurogenetics Study. Y-axis is the percent of variation in the outcome measure explained by CD PRS. Positive values indicate a positive association between CD PRS and the outcome. Negative values are for display purposes only and indicate a negative association. Shades of gray in legend indicate the P-value threshold at which the risk score was calculated.

* $p<0.05$, + $p<0.10$

Repetition of analyses with raw (i.e., non-winsorized, untransformed) data did not substantively alter results.

Despite significant associations with SRP-SF *Lifestyle* scores, rs12536973 genotype was not associated with differential neural activity to All Faces Blocks > Shapes Blocks when accounting for multiple comparisons at the voxel-wise level. Increased genome-wide polygenic risk for CD, however, was associated with decreased left insula activity (max voxel Montreal Neurological Institute [MNI] coordinates=[-32, 2, 14], $k=13$, $t=4.95$, whole-brain voxel-wise $p<0.05$ FWE; **Figure 4.6**).

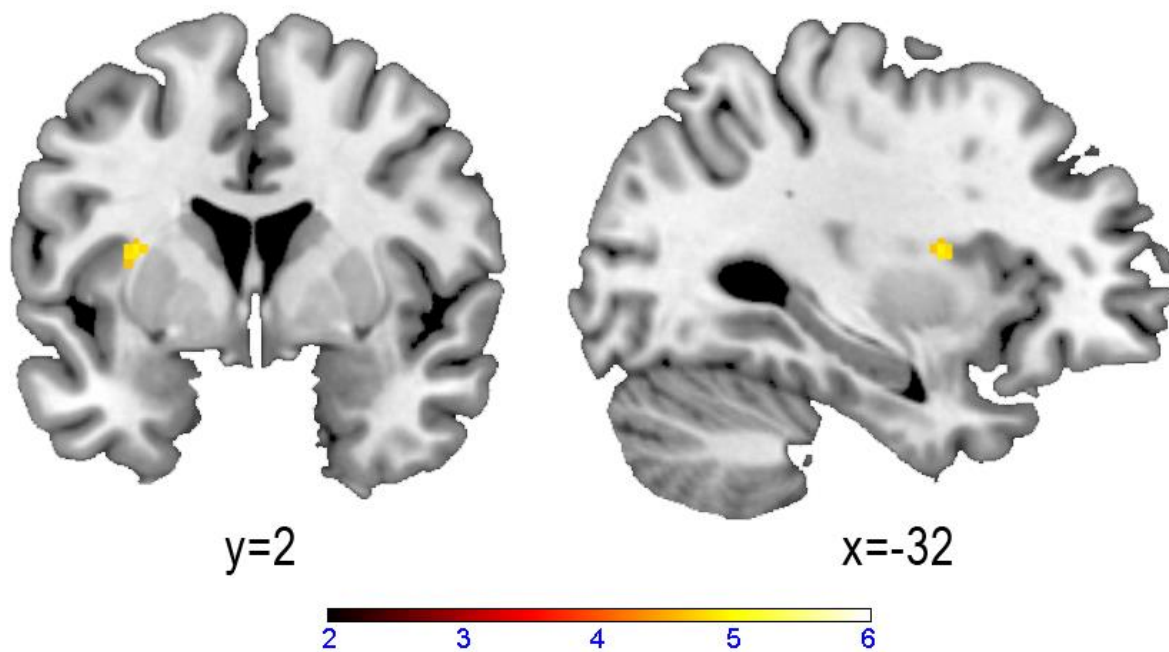


Figure 4.6 Associations between CD PRS and Whole-brain Activity to Emotional Faces. CD PRS were associated with decreased activity within a cluster in the left insula (max voxel MNI coordinates=[-32, 2, 14], $k=13$, $t=4.95$, $p<0.05$ FWE). No clusters were positively associated with PRS. PRS were calculated based on variants meeting a threshold of $P<0.50$ in the original GWAS.

Notably, post hoc analyses revealed that this cluster was associated with CD PRS at 7 of 10 P -value thresholds using a $p<0.005$ whole-brain uncorrected threshold (**Figure 4.7**).

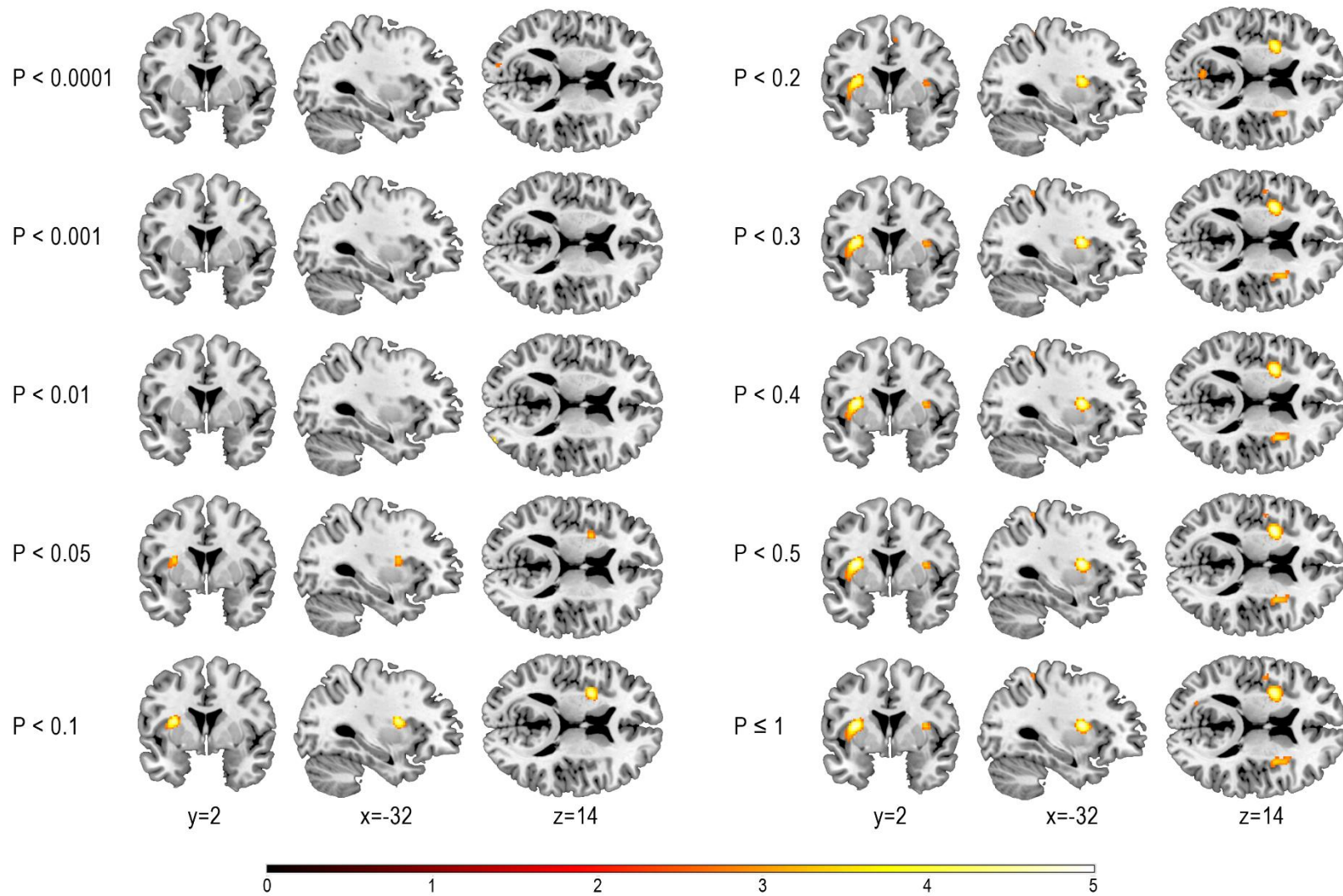


Figure 4.7 Associations between CD PRS and Whole-brain Activity to Emotional Faces Across All P-value Thresholds. Images are presented at the coordinates of the maximum voxel in our primary analysis (P-value threshold of $P < 0.05$: max voxel MNI coordinates= $[-32, 2, 14]$, $k=13$, $t=4.95$, $p < 0.05$ FWE) and are thresholded at $p < 0.005$, uncorrected.

Because PRS were associated with increased SRP-SF *Antisocial* subscale scores as well as decreased activity in the insula during the face-matching task, I undertook a post hoc conjunction analysis to investigate whether left insula activity, or activity in any other regions, may be associated with both CD PRS and SRP-SF *Antisocial* scores. As in prior research (Mioshi, Hodges, & Hornberger, 2013), I overlaid statistical maps, each thresholded at $p < 0.005$, uncorrected, from independent whole-brain regressions conducted in SPM8 with CD PRS and SRP-SF *Antisocial* scores as covariates of interest, respectively, in xjView (<http://www.alivelearn.net/xjview>), which revealed overlapping activation clusters in the bilateral insula and supramarginal gyri (**Figure 4.8**).

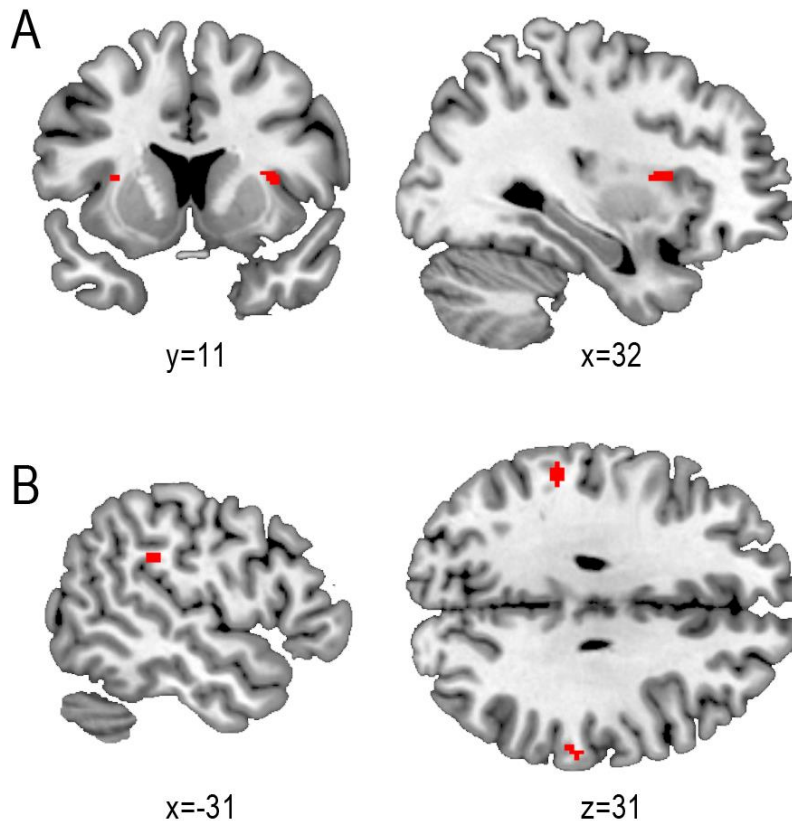


Figure 4.8 Brain Regions Associated with Both CD PRS and SRP-SF *Antisocial* Scores. The highlighted regions represent the conjunction of those clusters, within the bilateral A) insula and B) supramarginal gyri, negatively associated with CD PRS and SRP-SF *Antisocial* at a threshold of $p < 0.005$, uncorrected. No overlap occurred for positively-associated regions.

4.4 Discussion

In this study, analyses identified one genome-wide significant variant, rs12536973, and one gene-level significant gene, *GOLM1*, associated with conduct disorder in a discovery GWAS dataset of 1675 Australians of European ancestry ($n_{case}=680$, $n_{control}=995$). Providing preliminary evidence of extension to subclinical trait variation, rs12536973 genotype and PRS generated from GWAS summary statistics were also associated with individual differences in psychopathy among an independent sample of 406 non-Hispanic Caucasian U.S. college students who completed a neuroimaging protocol. Exploratory whole-brain analyses in the extension sample further demonstrated that polygenic risk for CD is associated with blunted left insula activity to emotional faces, and a post hoc conjunction analysis showed that decreased bilateral insula and supramarginal gyrus activation is linked to both CD polygenic risk and self-reported psychopathy, providing a putative neural mechanism through which polygenic risk for CD may be phenotypically expressed.

4.4.1 Genetic Associations with Conduct Disorder

The major A allele at rs12536973 was associated with increased rates of CD in the discovery GWAS, as well as with higher self-reported SRP-SF *Lifestyle* scores (e.g., “I’m a rebellious person” and “I rarely follow the rules”) in the extension sample. This locus resides in an intergenic region on chromosome 7 (7p21.3) between LOC340268, the C3 and PZP-like, alpha-2-macroglobulin domain containing 8 pseudogene, and HSPA8P8, the heat shock protein family A (Hsp70) member 8 pseudogene 8. Though the variant itself is noncoding, functional annotation using multiple brain tissue databases provided preliminary (i.e., nominally significant in two datasets without correction for multiple testing) evidence that rs12536973 may be associated with

differential *PHF14* expression in the prefrontal cortex. While the function of *PHF14* is poorly understood, it belongs to the class of highly conserved plant homology domain (PHD) finger transcription factor genes, which code for proteins structurally equipped to read the sequence-dependent methylation state of H3K4 histones (Sanchez & Zhou, 2011). However, the links between *PHF14* expression and externalizing behaviors are unknown, and the current results linking the CD GWAS-significant variant to *PHF14* expression should be considered speculative at best given the nominal nature of the association.

Gene-based analyses revealed one gene, *GOLM1*, the Golgi membrane protein 1, showing significant signal enrichment, and another nearby gene, *NAA35*, the N(alpha)-acetyltransferase 35, NatC auxiliary subunit, which was suggestive of association. Both are located at 9q21.33, and in gene-based analyses including ± 50 kb from defined gene boundaries, 9 SNPs were shared between analytic sets. However, repetition of analyses without extending gene boundaries to include promotor and flanking regions did not substantially alter empirical p-values (i.e., *GOLM1* $p=2.00\text{E-}06$, *NAA35* $p=2.91\text{E-}05$), indicating that the *NAA35-GOLM1* region as a whole is likely associated with CD. Interestingly, prior research has linked both *GOLM1* and *NAA35* to neuropsychiatric phenotypes: *GOLM1* with Alzheimer's disease (H. Li et al., 2008), schizophrenia (Mudge et al., 2008), and prefrontal cortex volume (Inkster et al., 2012), and *NAA35* with alcohol use (Pan et al., 2013; Rodd et al., 2008). These findings suggest that the *NAA35-GOLM1* region, and *GOLM1* in particular, may affect cognition in a broad manner, though the molecular mechanism remains to be explored.

None of the top GWAS hits (i.e., rs12536973, which was genome-wide significant, and the five other suggestive GWAS associations, $p<1\text{E-}05$; **Table 4.4**) or gene-level associations (i.e., *GOLM1* and the other nine most significant associations; **Table 4.5**) have been previously

identified in prior association or linkage studies of conduct disorder or related phenotypes (see **Table 4.1** for prior significant associations and the cited studies therein for lists of suggestive associations). Examination of previously-identified genes and SNPs in our sample revealed nominal evidence of replication (i.e., $p=0.022$) for rs4714329 (**Table 4.6**), which had originally been associated with ASPD diagnosis in a meta-analysis of two samples (Rautiainen et al., 2016). Given that CD is a diagnostic prerequisite for ASPD, and in our phenotypically extreme sample 96.1% of individuals with CD in childhood qualified for a diagnosis of ASPD at the time of the interview, replication of this association provides further evidence for a role of rs4714329 in antisocial behavior across the lifespan.

4.4.2 Neuroimaging Extension

Polygenic risk for CD was negatively associated with left anterior insula activation to the emotional face-matching task and positively coupled with SRP *Antisocial* subscale scores (e.g., “I have tricked someone into giving me money” and “I have broken into a building or vehicle in order to steal something or vandalize”). The insula is involved in interoceptive awareness, decision-making, empathy, and overall social-emotional processing (Craig, 2009; Gu et al., 2013; Lamm & Singer, 2010; Mutschler et al., 2013; Naqvi & Bechara, 2009), and has been repeatedly implicated in studies of conduct disorder and related phenotypes (Alegria et al., 2016; Hyde, Shaw, & Hariri, 2013; Noordermeer et al., 2016; Raschle et al., 2015; Rogers & De Brito, 2016; Rubia, 2011). One meta-analysis identified the left insular cortex as one of two regions (the other being the right dorsomedial prefrontal cortex) associated with differences across both structural and functional imaging modalities among adolescents exhibiting aggressive behavior (Raschle et al., 2015). Perhaps most relevant to the current study, the insula is essential to “affective empathy” (i.e., the vicarious experience of the emotions of others; Lockwood, 2016). For example, one study found

that in-scanner ratings of affective responses to emotional faces correlated with anterior insula activity, and, further, that such activity was negatively associated with SRP-SF *Lifestyle-Antisocial* scores, mirroring our subscale-specific observations (Seara-Cardoso, Sebastian, Viding, & Roiser, 2016). Moreover, passive viewing of emotional or painful video clips elicits relatively lower insula activation in psychopathic (Meffert, Gazzola, den Boer, Bartels, & Keysers, 2013) and offender (Arbuckle & Shane, 2016) populations, an effect that is normalized upon instruction to “feel” or empathize with the actors in the videos. In the context of these prior studies, the current results suggest that blunted left insula activation may be a neural mechanism through which genomic risk for CD confers less spontaneous affective empathy, and, in turn, increased antisocial behavior.

The post hoc conjunction analysis revealed that, in addition to the insula, activation in the supramarginal gyrus was also negatively associated with both CD polygenic risk and SRP-SF *Antisocial* scores. While this result should be interpreted with caution, as associations with CD PRS at the whole-brain level in this region did not survive family-wise error correction, several studies have linked CD and related phenotypes to differences in supramarginal gyrus structure (Huebner et al., 2008; Hyatt, Haney-Caron, & Stevens, 2012), function (Decety, Skelly, & Kiehl, 2013; Klapwijk et al., 2016; Meffert et al., 2013), and connectivity (Philippi et al., 2015). As with the insula, it has been associated with spontaneous vicarious representations of pain and emotion (Arbuckle & Shane, 2016; Meffert et al., 2013; Seara-Cardoso et al., 2016). In addition to affective representation, it also plays a role in self-other processing and perspective-taking (i.e., “cognitive empathy”; Bird & Viding, 2014; Hooker, Verosky, Germine, Knight, & D’Esposito, 2010), specifically in resolving conflicts between one’s own mental state and the mental states of others (Silani, Lamm, Ruff, & Singer, 2013). Taken together, while speculative, a genetic predisposition to hypoactivation of the insula and supramarginal gyrus in response to social-emotional signals

may result in a lack of affective representation combined with a diminished ability to process others' perspectives, causing a fundamental breakdown of empathic responding. Such an interpretation is strengthened by the fact that this relationship exists in the relatively healthy DNS sample, with minimal CD expression or enrichment of associated environmental confounds (e.g., childhood trauma, illicit drug use).

4.4.3 Limitations

These results must be interpreted in the context of several limitations. First, the discovery GWAS sample ($N=1675$) was underpowered to detect the small individual-variant effect sizes typically seen in studies of complex traits (Sullivan, Daly, & O'Donovan, 2012). However, because the CATS sample was recruited for opioid dependence, it is enriched for CD as well as extreme presentations (i.e., 40.6% cases in our study vs. 22.0% cases in Dick et al., 2011, and 2.1% in the general population, Polanczyk et al., 2015; cases reporting 5.49 ± 2.09 *DSM-IV* conduct disorder symptoms in this study vs. 4.62 ± 3.48 in Dick et al., 2011, $p < 0.001$). This may result in larger observed effect sizes than expected in non-extreme-case designs (CONVERGE Consortium, 2015). While this design potentially limits generalizability of the current results, the observation of a consistent association in a high-achieving college sample provides further support for the initial findings.

Second, regarding assessment, conduct disorder diagnosis was reached via retrospective self-report and may thus be subject to memory biases. Additionally, though conduct disorder was assessed in our neuroimaging extension sample, no participants met its criteria and minimal symptoms were endorsed. This precluded a more direct replication attempt of genetic associations with CD diagnosis and instead led me to examine individual differences in self-reported psychopathy. While this approach resulted in greater variation than using a binary diagnostic

variable, scores were still positively skewed in the DNS sample (**Figure 4.1**). Arguably, the lack of severe conduct disorder-related symptoms in the neuroimaging sample is a strength, particularly alongside the severe CD presentation in CATS, as it allowed me to explore relationships with neural regions without the confound of disorder expression. Nonetheless, it remains a caveat, along with small sample size ($N=406$), in any attempts to frame my extension results, though consistent with those of the original CD GWAS, as a replication.

Third, the analyses performed in this study were exploratory in nature and thus require future replication. Though I have attempted to control the Type I error rate in all mass univariate analyses within this study (i.e., genome-wide, gene-level, and whole-brain association) and denoted where results did not meet stringent correction thresholds (i.e., polygenic association with psychopathy subscales and brain-tissue-specific gene expression at the top GWAS locus), replication remains the gold standard of evidence, particularly in fields such as psychiatric and imaging genetics that have been plagued by recent high-profile replicability crises (Ryan Bogdan et al., 2017; Duncan & Keller, 2011). Overall, the results presented in this study should be considered convergent and suggestive yet preliminary.

Finally, the phenotypic and genetic architecture of CD is complex and likely reflects multiple possibly independent factors. A prior twin study, for example, suggested that two genetic factors, reflecting rule-breaking and aggression, influence risk for conduct disorder (K. S. Kendler, Aggen, & Patrick, 2013), consistent with a prior factor analysis indicating the presence of aggressive and non-aggressive subtypes (Tackett, Krueger, Sawyer, & Graetz, 2003). Furthermore, fewer than half of individuals with CD meet criteria for the new *DSM-5* modifier “with limited prosocial emotions,” meant to reflect callous-unemotional traits such as lack of remorse/guilt and reduced empathy traditionally representative of psychopathy (Frick, Ray, Thornton, & Kahn,

2014). Children and adolescents exhibiting callous-unemotional traits are at increased risk for the development of ASPD in adulthood, as well as numerous related adverse outcomes (Blair, Leibenluft, & Pine, 2014). Due to limited discovery sample size and use of *DSM-IV* diagnostic criteria, it was not feasible to examine potentially different genetic influences on these subtypes, though association of the top GWAS hit and polygenic risk scores with the SRP-SF *Lifestyle* and *Antisocial* scales, respectively, but not *Interpersonal* or *Affective* scores, suggests some specificity to behavior. Future genetic and neuroimaging studies may wish to interrogate distinct CD-related presentations in order to identify general and subtype-specific risk markers.

4.4.4 Conclusion

In the current study, I linked a novel genome-wide significant locus, rs12536973, and the *NAA35-GOLM1* genomic region to CD based on a GWAS of severe CD presentations. The top locus and polygenic risk for CD derived from the discovery GWAS were further associated with individual differences in self-reported psychopathy constructs. Lastly, both polygenic risk for CD and self-reported psychopathy were associated with blunted activity in overlapping clusters within the bilateral insula and supramarginal gyri during an emotional face-matching paradigm. These results suggest that blunted neural responses to social-emotional stimuli in brain regions previously linked to individual differences in empathy may represent a potential neural mechanism through which genomic risk may promote the expression of CD.

Chapter 5:

General Discussion

5.1 Overall Summary

Overall, this series of three studies probed the neural and genetic risk mechanisms underlying externalizing spectrum disorders (**Figure 1.1**; Beauchaine & McNulty, 2013; Beauchaine et al., 2017), which are characterized primarily by disinhibition and thought to be linked by a continuous latent construct (Hicks et al., 2004; Krueger et al., 2002; Krueger et al., 2007; Krueger et al., 2005; Markon & Krueger, 2005; Young et al., 2000). In **Chapter 2**, increased reward-related ventral striatum activity was shown to be associated with both genetic risk for childhood ADHD and problematic alcohol use in young adulthood, consistent with these disorders arising from a common mechanism. **Chapter 3** complemented the results of **Chapter 2** by showing that heightened reward-related VS activity in early adolescence, a critical developmental period, prospectively predicts early versus late onset of drinking. In **Chapter 4**, genetic risk for conduct disorder, quantified based on a genomewide association analysis, also predicted self-reported psychopathy and blunted insula activity during social-emotional processing among healthy college students, indicative of a common genetic architecture underlying both normal and pathological variation. Taken together, these findings identify putative genetically influenced neural mechanisms underlying externalizing psychopathology, shed light on the nature of premorbid risk versus downstream disorder-related consequences, and provide further evidence for a continuous relationship between ESDs and normal trait variation.

5.1.1 Neurogenetic Mechanisms

Across the three studies, two neural mechanisms linking genetic risk to externalizing pathology emerged: heightened reward-related VS activity (**Chapters 2 and 3**), and blunted anterior insula activity during social-emotional processing (**Chapter 4**). In each case, polygenic risk for an ESD (i.e., ADHD in **Chapter 2** and CD in **Chapter 4**) was linked to differential neural activity (i.e., heightened reward-related VS activity and blunted anterior insula activity to emotional faces, respectively), which was then linked to subclinical externalizing behaviors (i.e., drinking patterns in **Chapters 2 and 3** and self-reported psychopathy in **Chapter 4**, respectively). As such, these differences in relative task-evoked regional activation represent strong candidate intermediate externalizing phenotypes.

Reward-related Ventral Striatum Activation

The ventral striatum is a subcortical region consisting of the nucleus accumbens, ventral portions of the caudate and putamen, and olfactory tubercle (Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004). It is a key component of the mesolimbic dopamine pathway, receiving input from the ventral tegmental area, basolateral amygdala, hippocampus, orbitofrontal cortex, ventromedial prefrontal cortex, anterior cingulate, and thalamus (Alexander, Crutcher, & DeLong, 1990; Cardinal, Parkinson, Hall, & Everitt, 2002; Haber & Knutson, 2010). It has been implicated in reward processing, motivation, incentive salience, and the subjective experience of pleasure (see Berridge & Kringelbach, 2015, and Haber & Knutson, 2010, for reviews). Given prior associations with multiple forms of externalizing pathology (e.g., ADHD, Plichta & Scheres, 2014; DBDs, Alegria et al., 2016, and Noordermeer et al., 2016; and AUD/SUD, Balodis & Potenza, 2015, and Hommer et al., 2011) and subclinical variation (e.g., drinking behavior, Weiland et al., 2016; and trait impulsivity, Kennis et al., 2013), as well as indirect links to two ESDs (i.e., ADHD through

genetic risk [**Chapter 2**] and AUD through normal variation in drinking behaviors [**Chapters 2 and 3**]) in the current studies, aberrant reward-related VS activation may represent a general transdiagnostic externalizing risk factor (see Beauchaine et al., 2017, for review).

Social-emotional Anterior Insula Activation

The insula is a lobe of the cerebral cortex situated deep within the Sylvian fissure, or lateral sulcus, which divides the frontal and parietal lobes from the temporal lobe (Reil, 1809; Türe, Yaşargil, Al-Mefty, & Yaşargil, 1999). It is further divided into anterior and posterior sections by the central insular sulcus (Flynn, 1999). The anterior portion has reciprocal connections to paralimbic regions including the anterior cingulate, ventromedial prefrontal cortex, temporal lobe, amygdala, and ventral striatum (Flynn, 1999; Naqvi & Bechara, 2009). Structurally, it has been linked to general psychopathology (Goodkind et al., 2015), including alcohol use disorders (Yang et al., 2016) and conduct problems (Raschle et al., 2015; Rogers & De Brito, 2016); functionally, it has been linked to a wide range of cognitions, including decision-making (Naqvi & Bechara, 2009), interoception (Craig, 2009), empathy (Mutschler et al., 2013), and overall social-emotional processing (Gu et al., 2013; Lamm & Singer, 2010; Mutschler et al., 2013). Consistent with prior literature showing blunted anterior insula activation to emotion-processing and empathy tasks among youth with conduct problems (Alegria et al., 2016; Noordermeer et al., 2016; Raschle et al., 2015), criminal offenders (Arbuckle & Shane, 2016), and adults with psychopathy (Meffert et al., 2013, but see also Decety et al., 2013), the results of **Chapter 4** indicate that it is further associated with genome-wide genetic risk for CD as well as self-reported antisocial behavior (e.g., “I have tricked someone into giving me money” and “I have broken into a building or vehicle in order to steal something or vandalize”). Though I only investigated social-emotional processing activation in the context

of CD genetic risk and psychopathy, prior reviews suggest this functional impairment may be specific to antisocial behavior and psychopathy relative to other ESDs (Rubia, 2011).

5.1.2 Premorbid Risk vs. Downstream Disorder Consequences

A major limitation of prior studies of ESDs, particularly in neurimaging, has been confounds of disorder expression. Cross-sectional case-control studies do not allow one to disentangle premorbid risk from “environmental” correlates of disorder expression (e.g., substance or medication use and experience of living with a disorder). The current studies relied upon two complementary approaches to circumvent such issues: longitudinal data (i.e., **Chapter 3**) and indices of genetic risk in healthy samples (i.e., **Chapters 2 and 4**). **Chapter 2**, for example, provided evidence that genome-wide genetic risk for childhood ADHD, which is fixed at birth, is associated with heightened ventral striatum activity to monetary reward, which is, in turn, related to greater problematic alcohol use. Supplemental analyses with baseline nondrinkers provided preliminary evidence that such activity prospectively predicted alcohol use initiation, and analyses with longitudinal data in **Chapter 3** demonstrated a prospective association between heightened VS activity and early vs. late age-at-first-drink, which has previously been shown to predict future problem drinking (DeWit et al., 2000).

Prior investigations of reward-related VS activation have yielded mixed results (Balodis & Potenza, 2015; Hommer et al., 2011), though mounting evidence suggests that heightened VS activity is associated with early stages of substance use (i.e., initiation and escalation; Heitzeg et al., 2014; Stice et al., 2013; Weiland et al., 2016), but that decreased VS activity is associated with later stages (i.e., compulsive use; Koob, 2013; Koob & Le Moal, 2005) and influenced by repeated use of substances (Martz et al., 2016; Volkow et al., 2004). Results from **Chapters 2 and 3** are consistent with such an interpretation, as heightened VS activation was associated with both age-

at-first drink (**Chapter 3**) and normal variation in problem drinking (**Chapter 2**), though I was unable to test whether sustained alcohol use may subsequently decrease activation (but see also **Appendix A** for some preliminary evidence). By using both genetically informed and longitudinal designs across two separate studies, I have provided additional evidence that increased reward-related VS activation is indeed a premorbid risk factor for alcohol (mis)use, and is also influenced by genetic risk for another ESD: ADHD.

5.1.3 ESDs as Quantitative Traits

In contrast to most prior studies of ESDs, which have relied upon diagnosed cases and matched controls, only one of the three samples utilized in the current studies (i.e., **Chapter 4**) was clinically ascertained. This choice reflects both a desire to eliminate confounds of disorder expression in the neuroimaging samples (see **Section 5.1.2**), as well as a growing understanding in the fields of neuroimaging and genetics that psychiatric disorders represent extremes of normal variation in quantitative traits (Marquand, Rezek, Buitelaar, & Beckmann, 2016; Plomin et al., 2009). Both genetic risk and phenotypic expression for disorders are thought to be normally distributed in the population, with those individuals with high enough genetic and phenotypic loading qualifying for a diagnosis. The externalizing *spectrum*, as its name implies, adopts this conceptualization of ESDs as representing extremes of quantitative traits; factor analyses have shown both ESD diagnoses and related continuous traits (e.g., impulsivity, aggression, and [lack of] empathy) to load on one higher-order continuous latent externalizing construct (Krueger et al., 2002; Krueger et al., 2007; Krueger et al., 2005; Krueger & South, 2009; Markon & Krueger, 2005).

Chapters 2 and **4** explicitly relied upon the assumption of continuous genetic and phenotypic risk through their use of polygenic risk scores (S. M. Purcell et al., 2009). In the

absence of family history and/or diagnostic data, risk scores generated based on results from a GWAS provide a quantitative measure of genome-wide genetic risk for a particular disorder. In **Chapter 2**, I demonstrated that PRS for childhood ADHD are associated with heightened VS activity to reward, which consequently was associated with greater self-reported problem drinking. In **Chapter 4**, based on the results of my discovery GWAS of CD, I showed that genome-wide polygenic risk, in addition to genotype at the top locus, was associated with both self-reported psychopathy and blunted insula activation to emotional faces. Notably, though both PRS were tested for association in a healthy college sample, results were consistent with prior disorder-specific studies. The results of **Chapter 2**, for instance, are consistent with studies linking childhood ADHD to future substance use (Charach et al., 2011; S. S. Lee et al., 2011) and a growing literature tying ADHD, as well as family-based genetic risk for ADHD, to heightened VS activity to reward receipt (Furukawa et al., 2014; Paloyelis et al., 2012; von Rhein, Cools, Zwiers, et al., 2015). The results of **Chapter 4** are similarly consistent with studies linking DBDs (Alegria et al., 2016; Noordermeer et al., 2016; Raschle et al., 2015), adult antisocial/criminal behavior (Arbuckle & Shane, 2016), and psychopathy (Meffert et al., 2013), to blunted social-emotional insula activity. Though ADHD and CD diagnoses or symptom counts were not available for the college sample, the association between CD polygenic risk, calculated based on a phenotypically extreme sample ascertained for opioid dependence, with self-reported psychopathy constructs among relatively affluent, high-functioning undergraduates indicates that genetic risk for severe CD pathology also influences non-pathological variation. Therefore, continuous indices of disorder-related genetic and phenotypic variability can be useful tools in investigating disorder etiology even within non-clinical samples.

Additionally, consistent with viewing externalizing psychopathology as existing along a spectrum encompassing both normal and extreme variation, the results presented here, in particular those of **Chapter 2**, provide evidence for a neurogenetic basis for ESD comorbidity and continuity. It has been hypothesized that the high degree of comorbidity (e.g., 30-50% of children with ADHD also meet criteria for CD; Biederman et al., 1991) and continuity (e.g., children diagnosed with ADHD are greater than 2.5-times as likely as nondiagnosed peers to meet criteria for an SUD in adulthood; Lee et al., 2011) across ESDs is due to differential expression of a continuous latent externalizing factor across the lifespan. **Chapter 2** demonstrated a potential link between two aspects of externalizing psychopathology, ADHD and problematic alcohol use, wherein both polygenic risk for ADHD and self-reported problem drinking were associated with heightened VS activity. Though I could not directly test such a hypothesis in the current data, such results suggest that genetic risk for ESDs, indexed by ADHD polygenic risk in **Chapter 2**, may express itself as ADHD in childhood and then problem drinking in young adulthood, linked neurobiologically through heightened VS response to reward (see **Section 5.1.1** for further discussion).

5.2 Limitations

Though individual study limitations are outlined in detail in each chapter, several limitations are common to all three studies and should be considered in interpreting findings as a whole. First, sample sizes were relatively small and likely underpowered to detect effect sizes typically seen in genetic and imaging studies of complex traits (Bogdan, Hyde, & Hariri, 2013; Poldrack, 2012; Sullivan et al., 2012). However, oversampling for at-risk individuals in the Teen Alcohol Outcomes Study (**Chapter 3**) and Comorbidity and Trauma Study (**Chapter 4**) boosted cell sizes and likely enriched for more severe presentations, thus increasing power relative to non-

ascertained samples. Relatedly, the significant effects reported across the studies are unlikely to be clinically informative on an individual level (e.g., polygenic risk for childhood ADHD in **Chapter 2** explained at most 1.7% of variance in reward-related VS activity). Nonetheless, effects identified reflect promising biological mechanisms that provide etiologic insight and are worthy of future study.

Second, despite convergent evidence of association within and across studies (i.e., association of CD genetic risk with self-reported psychopathy as well [**Chapter 4**]; and association of heightened VS activity to reward with problematic alcohol use [**Chapter 2**], future initiation of drinking [**Chapter 2**], and age-of-first drink [**Chapter 3**]), I was not able to directly replicate my largely exploratory findings using the available data. The fields of neuroscience (e.g., Button et al., 2013), genetics (e.g., Duncan & Keller, 2011), and imaging genetics (e.g., Bogdan et al., 2017), have faced criticism in recent years for high-profile replication failures, and may be particularly susceptible to false positives due to the large degree of multiple testing, traditionally small inter-individual effect sizes, and methodological flexibility. I attempted to reduce the chance to Type I error by applying stringent correction in individual analyses (e.g., permutation-based thresholding in **Chapter 2** and GWAS significance and family-wise error correction in **Chapter 4**); however, I did not control the error rate across studies and have also reported results not surviving strict correction, noting as such. These results, and especially those for which no convergent evidence was presented (e.g., association of *GOLM1* with CD), thus should be interpreted cautiously.

Third and finally, despite contextualizing these studies within the framework of the externalizing spectrum, the available archival data did not permit direct comparisons across disorders, as only one sample was recruited based on ESD diagnosis (i.e., opioid dependence in the Comorbidity and Trauma Study, **Chapter 4**). **Chapter 2** is the only study of the three which

explicitly tests whether a single mechanism may link multiple disorders, and, even then, proxy measures (i.e., childhood ADHD polygenic risk and self-reported problematic alcohol use) were used rather than diagnostic criteria. Conversely, it is worth noting that given the high level of other externalizing behaviors (i.e., **Table 4.2**) present in the sample in which the conduct disorder GWAS (**Chapter 4**) was performed, even among “controls,” it is possible that the genetic risk identified may be more specific to aggression and antisocial behavior. This is supported by the association of this genomewide genetic risk with blunted insula response to an emotional face-matching task, suggesting that it may be mechanistically related to empathy (Mutschler et al., 2013). The results of the studies are thus best thought of as complementary, with each providing insight into one component disorder, behavior, or trait of the externalizing spectrum that may, or may not, be relevant to other disorders as well.

5.3 Future Directions

The current findings inspire several future research directions that may prove fruitful in identifying additional mechanisms of ESD risk. Foremost, they highlight the need for additional large-scale neurogenetic consortia encompassing both pathological and normal/subclinical externalizing variation. As mentioned in **Section 5.2**, effects sizes in neurogenetics research are small (Ryan Bogdan et al., 2017) and require large samples to detect, which are often cost-prohibitive to accrue at one site. Though I was able to leverage data from three large independent studies—the Duke Neurogenetics Study in **Chapters 2 and 4** (Corral-Frias et al., 2015), Teen Alcohol Outcomes Study in **Chapter 3** (Bogdan et al., 2012), and Comorbidity and Trauma Study in **Chapter 4**, (Nelson et al., 2016)—for this project, even larger multimodal samples are needed yet. Consortia such as the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) network

(Thompson et al., 2014), Human Connectome Project (Van Essen et al., 2013), UK Biobank Imaging Study (Miller et al., 2016), Brain Genomics Superstruct Project (Holmes et al., 2015), and particularly longitudinal studies such as IMAGEN (Schumann et al., 2010) and the Adolescent Brain Cognitive Development (ABCD) study (Bjork, Straub, Provost, & Neale, 2017), promise to deliver sample sizes and data coverage that should adequately allow for both a priori and exploratory analyses of the externalizing spectrum, as well as for adequate cross-study replication.

Furthermore, in keeping with the spirit of National Institute of Mental Health’s Research Domain Criteria (RDoC; Insel et al., 2010) initiative and the conceptualization of a continuous spectrum of externalizing psychopathology (Beauchaine & McNulty, 2013), future studies should focus on quantitative transdiagnostic neural and behavioral phenotypes rather than “discrete” disorders. As demonstrated, for example, in **Chapter 2**, certain intermediate phenotypes such as heightened reward-related VS activity may be related to multiple externalizing disorders, especially when disorders exhibit high comorbidity and/or continuity. Conversely, within-disorder heterogeneity, as in, for instance, conduct disorder with and without the *DSM-5* “with limited prosocial emotions” specifier, may obscure key subtype-specific neural and genetic differences when using only a case-control diagnostic design. As mentioned in **Sections 5.1.1** and **5.2**, I was unable to establish disorder specificity vs. generality for the candidate genetically influenced neural mechanisms (i.e., heightened reward-related VS activity and blunted anterior insula activity during social-emotional processing) I identified. Perhaps a more useful future follow-up, instead of testing *disorder* specificity, would be to test which continuous externalizing traits (e.g., impulsivity, aggression, and [lack of] empathy) do and do not associate with such neural activity.

Finally, though genetic correlation methods such as polygenic risk scores, as used in **Chapters 2** and **4**, are useful in determining whether neural phenotypes are associated with whole-

genome risk for certain disorders, they do not allow for the identification of specific regions, systems, or pathways within the genome underlying such links. For example, it is unlikely that every childhood ADHD risk locus also contributes to heightened reward-related VS activity, as the PRS method assumes; instead, likely a subset, perhaps those coding for proteins in the dopaminergic system (e.g., as in Nikolova, Ferrell, Manuck, & Hariri, 2011), influences both. Newer statistical genetics methods have allowed for the partitioning of heritability (Finucane et al., 2015) and co-heritability (Shi, Mancuso, Spendlove, & Pasaniuc, 2016), such that areas of the genome exerting disproportionate influence on one or two correlated phenotypes may be identified. Additionally, weights from prior GWAS may be combined with a priori knowledge of likely related pathways to form pathway-specific PRS (Darst et al., 2017). Future use of such techniques will provide more insight into specific molecular pathways linking genes to brain to behavior, further refining targets for treatment.

5.4 Conclusion

In three complementary studies, I have identified promising genetic markers and genetically influenced functional neural phenotypes (i.e., reward-related ventral striatum and social-emotional anterior insula activation) for externalizing psychopathology and related traits. I have additionally provided evidence that they represent premorbid risk factors, rather than disorder- or substance-related effects, linked to normal trait variation in the population. Despite several notable limitations, including relative lack of power, need for direct replication, and inability to compare across disorders, these findings lay the groundwork for future neurogenetic research into general and specific risk mechanisms for externalizing pathology which may eventually lead to improved treatment and prevention strategies in halting potentially lifelong impairment.

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Appendix A:

Chapter 3 Supplemental Analyses

Though I had originally planned to investigate predictive neural markers of alcohol use escalation, as well as downstream neural consequences of use, among individuals who initiated drinking during the course the Teen Alcohol Outcomes Study (TAOS; Bogdan, Williamson, & Hariri, 2012), restricted variability in drinking behavior prohibited an adequate treatment of these hypotheses in the main document. As such, I limited **Chapter 3** to addressing alcohol use initiation in the form of early vs. late age-of-first-drink, for which sufficient variability was present in TAOS. However, I have summarized below the analyses I performed in assessing the current state of the drinking and longitudinal reward-related neuroimaging data in TAOS, in the hopes of inspiring additional discussion and adequate follow-up. Notably, a third wave of imaging data is currently being collected in the sample, and the results of these preliminary analyses may be used to inform future analyses related to neural predictors and consequences of alcohol use escalation and problem drinking.

A.1 Alcohol Use Escalation in TAOS

In an attempt to index alcohol use escalation among individuals who initiated use during the study ($n=66$ with usable baseline imaging data), I created three variables based on responses to the Substance Use Questionnaire (SUQ; Molina et al., 2007): slope of use across timepoints, last-month use among first-time drinkers, and time-to-first-drunkenness (**Figure A.1**). However, due to lack of variation in these measures, further analyses were not pursued. The process of creating and evaluating these variables is described below.

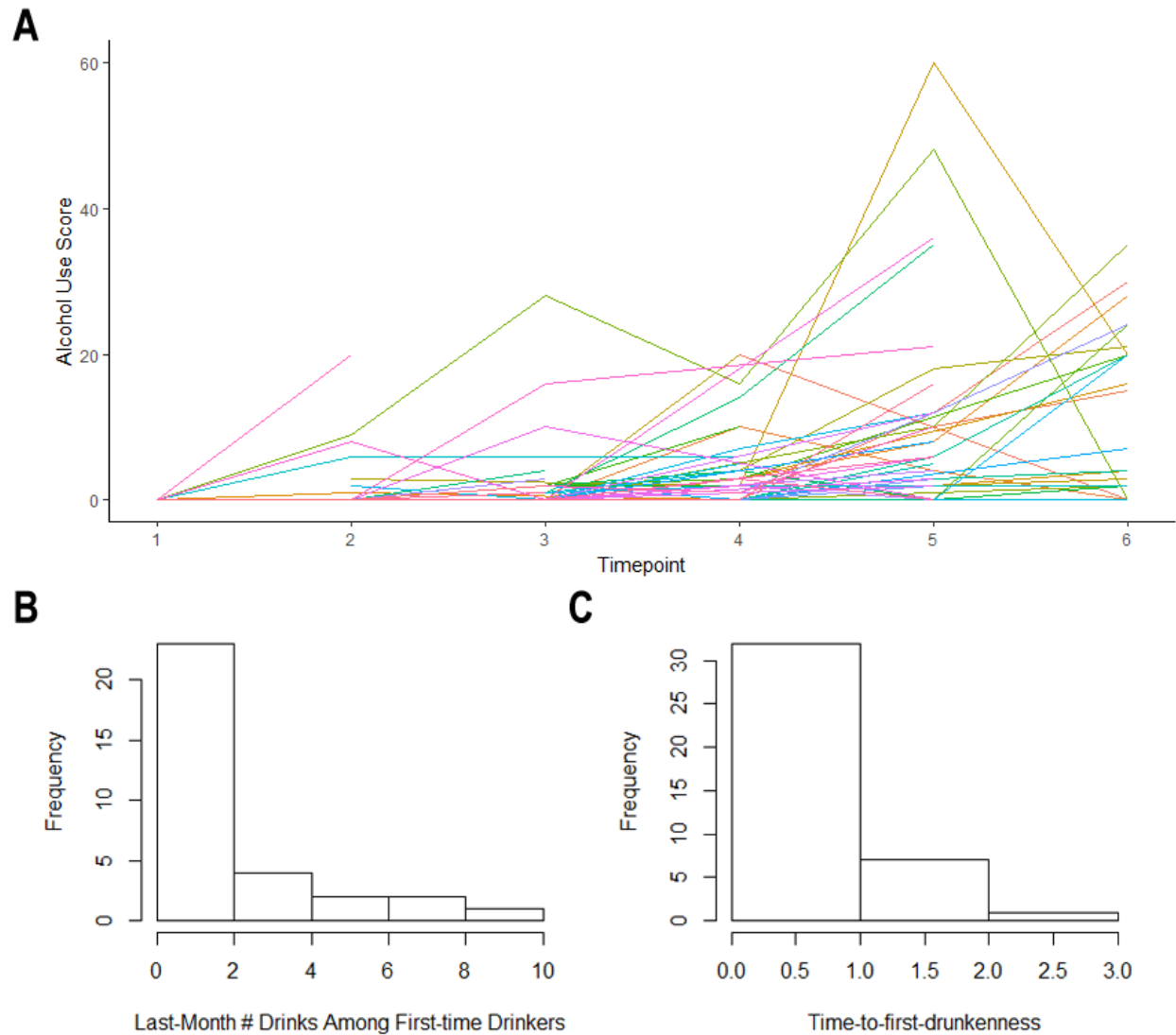


Figure A.1 Variables Developed to Index Alcohol Use Escalation. Panels correspond to **A)** Slope of Use, **B)** Last-month Use Among First-time Drinkers, and **C)** Time-to-first-drunkenness.

A.1.1 Escalation Variables

Slope of Use

As indicated in my proposal, to develop a “slope of use” metric, I first multiplied frequency-of-use (i.e., response to the SUQ question “In the past 12 months, how often did you drink beer, wine, wine coolers, or liquor?”), where answers among past-year drinkers may range from “1-3 times” to “Several times a day”) and quantity-of-use (i.e., response to the SUQ question “Think of all the

times you have had a drink in the past 12 months. How much did you usually drink each time?”, where answers among past-year drinkers may range from “Less than one can or glass” to “More than 25 drinks”) data to create an overall “use score,” as done previously by Ramage and colleagues (Ramage, Lin, Olvera, Fox, & Williamson, 2015), at each of the six assessment timepoints (i.e., baseline and five follow-up). I had then planned to enter these scores into a latent growth model, with the individual slopes from the resultant model representing the rapidness of alcohol use escalation across timepoints for each participant. However, given that most individuals with baseline imaging data who initiated drinking during the study did so at later timepoints ($M=3.95\pm1.21$), visual inspection of the data revealed patterns to be nonlinear (**Figure A.1**). Therefore, the slopes were not actually representative of rapidness of escalation in a linear fashion; instead, they were heavily influenced by timepoint-at-first-drink and thus were not appropriate for main analyses in **Chapter 3**.

Last-month Use Among First-time Drinkers

As an alternative to the use of slope to index rapidness of alcohol use escalation, I instead tried using self-reported last-month alcohol use at the timepoint at which an individual first reported drinking. For example, if an individual reported first drinking at timepoint 3 (i.e., “Did you have your very first drink since your last interview here?”), his or her past-month alcohol use frequency (i.e., “Think specifically about the past 30 days up to today. During the past 30 days, what is your best estimate of the number of days you drank one or more drinks of an alcoholic beverage?”, where answers among past-year drinkers may range from “0 days” to “All 30 days”) would be multiplied by his or her past-month alcohol use quantity (i.e., “On the days that you drank during the past 30 days, how many drinks did you usually have each day? Count as a drink a can or bottle of beer, a wine cooler or a glass of wine, champagne or sherry; a shot of liquor or a mixed drink

or cocktail.”) to create a metric of past-month number of drinks among first-time drinkers. Last-month, rather than last-year, number of drinks was selected to reduce potential bias introduced by individuals having begun to drink within only months of the interview, rather than during the entire 12-month period the past-year questions inquire about. However, not much inter-individual variation was captured by this measure ($M=2.03\pm2.76$), with most first-time drinkers reporting only 1-2 or fewer drinks in the past month (**Figure A.1.B**). As such, further analyses were not pursued.

Time-to-first-drunkness

Finally, hoping to index rapidness of escalation to problematic drinking specifically, I calculated “time-to-first-drunkness” by subtracting self-reported age-at-first-drunkness (i.e., “How old were you when you first got drunk or very, very high on alcohol?”) from age-at-first-drink (i.e., “How old were you the first time you had a drink, not just a sip or a taste?”). Of the 66 alcohol use initiators included in my imaging subsample, only 39 reported having also gotten drunk during the course of the study. Consistent with prior literature (Monshouwer, Smit, de Zwart, Spruit, & van Ameijden, 2003; Morean, Corbin, & Fromme, 2012), participants reported a short period from first drink to first drunkness, with the majority of those who reported getting drunk doing so within the first year of initiating alcohol use ($n=26$). Due to lack of variation in this measure ($M=0.58\pm0.87$), it was not deemed appropriate for main analyses (**Figure A.1.C**).

A.1.2 Interim Conclusions

As perhaps should have been expected in a sample of participants first interviewed in early adolescence, individual differences in problem drinking behaviors were insufficient to warrant further analyses for the current project (**Figure A.1**); initiation was the primary stage of alcohol

use captured within this sample, as became my focus in **Chapter 3**. One potentially more fruitful avenue of research, currently being pursued by our collaborators at Duke University, may be to characterize the *shape* of alcohol use trajectories, rather than assuming a linear trajectory and modelling slope. Despite a common overall developmental pattern of alcohol use initiation, escalation, and progression to problem drinking during adolescence (Chassin, Sher, Hussong, & Curran, 2013; Johnston, 2016; Substance Abuse and Mental Health Services Administration, 2015), past studies using latent class growth analysis (LCGA) and growth mixture modelling (GMM) have identified multiple discrete adolescent alcohol use trajectories, including stable low/no use, swift escalation to chronic high levels of drinking, and more gradual escalation (see Table 1 in Nelson, Van Ryzin, & Dishion, 2015, for review), and found individuals belonging to certain trajectories (e.g., rapid escalation) to be particularly susceptible to future addiction (Colder, Campbell, Ruel, Richardson, & Flay, 2002; Nelson et al., 2015). Notably, such an analysis addresses a fundamentally different question than does looking at initiation and escalation independently; however, in the absence of substantial individual differences in drinking behavior, it may be the only way to capture variation beyond initiation in adolescent samples.

A.2 Whole-brain Main Effects of Task

Though I decided to focus exclusively on one a priori region of interest, the ventral striatum, in **Chapter 3**, I also probed whole-brain activation to the monetarily incentivized number-guessing paradigm (Delgado, Nystrom, Fissell, Noll, & Fiez, 2000; Hariri et al., 2006) as part of this project. Notably, these analyses were not limited to baseline nondrinkers, as I was interested in overall main effects.

A.2.1 Methods

As described in **Section 3.2.3**, following preprocessing steps, the general linear model in Statistical Parametric Mapping 8 (SPM8; <http://www.fil.ion.ucl.ac.uk/spm>), employing canonical hemodynamic response functions, was used to estimate condition-specific (i.e., positive feedback, negative feedback, and control block) BOLD responses for each individual with usable imaging data (see **Section 3.2.3** for a description of imaging QC) at each scanning session ($N_{MRI1}=214$; $N_{MRI2}=193$; $N_{MRI1\&MRI2}=131$).

Main Effects at Each Scanning Session

As outlined in SPM8's random-effects analysis via summary statistics approach (Holmes & Friston, 1998; Penny, Holmes, & Friston, 2003), Positive Feedback > Negative Feedback contrast images (i.e., weighted sums of single-condition beta images) for each individual were used in second-level random effects models to determine mean contrast-specific responses at each scanning session ($N_{MRI1}=214$; $N_{MRI2}=193$) using one-sample t-tests.

Common Areas of Activation Across Sessions

A 2 session (scan 1, scan 2) x 2 condition (positive, negative) repeated-measures ANOVA with an explicitly modeled subject-level factor, implemented using SPM8's flexible factorial option (Henson & Penny, 2003), was used to model common areas of activation across participants with usable imaging data at both sessions ($N_{MRI1\&MRI2}=131$). Following model estimation, weights were assigned to the resulting whole-brain beta maps based on the recommendations of Gläscher and Gitelman (2008) to assess the main effect of condition (i.e., Positive Feedback > Negative Feedback) across both sessions.

Differences in Activation Across Sessions

To investigate systematic changes in activation across sessions, Positive Feedback > Negative Feedback contrast images for each session for each participant with usable imaging data at both scans ($N_{MRI1 \& MRI2}=131$) were entered into a paired samples t-test in SPM8.

Significance Assessment

Cluster-level significance was assessed at $\alpha=0.05$ using AFNI's 3dClustSim (Cox, 1996), as described in **Section 3.2.4**. The results of these simulations indicated that cluster sizes of 102 voxels for the session-specific analyses, 96 for the ANOVA, and 91 for the paired t-test at a p-threshold of $p<0.001$ would appropriately control the cluster-level family-wise error rate.

A.2.2 Results

Main Effects at Each Scanning Session

Across both scanning sessions, the contrast of positive feedback versus negative feedback elicited robust activation in the striatum (**Figure A.2**). At session 1, the only significant cluster (k [or cluster size]=125, $p<0.05$ FWE; max voxel MNI coordinates=[-4, 8, -6], $t=4.38$, ns FWE; **Figure A.2A**) encompassed the left ventral striatum (i.e., ventral portions of the caudate and putamen as well as the olfactory tubercle). At session 2, a larger, more lateral cluster emerged within the left striatum ($k=206$, $p<0.005$ FWE; max voxel=[-14, 0, -10], $t=4.85$, ns FWE; **Figure A.2B**), situated primarily within the lentiform nucleus (i.e., putamen and globus pallidus) but also extending into the ventral striatum (i.e., ventral putamen).

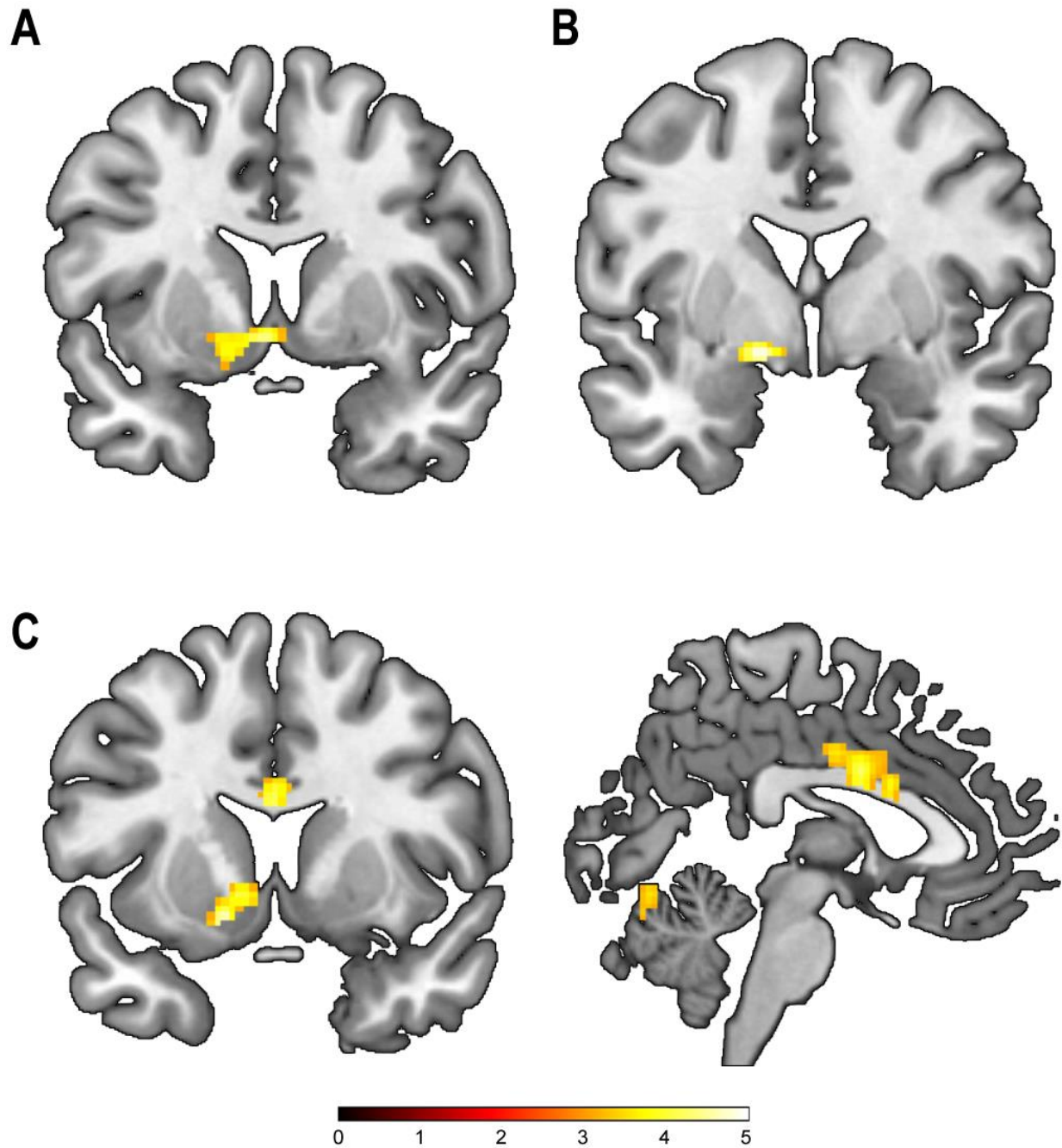


Figure A.2 Whole-brain Main-effect-of-task Analyses. Panels correspond to mean group-level Positive Feedback > Negative Feedback contrast **A)** at scanning session 1, **B)** at scanning session 2, and **C)** across both sessions. All images are displayed at the coordinates of the maximum voxel, with the exception of the midline image in order to highlight both significant clusters. All clusters displayed meet an FWE-corrected threshold of $p < 0.05$.

Common Areas of Activation Across Sessions

In the repeated-measures ANOVA, modelling both sessions simultaneously, a common region of activation within the ventral striatum was revealed ($k=113$, $p<0.05$ FWE; max voxel= $[-14, 10, -10]$, $t=4.64$, ns FWE; **Figure A.2C**). Additionally, clusters along the midline within the cingulate gyrus ($k=272$, $p<0.005$ FWE; max voxel= $[-2, 2, 24]$, $t=4.68$, ns FWE) and cerebellum ($k=111$, $p<0.05$ FWE; max voxel = $[0, -72, -12]$, $t=3.72$, ns FWE) were differentially activated to positive versus negative feedback across sessions.

Differences in Activation Across Sessions

No regions systematically differed in activation across sessions for the contrast of Positive Feedback > Negative Feedback, per the results of the paired t-test. Restricted analyses with bilateral prior-coordinate-based ventral striatum (i.e., based on maximum coordinates from Hariri et al., 2006) and dorsal striatum anatomical (i.e., bilateral caudate and putamen; Tzourio-Mazoyer et al., 2002) regions-of-interest (ROIs) also did not yield any differences in activation across the two sessions.

A.2.3 Interim Conclusions

Consistent with prior studies using this corticostriatal reactivity paradigm (Delgado et al., 2000; Hariri et al., 2006; **Chapter 2**), the Positive Feedback > Negative Feedback contrast revealed consistent activation differences within the left ventral striatum across participants and scanning sessions, indicating that such differences are present even in early and middle adolescence. Correspondingly, no significant effects of scanning session were found at either the whole-brain level or within a priori striatum ROIs, providing evidence that there is no systematic change in activation over time, at least in the TAOS sample, from early to middle adolescence. Visual

inspection of the statistical maps, however, suggested that there might be a shift in activation from medial to lateral areas of the striatum as adolescence progresses. Though its potential functional significance is at the moment unclear, it is worth noting that prior studies have suggested a ventromedial-dorsolateral, rather than purely dorsal and ventral, division of the striatum based on cytoarchitecture and patterns of connectivity (Burton, Nakamura, & Roesch, 2015; Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004), and that, using traditional dorsal-ventral divisions, functional connectivity between the dorsal and ventral striatum is strengthened in adolescence relative to both childhood and adulthood (Somerville, Hare, & Casey, 2011). As such, the “seat” of reward processing, in terms of both function and connectivity, may shift during the critical developmental period of adolescence. However, this impression is highly speculative and should be the subject of more rigorous future evaluation.

A.3 Longitudinal Effects of Initiation and Escalation

To shed light on the role of reward processing in different stages of substance use and addiction (Everitt & Robbins, 2016; Hommer, Bjork, & Gilman, 2011; Koob, 2013; Koob & Le Moal, 2005; Volkow, Fowler, Wang, Baler, & Telang, 2009; Wise & Koob, 2014), as well as reconcile previous discrepant findings on the directionality of the association between reward-related ventral striatum activity and drinking (Balodis & Potenza, 2015; Heitzeg et al., 2014; Hommer et al., 2011; Stice, Yokum, & Burger, 2013; Weiland, Zucker, Zubieta, & Heitzeg, 2016; **Chapter 2**), I utilized the longitudinal neuroimaging data within the TAOS sample to look at downstream consequences of alcohol use initiation and escalation. Due to my shift of focus within **Chapter 3** to prospective predictors of age-at-first-drink, I have decided to present the preliminary results of these analyses below.

A.3.1 Initiation and Escalation Groups

As reported in **Section A.2.1**, following exclusions for imaging quality issues, 131 participants had usable imaging data at both scanning sessions.

Initiation

Of these participants, 9 reported drinking at baseline and were thus excluded from longitudinal initiation analyses. By the second scanning session, 23 reported having initiated drinking, with 98 remaining abstinent, and one failing to report drinking status at the second scan.

Table A.1 Longitudinal Initiation Subsample Demographics

Variable	Abstainer	Initiator
N	98	23
Age at Scan 1	13.48(0.94)	13.87(0.89)
Age at Scan 2	15.51(0.98)	16.14(0.92)*
Years between Scans	2.03(0.33)	2.27(0.50)*
Tanner Stage >III at Baseline	54.1%	56.5%
Female	46.9%	30.4%
Non-Hispanic Caucasian	63.3%	65.2%
Hispanic	19.4%	34.8%

*Indicates a significant difference between groups.

“Escalation”

As discussed in **Section A.1**, there was little variation in post-initiation drinking behavior within TAOS. Additionally, there were only 9 individuals in the sample who reported drinking by the time of the first scan, so a longitudinal assessment of neural correlates of escalation *post*-initiation was not feasible. Instead, I decided to compare participants who reported binge drinking ($n=8$) to drinkers who did not report binge drinking ($n=20$) at scanning session 2, regardless of baseline drinking status. Binge drinking status was determined based on NIAAA criteria (i.e., 4+ drinks for females, and 5+ drinks for males, within a two-hour period; National Institute on Alcohol Abuse and Alcoholism), by binarizing responses to the following questions on the SUQ: “In the past 12

months, how often did you drink four or more drinks within about two hours?” and “In the past 12 months, how often did you drink five or more drinks within about two hours?”

Table A.2 Longitudinal Binge-drinking Subsample Demographics

Variable	Non-binging Drinker	Binge Drinker
N	20	8
Age at Scan 1	13.89(0.93)	14.02(0.68)
Age at Scan 2	16.13(0.96)	16.31(0.71)
Years between Scans	2.24(0.50)	2.29(0.46)
Tanner Stage >III+ at Baseline	60.0%	62.5%
Female	40.0%	62.5%
Non-Hispanic Caucasian	65.0%	62.5%
Hispanic	30.0%	37.5%

Note. No significant differences in demographics between groups.

A.3.2 Ventral Striatum

Methods

To index change in ventral striatum activity across timepoints, parameter estimates were extracted for the Positive Feedback > Negative Feedback contrast at each session from the maximum voxel (i.e., MNI coordinates=[-14, 10, -10], $t=4.64$) of the left VS cluster identified in the 2 session x 2 condition repeated-measures ANOVA described in **Section A.2**. Change scores across sessions were then generated for each participant by conducting a linear regression with scan 1 parameter estimates as the independent variable and scan 2 estimates as the dependent variable. Residuals from this model thus represented deviation from expected scan 2 VS activity as predicted by baseline activity. For example, a positive residual value would reflect a greater increase in activity over time relative to that expected. This approach has been previously utilized in longitudinal imaging analyses (e.g., Whittle et al., 2014), including within the TAOS dataset (Hanson, Hariri, & Williamson, 2015). Change scores were then entered into logistic regressions predicting initiation or binge drinking status, respectively. Covariates for all analyses included age and pubertal status at baseline, gender, race/ethnicity, and days from scan 1 to 2.

Results

Change in left reward-related VS activity over time was nominally associated with binge drinking ($\beta=-1.005$, $p=0.039$; **Figure A.3**), but not initiation status, at session 2 ($\beta=-0.185$, $p=0.454$). For each standard-deviation *decrease* in left VS activity relative to that expected, odds of belonging to the binge-drinking group increased by a factor of 2.732.

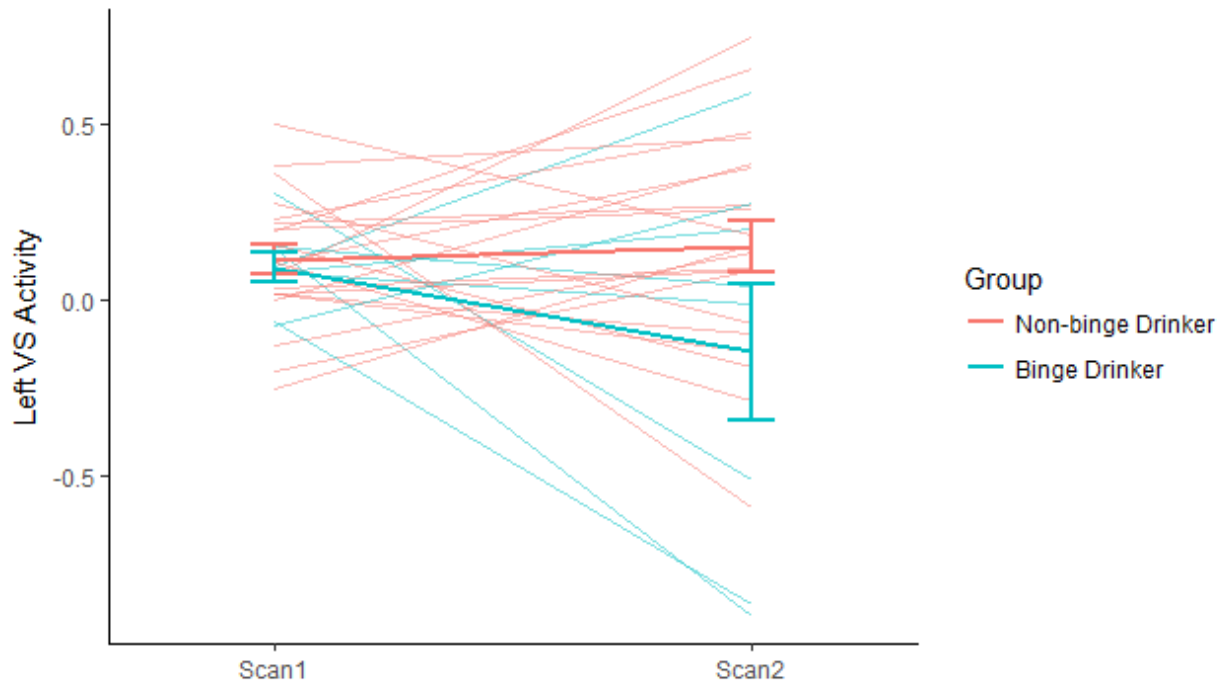


Figure A.3 Longitudinal VS Activity and Binge Drinking. Average trajectories for each group are shown in bold, with error bars representing standard error of the mean at each session. Individual trajectories, colored by group membership, are shown in the background. No significant differences emerged between the groups at individual scanning sessions.

A.3.3 Whole-brain

Methods

Two-group (abstainer vs. initiator in the first analysis, and binge drinker vs. non-binge drinker in the second) x 2 time (scan1 , scan 2) repeated-measures ANCOVAs with explicitly modeled subject-level factors, implemented using SPM8's flexible factorial option (Henson & Penny,

2003), were used to test for longitudinal differences in whole-brain activation to the Positive Feedback > Negative Feedback contrast. Covariates included age at each scan, pubertal status at baseline, gender, and race/ethnicity. Following model estimation, weights were assigned to the resulting whole-brain beta maps to test for group x time interactions. Cluster-level significance was assessed as described in **Section 3.2.4**, which resulted in minimum cluster size thresholds of 92 and 104 voxels, for initiation and binge-drinking analyses, respectively, at $p < 0.001$. In addition to probing whole-brain activation, I also performed analyses within bilateral prior-coordinate-based ventral striatum (i.e., based on maximum coordinates from Hariri et al., 2006) and dorsal striatum anatomical (i.e., bilateral caudate and putamen; Tzourio-Mazoyer et al., 2002) ROIs.

Results

No significant regions of activation emerged at the whole-brain or ROI-level for either model, FWE-corrected. However, inspection of the statistical map for the group x time interaction in the initiation analysis at the cluster-forming threshold of $p < 0.001$, uncorrected, revealed three clusters within the prefrontal cortex (PFC) separated by white matter. As activation can sometimes be present outside of grey matter due to the imprecision of BOLD fMRI, as well as spatial smoothing procedures, I reexamined the statistical map at $p < 0.001$ without using the probabilistic grey matter mask. As I had suspected, the original PFC clusters were now joined, and the full cluster survived FWE correction (revised $k=102$ at $p < 0.001$; PFC $k=107$, $p < 0.05$ FWE; max voxel= $[-16, 48, 10]$, $t=3.84$, *ns* FWE; **Figure A.4.A**).

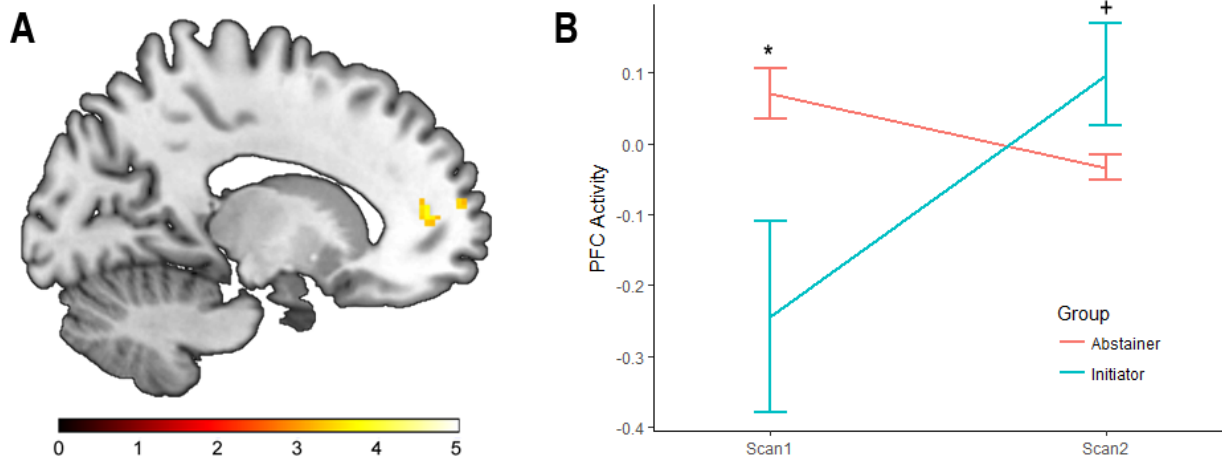


Figure A.4 Longitudinal PFC Activity and Alcohol Use Initiation. Shown in **A** is the significant cluster that emerged from the Group x Scan interaction of the alcohol use initiation ANCOVA, centered at the maximum voxel: [-16, 48, 10]. As can be seen in the visualization of the extracted values in **B**, future alcohol use initiators had less activation at baseline relative to abstainers, but by scan 2 the groups were statistically equivalent.
* $p < 0.05$, + $p < 0.10$

Follow-up independent samples t-tests using extracted parameter estimates averaged across the entire cluster revealed that at scan 1, future initiators had significantly decreased activation within the cluster relative to continuous abstainers ($t(25) = -2.255$, $p = 0.033$), but that at the time of scan 2, the two groups were statistically equivalent ($t(25) = 1.760$, $p = 0.091$), with a nonsignificant trend towards greater activation among initiators.

A.3.4 Interim Conclusions

Though preliminary at this point, results of longitudinal analyses suggest that alcohol use initiation is associated with an increase in reward-related (vm)PFC activity over time, while initiation of binge drinking specifically is associated with a relative decrease in VS activity. The latter finding is consistent with stage models of addiction, which posit that heightened reward sensitivity underlies initial stages of addiction such as initiation and escalation, but that later stages including progression to and maintenance of dependence may be driven by a revised reward-system homeostasis in which the substance itself replaces natural, non-substance rewards (Everitt &

Robbins, 2016; Hommer et al., 2011; Koob, 2013; Koob & Le Moal, 2005; Volkow et al., 2009; Wise & Koob, 2014). Such a role for chronic use of alcohol and other substances in repressing reward responsiveness has been documented in fMRI (Balodis & Potenza, 2015; Hommer et al., 2011) and PET (Volkow et al., 2009; Volkow, Fowler, Wang, & Swanson, 2004) studies of humans, as well as in animal models (Ahmed, Kenny, Koob, & Markou, 2002; Budygin et al., 2007; Zhou et al., 2007). Though binge drinking cannot be directly compared to dependence, it represents a problematic pattern of hazardous alcohol use, particularly during the sensitive developmental period of adolescence. If the association between heightened VS activity and prospective prediction of alcohol use initiation, but longitudinal association between decreasing VS activity over time among binge-drinkers, is replicated in additional larger samples, it could present a key piece of evidence for the role of reward processing in the progression through stages of addiction.

The association between alcohol use initiation and increasing reward-related PFC activity over time, particularly within the vmPFC, where the maximum voxel was located, was admittedly unexpected. However, the PFC in general, and vmPFC in particular, has been previously associated with reward processing and undergoes significant development during adolescence (see Casey & Jones, 2010, for a developmental review). Notably, only two other studies to date, at least to my knowledge, have examined neural activation both pre- and post-initiation of alcohol use (Squeglia et al., 2012; Wetherill, Squeglia, Yang, & Tapert, 2013); in them, the authors reported similar crossover effects within the right inferior parietal lobe and left medial frontal cortex during a working memory task, and right middle frontal gyrus and inferior parietal lobule during a response inhibition task, respectively, wherein future heavy drinkers had lesser activation than continuous nondrinkers at scan 1, but by scan 2 their relative activation in these regions increased,

while that of continuous nondrinkers decreased. Given that so little research has been previously conducted regarding neural activity pre- and post- alcohol use initiation, the current preliminary finding is worthy of further follow-up both within this sample, especially as data from the third neuroimaging session becomes available, and in additional longitudinal samples.

A.4 Lessons Learned and Looking Forward

Overall, my work with alcohol use and longitudinal imaging data in the TAOS dataset has produced interesting preliminary results regarding the role of heightened reward-related VS activity in predicting age-at-first drink (**Chapter 3**), as well as potential effects of initiation and binge drinking on activity within the PFC and VS (**A.3**), respectively. Additionally, it has identified specific methodological issues when working with substance use and imaging data within an adolescent population, including lack of variability in post-initiation drinking behaviors (**A.1**) and lack of adequate characterization of the developmental timecourse of activation to functional imaging tasks previously used and validated primarily in adults (**A.2**). The “grunt” work involved in such initial analyses often does not make it into final manuscripts or the main chapters of dissertations such as this one; nonetheless, as discussed in the individual conclusions to each subsection, they provide valuable information relevant to the design of future studies as well as future analyses. As additional longitudinal data become available, both in the TAOS sample and large-scale collaborations such as the Adolescent Brain and Cognitive Development project, which seeks to provide detailed longitudinal neuroimaging, genetic, and phenotypic data on 10,000 9- to 10-year-olds as they progress through adolescence and young adulthood (Bjork, Straub, Provost, & Neale, 2017), a more thorough treatment of questions regarding predictors and consequences of alcohol use initiation and escalation will become feasible.

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