2017

Integrating Genetics and Neuroimaging to study Subtypes of Binge Drinkers

Megan E. Cooke
Virginia Commonwealth University

Integrating Genetics and Neuroimaging to study Subtypes of Binge Drinkers

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

By

Megan Cooke
Bachelor's of Science
Furman University
2008

Director: Danielle Dick, PhD
Professor
Departments of Psychology, and Human & Molecular Genetics

Virginia Commonwealth University
Richmond, Virginia
August 2017

Acknowledgement

When I began my PhD training and later this dissertation, I was unaware what a challenging, exciting, frustrating, and fulfilling process it would be. I have been fortunate that throughout this process there have been many people who have assisted and supported me, my training, and this work. The following acknowledgements recognize many of these key individuals.

First, this research would not be possible without the individuals who choose to participate. I would like to thank the Spit for Science participants who contributed their time and DNA, especially those individuals who completed the neuroimaging S4S spin-off.

I would like to thank my advisor, Danielle Dick for helping me to decide to come to VCU, her mentorship over the last 6 years, and providing me with countless opportunities. I would like to thank my dissertation committee (Mike Neale, Ken Kendler, Jim Bjork, and Alexis Edwards) for their guidance and patience during the dissertation process. More specifically, I would like to thank Jim Bjork for teaching me everything I now know about neuroimaging from data collection to analysis to interpretation. Chapter 4 of this document would not have been possible without his intensive mentorship. Outside of her role as a committee member, I would like to additionally thank Alexis Edwards who over the last 6 years has spent many hours mentoring me on statistical analyses, scientific writing, genetics, and navigating the world of academia.

While I have had many unofficial mentors over the past 6 years, none have been as influential or enduring as Jessica Salvatore. I would like to thank Jessica for being able to seamlessly play the role of both mentor and friend, offering advice on any type of problem I brought to her and being an excellent role model for all aspects of my life.

Early on in graduate school it became clear that I had underestimated the challenges that come with being part of the first cohort of a PhD program. Later, I realized I had also not anticipated the benefits of a blank slate of student dynamics on which we could cultivate an environment of support, collaboration, and at times commiseration. Thank you to Jim, Neeru, and Mo who taught me the importance of peer support early, to Elizabeth and Jeanne for being an unwavering support system throughout and to the rest of the PBSG students (and honorary PBSG students) for continuing to create such a supportive and collaborative group. I am excited to continue to watch all of your careers grow from afar.

I want to thank what often felt like an army of friends supporting me from near and far over the last 6 years. Thank you for helping me celebrate my achievements along the way but possibly more importantly for the support through texts, calls, visits, and distractions when things were stressful, daunting, and challenging.

Finally, but most importantly, I would like to thank my parents and brother for being my biggest supporters throughout my life and for providing me with both the genes and environment to be successful.

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List of Abbreviations

AFNI……..….Analysis of Functional NeuroImages
AFR……...……African ancestry group
AMR……..American ancestry group
AUD……..……..Alcohol Use Disorder
BOLD……...…….……Blood oxygen level dependent
BP……..Base pair position
CFI…….Comparative Fit Index
CHR……..Chromosome
DLPFC……..Dorsolateral Prefrontal Cortex
EAS……..…East Asian ancestry group
EF……..…...Emotional faces
EUR……European ancestry group
fMRI……...…..Functional Magnetic Resonance Imaging
FWE…….....Family-Wise Error
GCTA…….……Genome-wide complex trait analysis
GWAS……..……Genome-wide association studies
GWS……...…...Genome-wide significant
LD……..…Linkage disequilibrium
MAC…….…Minor allele count
MAF……..……Minor allele frequency
MID……..…Monetary Incentive Delay
NAcc……...……..Nucleus Accumbens
NIAAA……..…National Institutes of Alcohol Abuse and Alcoholism
OFC……...……Orbitofrontal Cortex
PET……..…Positron Emission Topography
RIFG…….Right Inferior Frontal Gyrus
RMSEA……..…...Root Mean Square Error of Approximation
S4S……..…….Spit for Science
SAS……South Asian ancestry group
SD…….…….……..Standard deviation
SE……..……..Standard error
SNP…….…..Single Nucleotide Polymorphism
TE……..……Echo time
TLI…….……Tucker Lewis Index
TR……..…Repetition time
vmPFC……...….Ventromedial Prefrontal Cortex
VS……Ventral Striatum
XYGNG……...…XY Go/Nogo

Abstract

INTEGRATING GENETICS AND NEUROIMAGING TO STUDY SUBTYPES OF BINGE DRINKERS

Megan Elizabeth Cooke, B.S.

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

Virginia Commonwealth University, 2017

Danielle Dick, PhD
Professor, Departments of Psychology, and Human & Molecular Genetics

Risky alcohol use is a major health concern among college students, with 40.1% reporting binge drinking (5 or more drinks in one occasion) and 14.4% reporting heavy drinking (binge drinking on 5 or more occasions) in the past month. Risky alcohol use is thought to be the result of a complex interplay between genes, biological processes, and other phenotypic characteristics. Understanding this complex relationship is further complicated by known phenotypic heterogeneity in the development of alcohol use. Developmental studies have suggested two pathways to risky alcohol use, characterized by externalizing and internalizing characteristics, respectively. However, the underlying biological processes that differentiate these pathways are not fully understood. Neuroimaging studies have assessed reward sensitivity, emotion reactivity, and behavioral inhibition using fMRI and separately demonstrate associations in externalizing and internalizing disorders more broadly. In addition, previous genetic studies have found associations

between specific polymorphisms and these externalizing and internalizing subtypes. Therefore, we sought further characterize the biological influences on binge drinking subtypes through the following specific aims: 1) determine the genetic relationship between externalizing and internalizing characteristics in binge drinkers, 2) test whether externalizing and internalizing binge drinkers show differences in brain activation in response to tasks measuring emotion reactivity, reward sensitivity, and behavioral inhibition. In order to achieve these aims, we conducted a series of genetic analyses assessing differences in overall SNP-based heritability and specific associated variants between the externalizing and internalizing subtypes. There were a few variants that reached genome-wide significance, the most notable being a cluster of SNPs associated with internalizing characteristics that were located in the *RP3AL* gene. In a subset of these binge drinking young adults, brain activation was measured on tasks assessing behavioral inhibition, reward sensitivity, and emotion reactivity. We found some preliminary differences with regard to emotion reactivity, that suggest internalizing binge drinkers are more reactive to faces overall but have blunted reaction to sad faces compared to externalizers. These findings provide an initial step to better understanding the underlying biology between the classic externalizing and internalizing alcohol use subtypes, which has the potential to elucidate new subtype specific targets for prevention and intervention.

Chapter 1: Global Introduction

Prevalence of Alcohol Use/Misuse

Alcohol use is prevalent in the U.S., with 86.4% of people over the age of 18 having used in their lifetime and 56.0% having used in the past month (SAMHSA, 2014). The National Institute on Alcohol Abuse and Alcoholism (NIAAA) recommends 3 or fewer drinks per day (fewer than 7 drinks per week) for women and 4 or fewer drinks per day (fewer than 14 drinks per week) for men. While many people are able to consume alcohol at these healthy amounts, others find that their use causes problems in their life. In the U.S., an estimated $249.0 billion was lost in 2010 due to the effects of alcohol misuse (Sacks, Gonzales, Bouchery, Tomedi, & Brewer, 2015). In 2013, an estimated 14.7 million people met criteria for an alcohol use disorder (AUD) within the past year, which is defined by the *Diagnostic and statistical manual of mental disorders : DSM-5* 2013) as endorsing two or more symptoms in the past year. Symptoms include experiencing tolerance, withdrawal, craving, problems with family and friends, physical or psychological problems from drinking, spending a lot of time drinking or cutting back on other activities in order to drink. However, only a small proportion of these individuals (7.9%) receive treatment (SAMHSA, 2014). The fact that a large portion of individuals go untreated is problematic due to the widespread consequences on the familial, community, and societal level due alcohol misuse.

Alcohol Use in the College Population

College is an especially crucial time to study substance use, as risky alcohol use is prevalent among college students (Johnston, O'Malley, Bachman, & Schulenberg, 2010) with 40.1% reporting binge drinking in the past month and 14.4% reporting binge drinking on 5 or more occasions in the past month (SAMHSA, 2014). Binge drinking is defined as 4 or more drinks for women and 5 or more drinks for men. Additionally, lifetime, annual, and past 30-day alcohol use as well as binge drinking is higher in college students compared to their non-college-attending peers (Johnston et al., 2010). This frequent heavy use is associated with a range of academic, legal, physical and interpersonal consequences (Hingson, Zha, & Weitzman, 2009; McCarty, Morris, Hatz, & McCarthy, 2017; Orchowski, Mastroleo, & Borsari, 2012; Perkins, 2002). Specifically, binge drinkers were more likely to miss class/get behind in school work, get in trouble with the police, forget or regret something they did, get hurt or injured, and engage in unplanned sexual activity without protection than their non-binge drinking peers (Wechsler, Davenport, Dowdall, Moeykens, & Castillo, 1994).

Besides the increased prevalence and consequences, the college years represent a unique period to study heavy alcohol use. For many students this is the first time they are living on their own. This new freedom and reduced supervision coincides with increased genetic influences on alcohol use observed during late adolescence/young adulthood (Bergen, Gardner, & Kendler, 2007; Edwards & Kendler, 2013; Kendler, Schmitt, Aggen, & Prescott, 2008) which is hypothesized to reflect an increased ability for an individual to express their underlying propensity to binge drink or engage in other deviant behavior. Also during this time period, this age group is developing/formulating their long-term alcohol use patterns (Jackson, Sher, Gotham, & Wood,

2001), with the median age of onset for substance dependence being 19 to 23 years old (Kessler et al., 2005). This makes the college years a critical time for prevention and education efforts.

Alcohol Use Typologies

Although alcohol use disorder (AUD), previously referred to as alcohol dependence, is diagnosed as a single comprehensive disorder, even though many have theorized that there are actually different types of AUDs. Early research suggested two primary types of alcohol dependence. Cloninger, Bohman, and Sigvardsson (1981) called these Type 1 and Type 2. Type 1 was characterized by a later age of onset, genetic and environmental influences, affecting both men and women, low novelty seeking, and drinking to cope with anxiety. Conversely, Type 2 is characterized by an earlier age of onset, primarily genetic influences, affecting mostly men, and high novelty seeking. Babor et al. (1992) proposed Types A and B which have both similarities and differences to Types 1 and 2. Type A is characterized by a later onset and overall lower severity than Type B (fewer consequences, fewer treatment episodes, less psychological dysfunction). Type B is characterized by earlier onset, more familial risk factors, polydrug use, greater psychological dysfunction, and more treatment history.

Subsequent studies have expanded on these early binary classifications of alcohol dependence. Using the same data as Babor et al. (1992), Del Boca and Hesselbrock (1996) examined whether gender dependent subtypes would emerge. They found four subtypes, two of which represented either high or low risk/severity, similar to Types A and B. However, they also found internalizing and externalizing subtypes. The internalizing subtype was characterized by high symptoms of anxiety and depression, low antisocial personality, and low polydrug use. Conversely, the externalizing subtype was characterized by high rates of antisocial personality, increased polydrug use, and lower symptoms of anxiety and depression. These two subtypes were

somewhat gender specific with a greater proportion of females in the internalizing subtype and a greater proportion of males in the externalizing subtype. Windle and Scheidt (2004) analyzed data from a diverse group of in-patient alcoholics and also found four subtypes, which they described as mild, polydrug, negative affect and chronic/antisocial personality. The negative affect subtype is similar to Del Boca and Hesselbrock (1996)'s internalizing subtype, in that these individuals report high levels of anxiety and depression. In addition, there was about twice the proportion of women in this subtype than men. The chronic/antisocial subtype was similar to Del Boca and Hesselbrock's externalizing subtype in that they both demonstrated high levels of antisocial personality symptoms. Finally, Sintov et al. (2010) found that a "depressed" class made up 50% of their sample of Irish siblings with alcohol dependence. This class was characterized by high levels of depression and neuroticism, low levels of other substance dependence, and moderate levels of novelty seeking and antisocial personality disorder.

Some studies have looked at alcohol use typologies in college students often using either alcohol consumption and diagnostic criteria as indicators. Most of these studies have found subtypes based on levels of severity but a handful of these have further examined how externalizing and internalizing characteristics are associated with these types. For example, Beseler, Taylor, Kraemer, and Leeman (2012) found three classes indicated by number of diagnostic criteria endorsed. Sensation seeking, a type of impulsivity, was higher in the two riskier classes than in the class where no members met criteria for an AUD. Similarly, drinking to cope, a potential mechanism through which internalizing symptoms are related to alcohol use and problems, was higher among the two riskier classes. Kuvaas, Dvorak, Pearson, Lamis, and Sargent (2014) examined the effect of behavioral and emotional regulation on subtypes of college alcohol users indicated by alcohol consumption and problems. All of the heavier using classes had higher

sensation seeking than light drinkers. Higher sensation seeking also differentiated heavy drinkers from moderate drinkers. But emotional instability was significantly higher in the problem drinkers compared to the other use classes.

Externalizing Characteristics and Alcohol Use

Similar to the various subtypes of alcohol dependence that have been identified, there are also multiple developmental pathways to problem alcohol use. Mapping on to the alcohol dependence subtypes, the two most commonly studied developmental pathways are an externalizing and internalizing pathway (Hussong, Jones, Stein, Baucom, & Boeding, 2011; Zucker, 2008; Zucker, Heitzeg, & Nigg, 2011). The externalizing pathway has been more robustly associated with alcohol use and dependence than the internalizing pathway (Edwards, Gardner, Hickman, & Kendler, 2016; Farmer et al., 2016). According to Zucker (2008), the externalizing pathway is characterized by a lack of control and disinhibition manifested in aggressiveness, sensation seeking, impulsivity, delinquency and antisocial behavior. These types of externalizing behaviors have been shown to predict early initiation of alcohol use (Johnson, Arria, Borges, Ialongo, & Anthony, 1995; Mayzer, Fitzgerald, & Zucker, 2009; McGue, Iacono, Legrand, Malone, & Elkins, 2001), heavy alcohol use (Hussong, Curran, & Chassin, 1998) and general substance use (King, Iacono, & McGue, 2004) in adolescence.

The term "externalizing" is used to encompass a range of disorders, behaviors, and personality characteristics. Psychiatric disorders such as antisocial personality disorder, conduct disorder, oppositional defiant disorder, and substance use disorders are often classified as externalizing disorders. Externalizing disorders, specifically conduct disorder, antisocial behavior, and other substance use disorders, are highly comorbid with alcohol use disorders with odds ratios for having any comorbid externalizing disorder of 5.01 to 7.73 for men and 6.10 to

14.12 for women (Kendler, Prescott, Myers, & Neale, 2003; Kessler et al., 1997). Additionally, subclinical antisocial and delinquent behavior has been associated with increased risk of alcohol initiation, consumption, and problems (Dick et al., 2014; Nation & Heflinger, 2006).

Impulsivity is a core characteristic of most externalizing disorders. However, impulsivity is a heterogeneous construct itself which more generally can be divided into an increased propensity to reward seeking and a decreased ability to inhibit behavior (Gullo, Loxton, & Dawe, 2014). Both of these processes have been positively associated with a variety of alcohol outcomes and are thought to play a role in the progression to from use to addiction (Coskunpinar, Dir, & Cyders, 2013; MacKillop et al., 2011) and measured through a variety of medium (questionnaires, laboratory tasks, and animal models) (Dick et al., 2010).

Internalizing Characteristics and Alcohol Use

In contrast, evidence for a relationship between internalizing behavior and alcohol use has been mixed. Hussong et al. (2011) described an internalizing pathway whereby internalizing symptoms play an essential role in the progression to alcohol abuse. Additionally, Hussong et al. (2011) hypothesized that these internalizing symptoms can be present at any stage during development, and that they impact adolescent and adult risk for alcohol use disorders. Comorbidity has been shown between alcohol use disorders and internalizing disorders, such as anxiety and depressive disorders (Hasin, Stinson, Ogburn, & Grant, 2007; Kendler, Heath, Neale, Kessler, & Eaves, 1993), with odds ratios of 2.22 to 3.16 for men and 3.08 to 4.36 for women (Kessler et al., 1997). Developmentally, internalizing symptoms have been associated with earlier age of initiation and shortened time to first binge and first alcohol dependence symptom (Menary, Corbin, & Chassin, 2017). In a college sample, internalizing symptoms are significantly associated with

alcohol problems cross-sectionally but there is less conclusive evidence for their relationship longitudinally (Homman, Edwards, Cho, Dick, & Kendler, 2017).

However, other studies only identify a relationship between internalizing and alcohol use when controlling for externalizing behavior: King et al. (2004) demonstrated that major depressive disorder predicted alcohol use initiation and regular use only after controlling for externalizing symptoms. Even within this context, externalizing disorders alone had a stronger effect on subsequent alcohol use. One theory proposes that the increased comorbidity between alcohol use disorders and mood disorders is a result of drinking to regulate emotions (Cooper, Frone, Russell, & Mudar, 1995). There is evidence that this could explain the inconsistency in results across studies, as drinking to cope has been shown to moderate the relationship between internalizing symptoms and alcohol outcomes (Anker et al., 2017).

Biological Influences on Alcohol Use

Alcohol use, ranging from consumption to dependence, is known to be under genetic influence with genetic factors accounting for 50-60% of the variance (Kendler et al., 2008; Prescott & Kendler, 1999; Verhulst, Neale, & Kendler, 2015). However, discovering the specific genetic variants involved in problem alcohol use has largely been unsuccessful and identified variants currently explain very little of the genetic variance indicated by twin studies(Rietschel & Treutlein, 2013). The majority of gene finding studies treat alcohol misusers as a homogeneous group ignoring the possibility of potential genetic subtypes that correlate with the phenotypic subtypes described in the literature. Additionally, both externalizing and internalizing characteristics have been shown to be under modest to moderate genetic influence.

Previous cross-sectional neuroimaging studies have reported neurobiological differences in reward sensitivity, emotion reactivity, and behavioral inhibition among problem alcohol users

compared to healthy controls (Ahmadi et al., 2013; Ames et al., 2014; Gilman, Ramchandani, Davis, Bjork, & Hommer, 2008). These constructs have also been implicated in neuroimaging studies of internalizing and externalizing characteristics (Bjork, Chen, & Hommer, 2012; Buckholtz et al., 2010; Donegan et al., 2003; Hägele et al., 2014; Jones, Laurens, Herba, Barker, & Viding, 2009). While there has been some initial research to show that the phenotypic heterogeneity among drinkers can also be represented at the neurological level (Andrews et al., 2011; Beck et al., 2009; Gilpin, Herman, & Roberto, 2014; Nikolova & Hariri, 2012), direct comparisons need to be made using the same tasks, equipment and population.

Present Study

As reviewed in this chapter, there is a wealth of research on the phenotypic relationship between externalizing characteristics, internalizing characteristics, and alcohol use. This phenotypic research is already being used to improve efficacy in prevention/intervention by increased personalization (Conrod, Castellanos, & Mackie, 2008; Conrod, Castellanos-Ryan, & Mackie, 2011; Savage et al., 2015; Schuckit, Kalmijn, Smith, Saunders, & Fromme, 2012). However, far less research has investigated how these relationships may be influenced by genetic or neurobiological factors, despite the evidence described above that biological factors impact risk for both alcohol use/misuse and internalizing/externalizing characteristics. This dearth of research exists in spite of the knowledge that personalized treatment has also been shown to be effective at a biological/pharmacological level (Heilig & Egli, 2006; Heilig, Goldman, Berrettini, & O'Brien, 2011; Kranzler, Burleson, Brown, & Babor, 1996; Lesch & Walter, 1996).

Therefore, the purpose of this dissertation is to characterize the genetic and neurobiological mechanisms that underlie externalizing and internalizing subtypes of binge drinkers. There are three primary components to this overarching goal: 1) to phenotypically characterize externalizing

and internalizing characteristics within the sample, 2) to determine the genetic relationship between externalizing and internalizing characteristics among binge drinkers, 3) to test whether externalizing and internalizing binge drinkers show differences in brain activation in response to tasks measuring emotion reactivity, reward sensitivity, and behavioral inhibition.

In Chapter 2, a description of the sample to be used for all analyses is provided, along with a description of the phenotypes examined (internalizing, externalizing, binge drinking). This chapter also provides descriptive statistics for the phenotypes of interest, empirically characterizes internalizing and externalizing in the sample, and examines the relationship between internalizing/externalizing and binge drinking.

Chapter 3 further reviews the literature on genetic influences underlying alcohol, externalizing, and internalizing, providing the context for a series of genetic analyses of externalizing and internalizing in binge drinkers. Results from SNP heritability, genome wide associations, and secondary analyses of promising SNP associations are also presented.

The primary analytic goal of the analyses presented in Chapter 4 was to explore potential differences in brain activation as a function of internalizing/externalizing within binge drinkers. In order to fulfill this goal a complete neuroimaging study was conducted for this dissertation. The process is detailed in this chapter including, relevant literature, recruitment, data collection, and results.

Finally, Chapter 5 summarizes the overall findings of the dissertation and positions these results within the context of the existing literature. Limitations and opportunities for extending these analyses are discussed. Therefore, these analyses provide a uniquely comprehensive perspective due to the integration of both psychologically meaningful constructs and multiple

levels of biological influences of alcohol misuse, and may be helpful in developing programming for intervention, prevention, and treatment.

Chapter 2 Sample Description and Phenotypic Analysis of Externalizing and Internalizing Characteristics

As discussed at the end of the previous chapter, the goal of this dissertation is to examine the genetic and neurobiological factors influencing binge drinkers with comorbid externalizing or internalizing behavior. Externalizing and internalizing characteristics are known to be highly comorbid with heavy alcohol use (Hussong et al., 2011; Kendler et al., 2003; Kessler et al., 1997; Zucker, 2008).

Internalizing encompasses symptoms and disorders related to anxiety and depression. Internalizing disorders have been shown to increase the an individual's risk of developing an AUD (Kessler et al., 1997). However, anxiety and depression are complex constructs in themselves and their relationship to alcohol use is more nuanced than that of externalizing characteristics. For example, Nichter and Chassin (2015) examined two subfacets of anxiety (worry and physiological anxiety) and their relationships with alcohol use in male juvenile offenders. While these subfacets were moderately correlated with each other (0.58), they had unique relationships with alcohol use. Worry was negatively associated with quantity, frequency of binge drinking, and dependence symptoms, while physiological anxiety was positively associated with each of these alcohol outcomes. Edwards et al. (2014) examined childhood internalizing symptoms on adolescent alcohol use and found that fear, separation anxiety, and less robustly, worry were protective against alcohol outcomes while depressive symptoms increased an individual's likelihood of ever having binged. More relevant to the current study, Homman et al. (2017) combined symptoms of anxiety

and depression to show that this combined measure significantly predicted alcohol problems in college students within each timepoint.

As discussed in Chapter 1, externalizing is a broad term that manifests as both deviant behavior, such as antisocial behavior and conduct problems, and in ways that are less defiant such as impulsivity. Similarly, impulsivity itself is also a multifaceted construct. Whiteside and Lynam (2001) proposed a five factor model of impulsivity composed of urgency, lack of perseverance, lack of premeditation, and sensation seeking (UPPS). Urgency was then subsequently split into negative and positive urgency representing rash action in the context of negative or positive emotions (Cyders & Smith, 2008; Cyders et al., 2007). Overall, sensation seeking, positive, and negative urgency have consistently demonstrated the strongest associations with alcohol outcomes (Berg, Latzman, Bliwise, & Lilienfeld, 2015; Cyders, Flory, Rainer, & Smith, 2009; Jones, Chryssanthakis, & Groom, 2014; Magid & Colder, 2007; Stojek, Fischer, Murphy, & MacKillop, 2014; Whiteside & Lynam, 2009). More specifically, sensation seeking has consistently been associated with increased alcohol consumption (Cyders et al., 2009; Jones et al., 2014) and to a lesser extent alcohol problems (Stojek et al., 2014) in college students. However, Whiteside and Lynam (2009) found increased sensation seeking only in individuals with comorbid antisocial personality disorder and alcohol dependence. Individuals with alcohol dependence alone were not different from controls on levels of sensation seeking. Conversely, urgency is consistently associated with alcohol problems and disorder (Cyders et al., 2009; Jones et al., 2014; Magid & Colder, 2007; Stojek et al., 2014).

In order to examine a general disposition of externalizing or internalizing characteristics in the context of heavy drinking, initial data reduction analyses were conducted in the sample to capture each individual's overall level of externalizing and internalizing. Therefore, in this chapter

we describe the preliminary analyses necessary to complete the overarching goal of understanding genetic and neurobiological influences on externalizing and internalizing subtypes of binge drinkers.

Description of Sample and Measures

Full Sample

The sample used in all analyses for this dissertation is derived from the Spit for Science sample which was collected at a large, public university in an urban area in the eastern United States (Dick et al., 2014). This study is a longitudinal cohort study of university students. Eligible participants (freshman 18 years or older) are invited to fill out an online survey early in the fall semester of their freshman year and paid $10 as compensation for their participation. Freshman who are not yet eligible in the fall or do not participate in the fall are eligible to complete an entry survey in the spring. Any individual who fills out at least one survey during their freshman year becomes a part of the Spit for Science Registry and is eligible to complete subsequent surveys and spin-off projects. These individuals are then invited to fill out another online survey each spring. Since the study began in fall 2011, 4 cohorts have been enrolled in the study for a total of 9892 participants, or 66% of those eligible (Cohort 1 – 2,707, Cohort 2 – 2,483, Cohort 3 – 2,392, Cohort 4 – 2,310). When participants picked up their payment they were given the option of providing a saliva sample in order to genotype their DNA and earn an additional $10 (additional details in Chapter 3). Of the current sample, 91.3% of individuals (N = 9,036) have provided a DNA sample. Study data were collected and managed using REDCap (Research Electronic Data Capture) tools hosted at Virginia Commonwealth University (Harris et al., 2009).

The analyses presented in this dissertation use a subset of the Spit for Science sample. The sample was restricted to data from only cohorts 1 through 3 (N= 7,582) since at the time of these analyses, cohort 4 had not yet been genotyped. The sample was further reduced such that only individuals who were under 21 during their freshman year were included in the analysis (N = 7,501). This was to ensure homogeneity in the sample with regard to drinking behavior as well as internalizing and externalizing characteristics since nontraditional students represent a small proportion of the sample but may differ significantly on these variables.

Measures

Internalizing. In each survey, participants were asked about their symptoms of anxiety and depression using a subset of questions from the Symptom Checklist-90 (SCL-90; Derogatis, Lipman, & Covi, 1973). Participants were asked to indicate how much each symptom bothered them in the past 30 days, using five levels ranging from "not at all" to "extremely". The symptoms of anxiety that were measured were: nervousness or shakiness inside; suddenly scared for no reason; feeling fearful; and spells of terror or panic. The symptoms of depression that were measured were: feeling blue; worrying too much about things; feeling no interest in things; and feeling hopeless about the future.

Externalizing. In order to capture a range of externalizing characteristics, participants were asked about different types of impulsivity and their antisocial behavior. Impulsivity was assessed using a subset of three questions from each of the five subscales of the Urgency, lack of Premeditation, lack of Perseverance, and Sensation seeking – Positive urgency Impulsive Behavior Scale (UPPS-P), which measures the five facets of impulsivity it is named after (Magid & Colder, 2007; Whiteside & Lynam, 2001). Participants were asked to rate the extent to which they agreed

or disagreed that a statement represented who they generally are as a person. Negative urgency was measured by the statements: "when I feel bad, I will often do things I later regret in order to make myself feel better now"; "when I am upset I often act without thinking"; and "when I feel rejected, I will often say things I later regret". Lack of perseverance was measured by the statements: "I generally like to see things through to the end"; "unfinished tasks really bother me"; and "I finish what I start". Lack of premeditation was measured by the statements: "my thinking is usually careful and purposeful"; "I like to stop and think things over before I do them"; and "I usually think carefully before doing anything". Items representing lack of perseverance and lack of premeditation were reverse coded. Positive urgency was measured by the statements: "I tend to lose control when I am in a great mood"; "others are shocked or worried about what I do when I am feeling very excited"; and "I tend to act without thinking when I'm really excited". Sensation seeking was measured by the statements: "I quite enjoy taking risks"; "I welcome new and exciting experiences, even if they are a little frightening and unconventional"; and "I would enjoy the sensation of skiing fast down a mountain slope".

Participants were also asked about their antisocial behavior at each survey. Items were a subset of those used to measure antisocial behavior in the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA; Bucholz et al., 1994). Participants were asked how many times over the last year had they "deliberately damaged or destroyed property that did not belong to them"; "broken into a car or van to try and steal something out of it"; and "carried knife or other weapon with you for protection or in case you needed it in a fight".

Binge Drinking Phenotype. The National Institute of Alcohol Abuse and Alcoholism provides gender-based guidelines for binge drinking: for women, 4 or more drinks in a day is characterized as binge drinking, while the corresponding number for men is 5 or more drinks in a

day. In the current study, participants were asked at each survey, "How many drinks containing alcohol do you have on a typical day when you are drinking?" Participants chose from five options: "1 or 2"; "3 or 4"; "5 or 6"; "7,8, or 9"; or "10 or more". If on any survey a participant endorsed their typical drinking to be "5 or 6" drinks or more they were considered a binge drinker. In Cohort 1's sophomore and junior years and Cohort 2's sophomore year, a sex specific question was asked on binge drinking "How often do you have five (four for females) or more drinks in a single sitting (considered about a 2 hour period)?" Participants choose from five options: "Never"; "Monthly or less"; "2 to 4 times a month"; "2 to 3 times a week"; or "4 or more times a week". If at any time point participants in Cohorts 1 and 2 endorsed binge drinking "2 to 4 times a month" or more frequently they were considered a binge drinker. This resulted in a sample size of 3,079 binge drinkers (57.3% female).

Table 1: Alcohol items to select binge drinkers

	Y1F		Y1S		Y2S		Y3S		Y4S		Max	
	N	%	N	%	N	%	N	%	N	%	N	%
1 or 2	831	33.03	1110	27.43	828	29.89	603	37.34	275	36.28	1335	22.77
3 or 4	839	33.35	1453	35.91	1008	36.39	555	34.37	296	39.05	1921	32.76
5 or 6	555	22.06	932	23.04	608	21.95	305	18.89	134	17.68	1556	26.54
7,8, or 9	234	9.30	415	10.26	259	9.35	122	7.55	40	5.28	789	13.46
10 or more	57	2.27	136	3.36	67	2.42	30	1.86	13	1.72	262	4.47
Total	2516	61.89	4046	68.68	2770	74.18	1615	82.23	758	86.63	5863	77.54
ICN	154	3.79	176	2.99	99	2.65	66	3.36	21	2.4	110	1.45
Skip	1395	34.32	1669	28.33	865	23.17	283	14.41	96	10.97	1588	21.00
Total	4065		5891		3734		1964		875		7561	

Note: Participants had the option of endorsing "I choose not to answer" (ICN) for any question. Participants skipped these questions if they indicated they had not had at least one full alcoholic beverage. Percentages for number of typical drinks are out of individuals who endorsed an option.

Analytic Plan

We used factor analysis to create factors scores in the full S4S sample representing externalizing and internalizing characteristics. Factor analysis describes the underlying relationship between observed items using a latent continuous variable called a factor or latent construct. Factor analysis is frequently used as a data reduction tool because a large number of items can be represented by a fewer number of factors. Factor loadings describe the strength of the relationship between the observed items and the latent construct. By utilizing these factor loadings and observed items, individual-level estimates (factor scores) of the value on the factor can be obtained and used in subsequent analyses; in our case, as phenotypes in genetic analyses (Chapter 3) and selection criteria for the neuroimaging sample (Chapter 4).

For internalizing factor analysis, indicators were the 8 items assessing symptoms of anxiety and depression. For each item, a participant's response was averaged across the number of surveys they had taken in order to get a measure of their general level of internalizing over time. Previous research in this sample has demonstrated significant correlations between these items across waves (Homman et al., 2017). For externalizing, factor analysis indicators were the three items measuring sensation seeking and the three measures of antisocial behavior. The sensation seeking items were selected due to their consistent relationship with alcohol outcomes (Cyders et al., 2009; Jones et al., 2014) and association with antisocial behavior among individuals with alcohol dependence (Whiteside & Lynam, 2009). A subset of participants answered the sensation seeking items twice; for those individuals, responses were averaged across the time points for each item. For antisocial behavior the maximum response per item across surveys was used, since unlike the other items these represent specific behaviors the participant may or may not have engaged in, while sensation seeking items represent a general disposition or personality. Additionally, as seen

in Table 1 which shows the means of each item across individuals and across surveys, the overall endorsement of antisocial behavior is low so we were interested in capturing when those individuals engaged in this behavior the most.

We hypothesized that each behavioral construct (internalizing behavior, externalizing behavior) would comprise a single factor. We therefore ran confirmatory factor analysis (CFA) with sex as a covariate to ensure that one factor provided a good fit to the data. The fit of these factors was evaluated by the comparative fit index (CFI; Bentler, 1990), Tucker-Lewis index (TFI; Tucker & Lewis, 1973) and the root mean square error of approximation (RMSEA; Steiger, 1990). From these factors we then extracted factor scores to be used in the subsequent genetic analyses and serve as the basis for participant selection in the neuroimaging study. All analyses were conducted using Mplus 7.1 (Muthén & Muthén, 1998 - 2012).

Table 2. Descriptive statistics for the externalizing and internalizing items

	Y1F			Y1S			Y2S			Y3S			Y4S			Total			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	Range
Externalizing																			1 - 4
Damaged property	N/A	N/A	N/A	5690	1.10	0.40	3618	1.10	0.40	1900	1.11	0.39	848	1.08	0.34	6135	1.17	0.50	1 – 4
Broken into car	N/A	N/A	N/A	5707	1.02	0.22	3622	1.02	0.17	1908	1.01	0.16	851	1.02	0.15	6142	1.04	0.26	1 – 4
Carried a knife	N/A	N/A	N/A	5656	1.39	0.92	3597	1.33	0.86	1902	1.32	0.85	848	1.30	0.83	6119	1.53	1.05	1 – 4
Enjoy taking risks	3992	2.84	0.84	5672	2.67	0.87	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	6927	2.75	0.81	1 – 4
New/exciting experiences	3983	3.20	0.74	5669	3.04	0.80	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	6930	3.11	0.73	1 – 4
Enjoys skiing fast	3944	2.86	1.08	5593	2.76	1.08	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	6857	2.80	1.05	1 - 4
Internalizing																			
Nervousness	5922	2.10	1.06	5664	2.11	1.07	3605	1.95	1.03	1898	2.00	1.04	846	1.99	1.07	7428	2.08	0.92	1 - 5
Suddenly scared	5931	1.50	0.89	5664	1.55	0.91	3603	1.45	0.82	1899	1.47	0.84	847	1.44	0.83	7437	1.51	0.76	1 – 5
Feeling blue	5931	2.20	1.12	5665	2.44	1.16	3605	2.38	1.16	1900	2.36	1.14	846	2.31	1.13	7436	2.33	1.00	1 – 5
Worrying about things	5929	2.75	1.28	5674	3.00	1.24	3602	2.94	1.28	1902	2.92	1.29	846	2.89	1.30	7434	2.88	1.10	1 – 5
Feeling no interest in things	5925	1.93	1.10	5658	2.18	1.17	3606	2.10	1.17	1898	2.09	1.19	844	2.06	1.15	7433	2.07	0.99	1 – 5
Feeling fearful	5931	1.75	0.99	5662	1.72	0.98	3604	1.67	0.96	1900	1.67	0.98	845	1.72	1.03	7435	1.72	0.83	1 – 5
Feeling hopeless	5927	1.78	1.11	5668	2.09	1.23	3602	2.08	1.23	1902	1.99	1.19	845	2.07	1.26	7431	1.97	1.02	1 – 5
Spells of terror or panic	5924	1.39	0.85	5668	1.45	0.89	3602	1.45	0.87	1901	1.46	0.87	843	1.49	0.91	7431	1.43	0.75	1 – 5

Results

Table 2 shows the sample size, means, standard deviations, and ranges for each item at each time point as well as collapsed across time points. Confirmatory factor analyses were conducted on 7,501 participants who were younger than 21 in their initial survey and in cohorts 1-3. The sample was 60% female. Representative of the VCU student body as a whole, the ethnic/racial breakdown is as follows: 49.5% white (3,718), 19.3% black/African American (1,449), 16% Asian (1,201), 6.2% more than one race (465), 6% Hispanic (447), 0.6% Hawaiian/Other Pacific Islander (50), 0.5% American Indian/Alaska Native (35). The results generally supported a one factor solution for both externalizing and internalizing characteristics. The CFI, TLI, and RMSEA were 0.871, 0.828, and 0.118, respectively. The CFI and TLI are just below the desired value of 0.9. The RMSEA is much greater than the ideal value of 0.06. However, greater values of RMSEA are both sensitive to sample size and often indicate high correlations between items (redundancy of information). In this analysis, since these items are from previous scales hypothesized to capture similar constructs it is not surprising (and less concerning) that the RMSEA is higher than typical cutoffs. The factor loadings and standard errors are shown in Table 3. The factor loadings of each item were significantly different from zero. Solutions were also tested with nicotine use items loading on the externalizing factor and items measuring drinking to cope on the internalizing factor. Both these solutions resulted in a poorer fit and lower item loadings for the nicotine and drinking to cope items. The internalizing and externalizing factors are significantly, although modestly, negatively correlated at -0.066.

Table 3: Factor loadings and standard errors for the externalizing and internalizing factors

Item	Loading	S.E
Externalizing		
Damaged property	.683	.078
Broken into car	.664	.152
Carried a knife	.394	.048
Enjoy taking risks	.750	.014
New/exciting experiences	.606	.012
Enjoys skiing fast	.546	.014
Internalizing		
Nervousness	.755	.007
Suddenly scared	.745	.009
Feeling blue	.760	.007
Worrying about things	.716	.007
Feeling no interest in things	.665	.009
Feeling fearful	.777	.008
Feeling hopeless	.708	.009
Spells of terror or panic	.729	.009

Note: All factor loadings were significantly different from zero, $p<0.05$

Table 4 shows the mean, SD, and range for the externalizing and internalizing factor scores for binge drinkers and non-binge drinkers in the sample. Levels of externalizing and internalizing were statistically higher for binge drinkers compared to non-binge drinkers.

Table 4: Group differences on Externalizing and Internalizing factor scores

	Non-Binge Drinkers			Binge Drinkers				
	Mean	SD	Range	Mean	SD	Range	t-statistic	p-value
Externalizing	-0.56	0.58	-2.17 – 0.74	-0.35	0.56	-2.17-0.83	-10.929	2.2e-16
Internalizing	0.42	0.65	-0.46 – 3.02	0.47	0.65	-0.46-3.33	-2.206	0.027

Discussion

The goal of the current chapter was to characterize externalizing and internalizing within the sample of interest and determine the binge drinking subset of participants which will be used

in subsequent analyses (Chapters 3 and 4). The externalizing and internalizing items used in this chapter (and subsequent analyses) have been previously associated with alcohol use in this sample (Cho et al., 2015; Homman et al., 2017). However, the goal of our analyses moving forward is not to test that these characteristics are associated with substance use but rather given their known association what can we learn about the underlying biology of individuals with the comorbid conditions.

We found that a single internalizing and a single externalizing factor were a good fit to the items of interest. This mirrors the analyses conducted by Homman et al. (2017) which also found a one factor solution to be a good fit to the internalizing items in this sample. The existence of a single externalizing or internalizing factor providing a good fit to psychopathology data is not unique to this study (Krueger, 1999; Lahey et al., 2012). While each of the symptoms, traits, or disorders used in this and previous research has its own field of study, they also index overarching factors that capture externalizing and internalizing more generally. Therefore, despite that externalizing and internalizing are heterogeneous constructs, the items measured in the current sample seems to represent two underlying predispositions.

The factor scores resulting from the externalizing and internalizing factors were significantly higher in binge drinkers in the current sample. This indicates that as hypothesized (and previously demonstrated) these characteristics are associated with problem drinking in our sample. While these analyses did not attempt to assess the directional relationship between binge drinking and externalizing/internalizing characteristics, the increased comorbidity underscores the importance of study this population as they are at an increase of exhibiting other problem behaviors/symptoms.

To summarize, externalizing and internalizing characteristics can each be captured by a single factor in this sample and the resulting factor scores are increased in those individuals with a history of frequent binge drinking. These scores and the binge drinking sample will be used in analyses in the subsequent chapters. In Chapter 3, externalizing and internalizing factor scores will serve as the phenotypes in a series of genetic analyses conducted in the binge drinking sample. In Chapter 4, participants are selected for a neuroimaging study from the binge drinking sample based on their externalizing and internalizing factor scores.

Chapter 3: Genetic Analyses in Externalizing and Internalizing Subtypes of Binge Drinkers

Alcohol phenotypes (from use to alcohol use disorder) are known to be under genetic influence. Twin studies, which estimate the proportion of genetic to environmental influences for a given trait in a given population, estimate a range of heritabilities from 40-60% (Kendler, 2001; Kendler et al., 2008; Prescott & Kendler, 1999). A recent meta-analysis of twin and adoption studies on alcohol use disorder (AUD) estimated the heritability at 49% (Verhulst et al., 2015). Due to the substantial portion of variance attributed to genetic influences, identifying the specific genes or markers influencing AUD and alcohol use generally has the potential to improve our understanding of the biological mechanisms underlying these complex behaviors.

Initial gene finding efforts for alcohol phenotypes included linkage and candidate gene association studies. Linkage studies are conducted in related individuals and exploit the overall increased shared genetic background between these individuals to narrow in on regions that are more commonly shared among affected relatives than unaffected. This technique is best suited to discover variants of modest effect and implicates large areas of a chromosome which require subsequent fine-mapping. Using this method there has been consistent support for a protective effect of the alcohol dehydrogenase genes on developing alcohol dependence (Prescott et al., 2006; Reich et al., 1998). Linkage analysis also implicated a region in the genes coding for the GABA-A receptor (Edenberg et al., 2004; Porjesz et al., 2002; Reich et al., 1998).

In contrast to linkage studies, candidate gene association studies examined one gene at a time, often one variant, that was hypothesized to have a biological relevance to alcohol

consumption or problems. Since alcohol dependence is a psychiatric disorder, most of the genes tested initially in candidate gene studies were involved in neurotransmitter systems. More recently, enough individual candidate gene association studies have been conducted on specific variants to allow for meta-analyses. In meta-analysis of 40 published studies, Munafo, Matheson, and Flint (2007) found that the A1 allele of the Taq1A polymorphism did increase one's risk for alcohol dependence. Although this polymorphism is located in the *ANKK1* gene, it alters the function of the dopamine D2 receptor gene nearby. The serotonin transporter gene (*5HTTLPR*) has been examined as a gene of interest across many psychiatric conditions. McHugh, Hofmann, Asnaani, Sawyer, and Otto (2010) conducted a meta-analysis of studies that examined the influence of the short allele on alcohol dependence. They found that across 22 studies those who carried the short allele were at an increased risk of alcohol dependence; the risk was even greater for those who were homozygous for the short allele. Not all candidate gene findings have been robust to meta-analysis. Although initial findings were significant, variants in both the dopamine transporter gene (*SCL6A3*) and the catechol-O-methyl transferase gene (*COMT*) were not significantly associated with alcohol dependence in meta-analyses (Du, Nie, Li, & Wan, 2011; Tammimaki & Mannisto, 2010; Xu & Lin, 2011). These meta-analyses represent the handful of the candidate genes that have been tested in a large number of samples. To facilitate this type of analysis arrays have been developed that focus on markers thought to be associated with addiction and related traits (Hodgkinson et al., 2008). However, the majority of these candidate genes have yet to be robustly replicated.

With candidate gene association studies proving to be largely unreproducible, there was a renewed interest in agnostic approaches similar to linkage analysis. Genome-wide association studies (GWAS) test the relationship between hundreds of thousands of common variants across

the genome and a trait of interest. Unlike linkage studies and candidate gene studies, GWAS can detect variants of small effect using an agnostic approach. In the first GWAS of alcohol phenotypes, Treutlein et al. (2009) examined alcohol dependence in a sample of all males and found two genetic variants in the *PECR* gene to be genome-wide significant (GWS). This finding fit with previous linkage studies which had indicated increased allele sharing in this region for alcohol dependence. Stemming from this initial success, there was an increase in the number of GWAS on alcohol traits being conducted and published. Many of these studies have had null results (Bierut et al., 2010; Edenberg et al., 2010; Heath et al., 2011). Bierut et al. (2010) found no GWS associations with alcohol dependence in the both European Americans and African Americans of the Study of Addiction: Genes and Environment (SAGE) sample. Edenberg et al. (2010), using the Collaborative Study on the Genetics of Alcoholism (COGA) sample, also found no GWS markers for alcohol dependence. Kendler, Kalsi, et al. (2011) performed one of the first GWAS of a quantitative alcohol dependence phenotypes using the control sample of the Molecular Genetics of Schizophrenia study but still found no GWS associations. Finally, Heath et al. (2011) examined three alcohol phenotypes in a sample of over 8,000 individuals and still found no GWS markers. Although previously discovered through linkage, the protective effect of genetic variants in alcohol metabolizing genes has been supported by GWS in Asian samples (Park et al., 2013; Quillen et al., 2014), European samples (Frank et al., 2012) and American samples of both European and African descent (Gelernter et al., 2014).

This lack of robustly significant genetic markers for alcohol traits is somewhat surprising given the well replicated moderate heritability of alcohol phenotypes. Additionally, the emergence of a large number of initial null GWAS was not unique to alcohol phenotypes but similarly seen across complex psychiatric traits. Generally, there have been two strategies taken to address this.

The first has been to drastically increase the sample size. Due to the fact that in these highly polygenic traits each individual associated variant is likely to have a small effect on a given alcohol phenotype, it is thought much larger sample sizes will be needed to detect these associations. Sample sizes of around 100,000 have been successful for some psychiatric phenotypes, like schizophrenia (Ripke et al., 2014), but less successful for other psychiatric phenotypes, such as major depression (Ripke et al., 2013). In fact, even with sample sizes greater than 300,000 there are still far fewer GWS variants associated with major depression than with schizophrenia (Hyde, Nagle, et al., 2016). Alcohol dependence falls between schizophrenia and major depression in terms of both prevalence in the population and heritability. Therefore, it is not surprising that even with sample sizes comparable to the schizophrenia there have been few robustly significant variants associated with alcohol dependence. In addition, the higher prevalence of alcohol dependence compared to schizophrenia implies that, unless carefully phenotyped, a cursory screening of participants could lead to controls who were missed as cases or eventually develop symptoms and cases who are misdiagnosed.

Recently much larger GWASs of alcohol phenotypes have been published with varying success. Sanchez-Roige et al. (2017) found no genome-wide significant hits associated with the alcohol use disorder identification test in a sample of over 20,000 alcohol users. Jorgenson et al. (2017) examined alcohol consumption in a trans ethnic sample of over 80,000 individuals. They replicated several previously associated variants including markers in the alcohol dehydrogenase genes but no novel variants reached genome wide significance. Schumann et al. (2016) conducted a GWA meta-analysis on alcohol consumption in non-dependent individuals. They discovered one novel loci and several other suggestive associations which they were able to replicate in another sample. Finally, the largest alcohol GWAS to date with over 112,000 individuals found

only 4 novel loci (Clarke et al., 2017). While there is some modest replication across these recent large scale GWAS, they have identified far fewer variants than identified with regard to schizophrenia at similar sample sizes. Therefore, alcohol phenotypes may require even larger sample sizes (N>100,000) or a more careful examination of what makes gene discovery for these phenotypes particularly intractable.

Another strategy in response to the initial null GWAS findings of complex psychiatric traits was to refine the phenotype of interest. CONVERGE refined their study of major depression by focusing on individuals of exclusively Han Chinese descent, using a diagnostic interview to identify cases, requiring cases to have had multiple depressive episodes, and restricting control participants to be past the vulnerability period for major depression. Furthermore, given prior evidence of sex-specific genetic effects (Kendler & Prescott, 1999), the sample was limited to women. By employing these restrictions, the CONVERGE study identified the first robust and replicated associations with major depression (Converge consortium, 2015). The goal of the current study is to also use the approach of phenotype refinement. As previously mentioned, the development of problem alcohol use is phenotypically heterogeneous. Similar to major depression, this heterogeneity may be adding to the difficulty in finding associated genetic variants in alcohol phenotypes. Therefore, an additional approach to increasing sample size is to directly examine these comorbid factors in the context of problem alcohol use.

As previously discussed the two more commonly studied pathways to problem alcohol use are an externalizing and an internalizing pathway. Externalizing phenotypes, such as antisocial behavior and impulsivity, are also genetically influenced. A recent meta-analysis estimated that 50% of the variance in impulsivity was due to heritable factors with a narrow sense heritability of 38% (Bezdjian, Baker, & Tuvblad, 2011). Specific to the current study, sensation seeking (a

subfacet of impulsivity) had a heritability of 34%. The co-occurrence of externalizing disorders and problematic alcohol use appears to be due, in part, to a shared genetic liability between these behaviors (Kendler et al., 2003; McGue, Iacono, Legrand, & Elkins, 2001). Khemiri, Kuja-Halkola, Larsson, and Jayaram-Lindstrom (2016) examined the genetic overlap between impulsivity and alcohol dependence and found that 80% and 53% of the correlation between impulsivity and alcohol dependence was due to genetic factors in males and females, respectively. Previous research has shown a shared latent genetic factor between antisocial behavior/personality disorder, conduct disorder and alcohol dependence (Kendler, Aggen, et al., 2011; Kendler et al., 2003; Krueger, 1999; Young et al., 2009).

Similarly, internalizing phenotypes, such as anxiety and depression, are also genetically influenced. Major depression has a heritability of about 37% (Sullivan, Neale, & Kendler, 2000) while generalized anxiety disorder has a slightly lower heritability of 32% (Hettema, Neale, & Kendler, 2001). Similar estimates are seen for the heritability of symptoms of anxiety and depression (Happonen et al., 2002). There is some evidence of genetic correlation between internalizing symptoms and alcohol use (Edwards, Sihvola, et al., 2011; Prescott, Aggen, & Kendler, 2000). However, other studies have shown that their comorbidity is explained by environmental factors (Edwards, Larsson, Lichtenstein, & Kendler, 2011; Kendler, Aggen, et al., 2011).

Even though externalizing and internalizing characteristics are under modest to moderate genetic influence, gene finding for these phenotypes has also progressed slowly. With the exception of the CONVERGE project, there have been few robustly significant genetic variants associated with either internalizing disorders (Otowa et al., 2016) or broad externalizing behavior

(McGue et al., 2013; Vrieze et al., 2014). Therefore, these disorders are likely to benefit from examining more refined phenotypes as well.

Thus the goal of this chapter is to use what we know about the heterogeneity of alcohol phenotypes (Chapter 1) to conduct genetic analyses on binge drinking subtypes (Chapter 2) with the hypothesis that a less heterogeneous phenotype will improve our ability to understand underlying biology and detect associated genetic variants. We focus on externalizing and internalizing subtypes since these are well known pathways to problem alcohol use (Hussong et al., 2011; Zucker, 2008) which are genetically influenced as well. Finally, we are studying these subtypes in college students as this is both when genetic influences peak (Kendler et al., 2008) and when problem drinking starts to manifest.

Methods

The binge drinking sample, described in Chapter 2, was used for theses analyses. Briefly, this sample is a subset of the parent Spit for Science sample who regularly binge drink (4 or more drinks for women and 5 or more drinks for men). The phenotypes used for the analyses are the externalizing and internalizing factor scores. The indicators and creation of the factor scores is described in Chapter 2 in detail. Of the 3,079 participants in the binge drinking sample, 2,618 provide a DNA sample and therefore were included in these analyses.

Genetic Data Cleaning and Imputation

Samples were genotyped on the Affymetrix Biobank Array at Rutgers University Cell and DNA Repository. This array contains 653K SNPs and InDels including a) 296K common variants used for GWAS and imputation and b) 357K rare, and likely functional, variants derived from

exome studies. Initial QC followed similar procedures as the Psychiatric Genomic Consortium (PGC; Ripke et al., 2014), removing off target variants found by SNPolisher, single nucleotide polymorphisms (SNPs) missing more than 5% of genotypes, samples missing more than 2% of genotypes, and SNPs missing more than 2% of genotypes after removing bad samples. This initial QC resulted in 6,325 samples and 560,138 variants brought forward to imputation. Imputation was conducted using SHAPEIT2 (Delaneau, Zagury, & Marchini, 2013) and IMPUTE2 (Howie, Donnelly, & Marchini, 2009) with 1000 genomes phase 3 as a reference panel (N=2,504; Sudmant et al., 2015).

Ancestry Principal Components (PCs)

Two types of ancestral principal components were created: cross ancestry PCs and ancestry specific PCs. For both types, EIGENSOFT/SmartPCA (Patterson, Price, & Reich, 2006; Price et al., 2006) was used to create the PCs, regions with high disequilibrium were excluded, and Plink 1.9 was used to prune variants ($r^2<0.1$). For the cross ancestry PCs, principal components analysis (PCA) was run in the 1KG phase 3 reference panel and then projected on to the S4S sample. For the ancestry specific PCs, PCA was run in each super population separately.

Assignment to Ancestry Super Population

The genotyped S4S sample was by design ethnically diverse with regards to self-identified census race/ethnicity (American Indian/Alaska Native, Asian, Black/African American, Hispanic/Latino, More than one race, Native Hawaiian/Other Pacific Islander, Unknown, and White). In order to reduce variance within ancestral groups and include individuals whose race/ethnicity is either unknown or a combination of races/ethnicities, each participant was assign to 1000 Genomes Project (1KGP) ancestry super population of African (AFR), American (AMR), East Asian (EAS), European (EUR), or South Asian descent (SAS). To do this, the 10 cross

ancestry PCs were used to calculate the Mahalanobis distance (Mahalanobis, 1936) between each sample and each of the 1KGP populations (N=26). Each S4S sample was then assigned to a 1KGP super population based on the minimum Mahalanobis distance and then further collapsed into the above super populations.

SNP-based Heritability

Genome-wide Complex Trait Analysis (GCTA) was used to estimate the proportion of phenotypic variance that is attributable to the observed (genotyped) genetic variants (SNP based heritability) of externalizing and internalizing characteristics among binge drinkers. Genetic relationship matrices (GRMs) were created within each of the five ancestry super populations using an ancestry specific minor allele frequency (MAF) cutoff of 0.01 and the associated ancestry specific PCs.

Genome-wide Association (GWA) Analyses

Five ancestry specific GWAS were performed for each phenotype (10 total) using SNPTEST 2.5.2 (Marchini, Howie, Myers, McVean, & Donnelly, 2007). Pre-GWAS filtering excluded markers with a MAF less than 0.005 and an INFO score less than 0.5. Post-GWAS filtering including ancestry specific violations of Hardy-Weinberg Equilibrium (p-value>10^{-6}) and ancestry dependent MAFs. A marker needed to have a minor allele count (MAC) of 40 within the ancestry group to be included in meta-analysis. Ancestry groups with a sample size of less than 400 individuals were not included in the meta-analysis. Ancestry specific GWASs were meta-analyzed using METAL (Willer, Li, & Abecasis, 2010).

Functional Mapping and Annotation of Genetic Associations (FUMA)

In order to further explore the results from the GWAS, FUMA (Watanabe, Taskesen, van Bochoven, & Posthuma, 2017) was used. FUMA is an online tool that functionally annotates

GWAS findings and prioritizes likely causal SNPs by combining information from 14 biological databases. FUMA has two main processes; SNP2GENE and GENE2FUNC. SNP2GENE takes summary statistics from GWAS and maps the SNPs to genes based on both their position and function. GENE2FUNC takes the prioritized genes from SNP2GENE and returns information on tissue expression patterns and enrichment in biological pathways.

Results

The phenotypes in the genotyped sample closely match those in the full binge drinking sample described in Chapter 2. The mean of the externalizing factor scores is -0.35 (SD = 0.57, range = -2.17 – 0.83). The mean of the internalizing factor scores is 0.46 (SD = 0.64, range = -0.46 – 3.33). Externalizing and internalizing factor scores were significantly correlated at -0.071 ($p = 0.001$).

SNP-based Heritability

Table 5 shows the SNP heritability for each ancestry group as well as the meta-analyzed estimate across ancestry groups. The meta-analyzed results show that the estimate of heritability of externalizing characteristics in binge drinkers are not significantly different from zero while the estimate of heritability for internalizing characteristics in binge drinkers is modestly heritable. Due to the small samples sizes of some of the ancestry groups (AMR, EAS, SAS), a meta-analysis including just the AFR and EUR ancestry groups was conducted. The results were similar to the full sample meta-analysis for both externalizing ($h^2 = 0.015$, S.E. = 0.228) and internalizing ($h^2 = 0.196$, S.E. = 0.226) characteristics.

Table 5: GCTA Results

Phenotype	Ancestry	h^2	S.E.	p-value	N	meta	meta_S.E.
Externalizing	AFR	0.000001	0.634003	0.5	449	0.064	0.2165
	AMR	0.721612	0.823059	0.4168	243		
	EAS	0.000001	1.867347	0.5	179		
	EUR	0.017609	0.244067	0.4689	1430		
	SAS	0.000002	1.852756	0.5	153		
Internalizing	AFR	0.000001	0.423701	0.5	448	0.25	0.21583
	AMR	0.999999	0.957031	0.02829	244		
	EAS	0.000001	1.460971	0.5	179		
	EUR	0.273349	0.267336	0.1502	1430		
	SAS	0.999999	1.708642	0.1598	152		

GWAS

After filtering and meta-analysis 12,028,511 and 12,020,239 markers were analyzed for externalizing and internalizing characteristics, respectively. The meta-analyses showed no evidence of genomic inflation with λ_{1000} of 0.9988 for externalizing characteristics and 0.9978 for internalizing characteristics. Figures 1 and 2 show the quantile-quantile plots. Figures 3 and 4 show meta-analyzed results for externalizing and internalizing characteristics respectively in manhattan plots. There were three markers with an FDR q <0.5 for externalizing characteristics (Table 6). There were 32 markers with an FDR q<0.5 and 13 markers with an FDR q <0.1 for internalizing characteristics (Table 3).

Figure 1: Q-Q plot for Externalizing Characteristics in Binge Drinkers

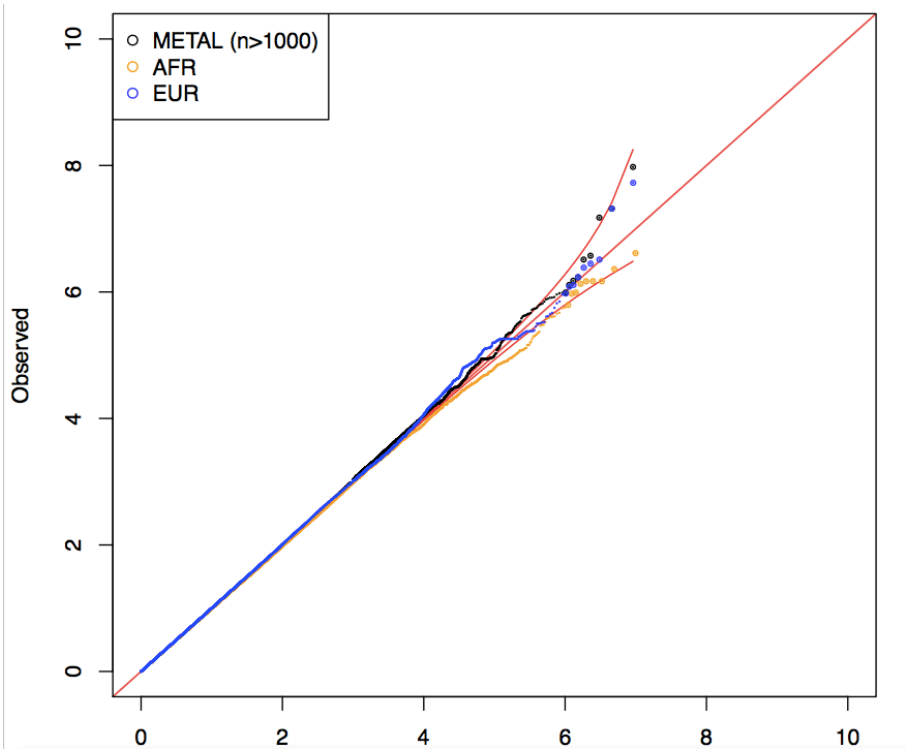


Figure 2: Q-Q Plot for Internalizing Characteristics in Binge Drinkers

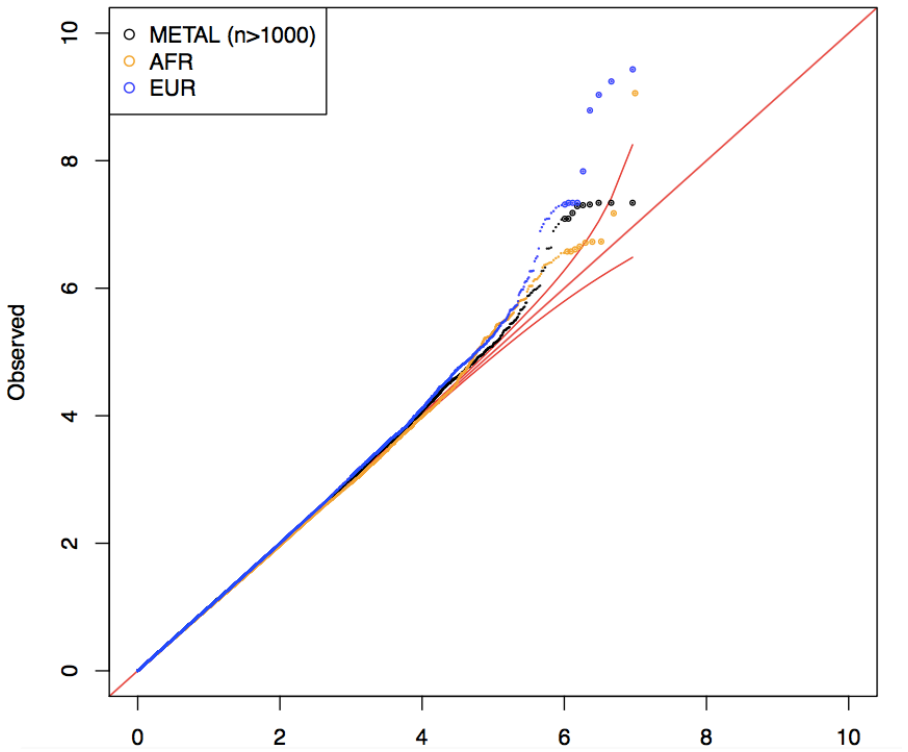


Figure 3: Manhattan Plot for Externalizing Characteristics in Binge Drinkers

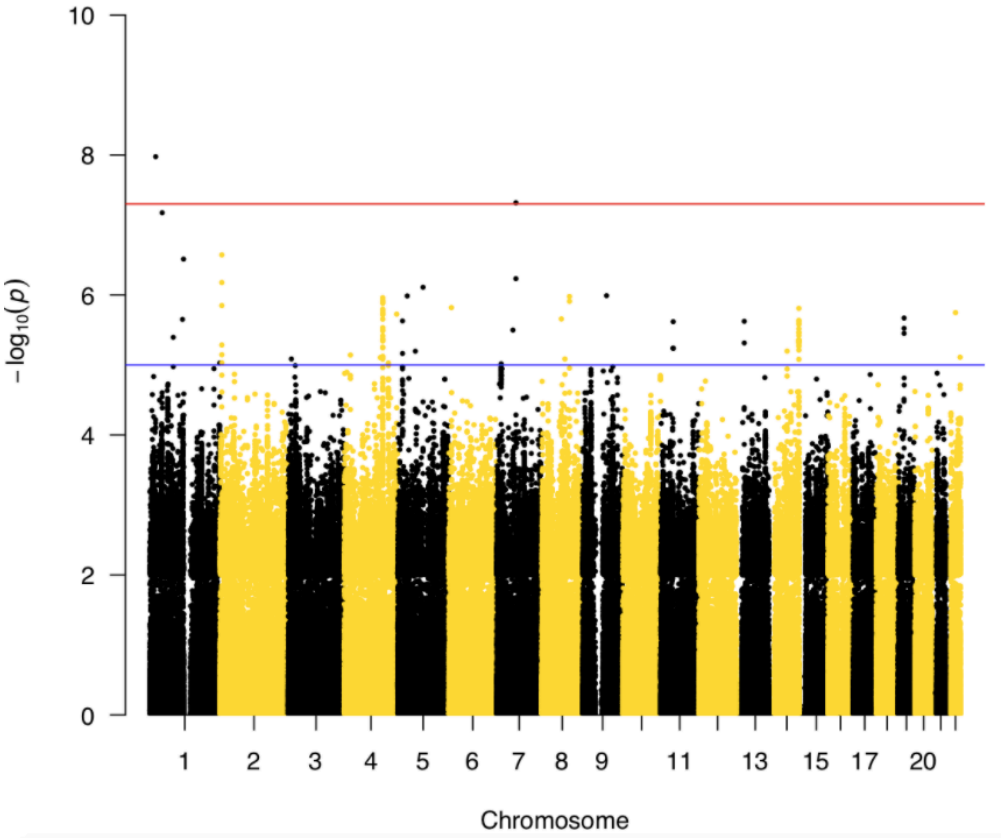


Figure 4: Manhattan Plot for Internalizing Characteristics in Binge Drinkers

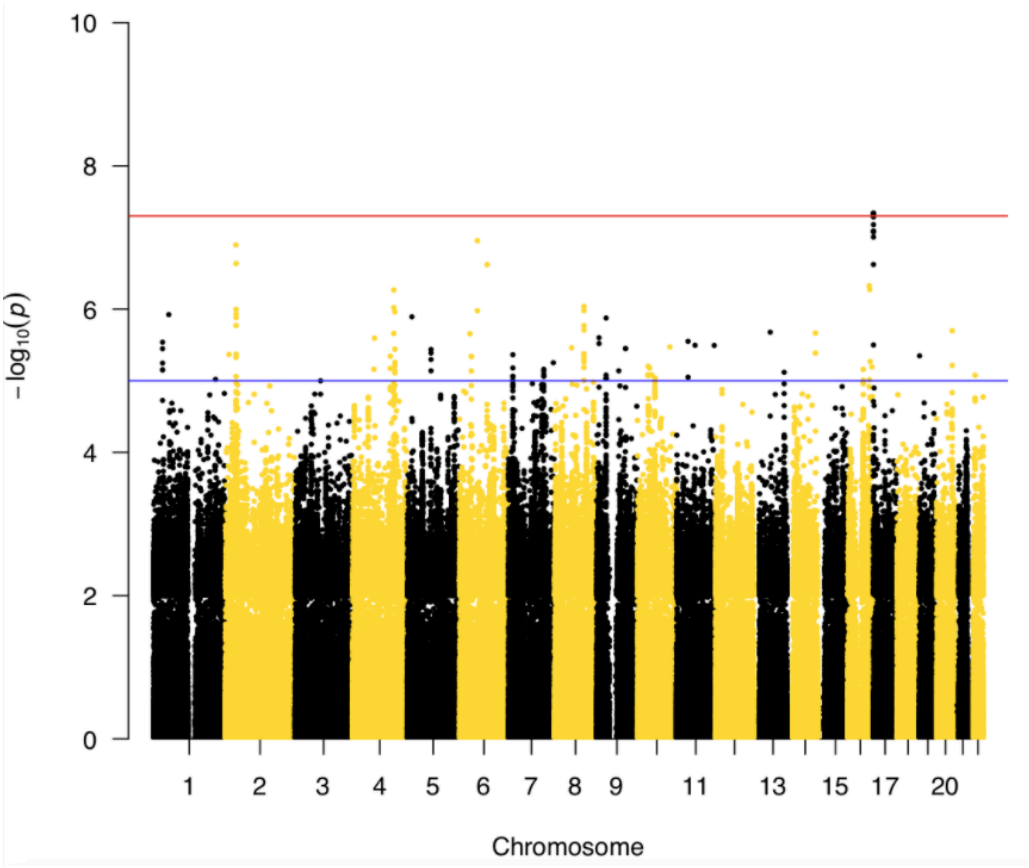

10
8
6
4
2
0
$-\log_{10}(p)$
1
2
3
4
5
6
7
8
9
11
13
15
17
20
Chromosome

Table 6: Genetic Markers with q>0.5 for GWAS of Externalizing Characteristics in Binge Drinkers

SNP	CHR	BP	MAF AFR	Pvalue AFR	MAF EUR	Pvalue EUR	Allele1	Allele2	Weight	Zscore	P.value	Direction	q_1k
rs71569819	1	21536624	0.312	0.008	0.127	4.12E-07	a	attatttat	1879	-5.722	1.06E-08	--	0.096
rs35728229	1	44516588	0.354	0.312	0.141	1.88E-08	t	ta	1879	5.4	6.68E-08	++	0.203
rs79401837	7	67832139	0.005	0.756	0.040	4.80E-08	a	c	1430	5.458	4.81E-08	?+	0.203

Table 7: Genetic Markers with q>0.5 for GWAS of Internalizing Characteristics in Binge Drinkers

SNP	CHR	BP	Maf AFR	Pvalue AFR	Maf EUR	Pvalue EUR	Allele1	Allele2	Weight	Zscore	P.value	Direction	q_1k
rs76897114	1	53799050	0.065	8.74E-10	0.044	0.033	t	c	1878	-4.857	1.19E-06	--	0.402
2:37261641:A:C	2	37261641	0.014	0.373	0.016	1.27E-07	a	c	1430	5.283	1.27E-07	?+	0.089
rs10549702	2	38153237	0.202	0.009	0.064	5.59E-05	a	aaact	1878	-4.788	1.69E-06	--	0.484
rs1446299	2	38153593	0.202	0.009	0.065	4.32E-05	a	g	1878	4.838	1.31E-06	++	0.404
rs7584626	**2**	**38161778**	**0.046**	**0.002**	**0.064**	**2.44E-05**	**t**	**c**	**1878**	**-5.173**	**2.30E-07**	**--**	**0.136**
rs139166299	2	38163952	0.080	0.014	0.064	2.38E-05	a	t	1878	4.89	1.01E-06	++	0.398
rs59738114	2	38192028	0.082	0.026	0.072	1.51E-05	t	g	1878	4.861	1.17E-06	++	0.402
rs77583243	4	144426869	0.007	0.362	0.020	9.52E-07	c	g	1430	4.901	9.52E-07	?+	0.398
rs75599877	4	144428647	0.007	0.401	0.018	5.39E-07	a	g	1430	-5.012	5.40E-07	?-	0.259
rs1395820	**4**	**148032488**	**0.499**	**0.002**	**0.196**	**0.0001**	**a**	**g**	**1878**	**4.875**	**1.09E-06**	**++**	**0.398**
rs112050397	5	16113885	0.003	0.947	0.019	1.28E-06	t	c	1430	-4.843	1.28E-06	?-	0.404
rs150847810	6	62893066	0.005	0.174	0.032	1.11E-07	a	g	1430	5.308	1.11E-07	?+	0.084
rs143991214	6	62944608	0.008	0.203	0.039	1.05E-06	a	g	1430	4.882	1.05E-06	?+	0.398
rs149499893	**6**	**96270587**	**0.071**	**0.034**	**0.154**	**2.20E-06**	**a**	**aac**	**1878**	**5.166**	**2.39E-07**	**++**	**0.136**
rs500000	8	102465957	0.438	0.039	0.499	1.51E-05	a	g	1878	-4.787	1.70E-06	--	0.484
rs618854	8	102467706	0.437	0.025	0.499	1.40E-05	t	c	1878	4.883	1.05E-06	++	0.398

rs543427	**8**	**102468599**	**0.454**	**0.019**	**0.499**	**1.58E-05**	**c**	**g**	**1878**	**4.909**	**9.15E-07**	**++**	**0.398**
rs10971108	9	32729457	0.460	0.036	0.202	1.26E-05	t	c	1878	-4.836	1.33E-06	--	0.404
rs11383665	16	75649082	0.119	0.029	0.046	5.31E-06	g	ga	1878	5.038	4.72E-07	++	0.253
rs115570676	16	77534851	0.023	0.958	0.016	5.31E-07	a	g	1430	-5.015	5.31E-07	?-	0.259
rs375077981	17	93102	0.020	0.379	0.041	9.86E-08	t	c	1430	-5.329	9.86E-08	?-	0.082
17:93710:A:ACT	17	93710	0.017	0.463	0.039	4.58E-08	a	act	1430	5.467	4.58E-08	?+	0.076
rs145520862	17	94227	0.018	0.434	0.040	4.58E-08	a	at	1430	-5.467	4.58E-08	?-	0.076
rs186729453	**17**	**94232**	**0.018**	**0.434**	**0.040**	**4.57E-08**	**t**	**g**	**1430**	**-5.467**	**4.57E-08**	**?-**	**0.076**
rs75336375	17	94595	0.017	0.530	0.040	4.86E-08	a	g	1430	-5.456	4.86E-08	?-	0.076
17:94869:C:T	17	94869	0.019	0.332	0.040	4.98E-08	t	c	1430	-5.452	4.99E-08	?-	0.076
17:94873:C:T	17	94873	0.018	0.425	0.040	8.37E-08	t	c	1430	-5.359	8.37E-08	?-	0.076
rs367726249	17	95191	0.018	0.384	0.040	5.17E-08	a	agtgt	1430	-5.445	5.17E-08	?-	0.076
rs76409107	17	97350	0.016	0.291	0.041	2.38E-07	t	c	1430	-5.167	2.38E-07	?-	0.136
rs11651220	17	98244	0.020	0.444	0.040	8.11E-08	c	g	1430	5.365	8.11E-08	?+	0.076
rs11651297	17	98409	0.015	0.271	0.040	8.15E-08	t	c	1430	-5.364	8.15E-08	?-	0.076
rs143909369	17	100194	0.036	0.172	0.040	6.61E-08	t	c	1430	-5.401	6.61E-08	?-	0.076

For externalizing characteristics two markers reached genome wide significance ($p < 5 \times 10^{-8}$). Figures 5 and 6 show the regional association plots for these two markers on chromosome 1 (rs71569819, $p = 1.6 \times 10^{-8}$, $q = 0.096$) and 7 (rs79401837, $p = 4.8 \times 10^{-8}$, $q = 0.203$). The marker on chromosome 1 is located in the gene endothelium converting enzyme 1 (*ECE1*) and was tested in the AFR and EUR samples. The marker on chromosome 7 is located in an intergenic region and was tested only in EUR sample.

Figure 5: Regional Association Plot for rs71569819

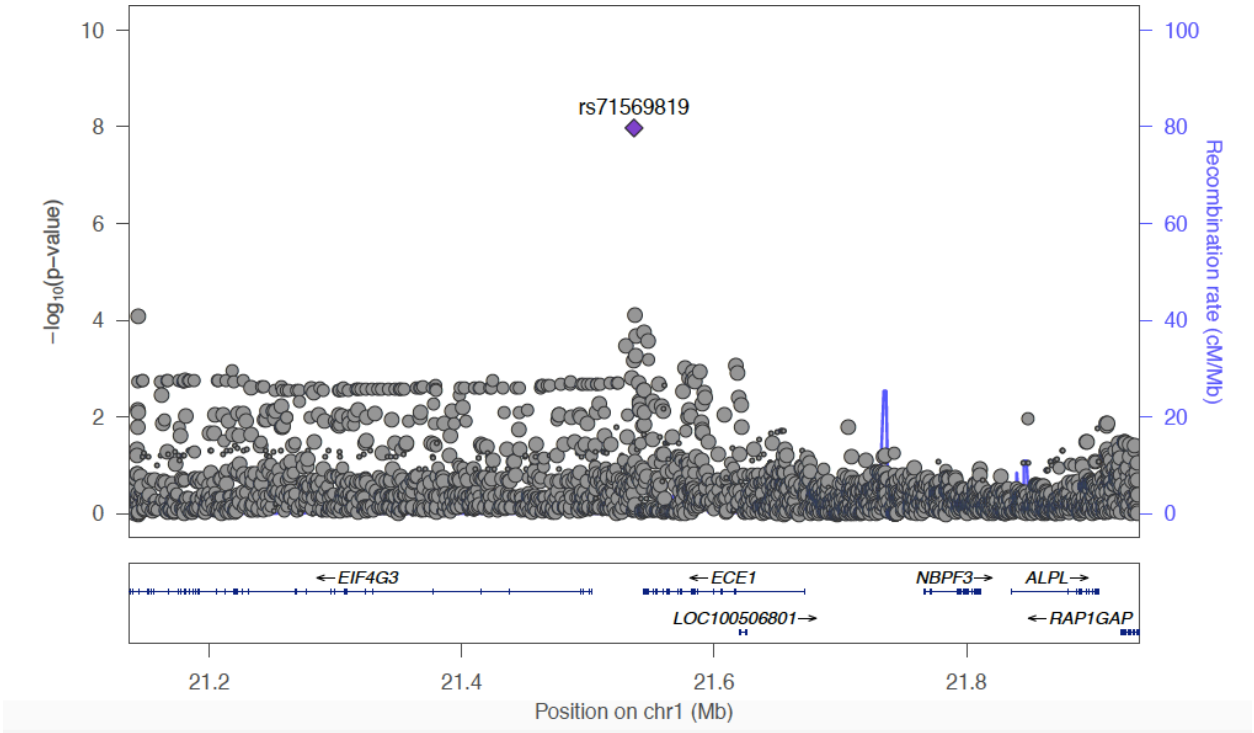
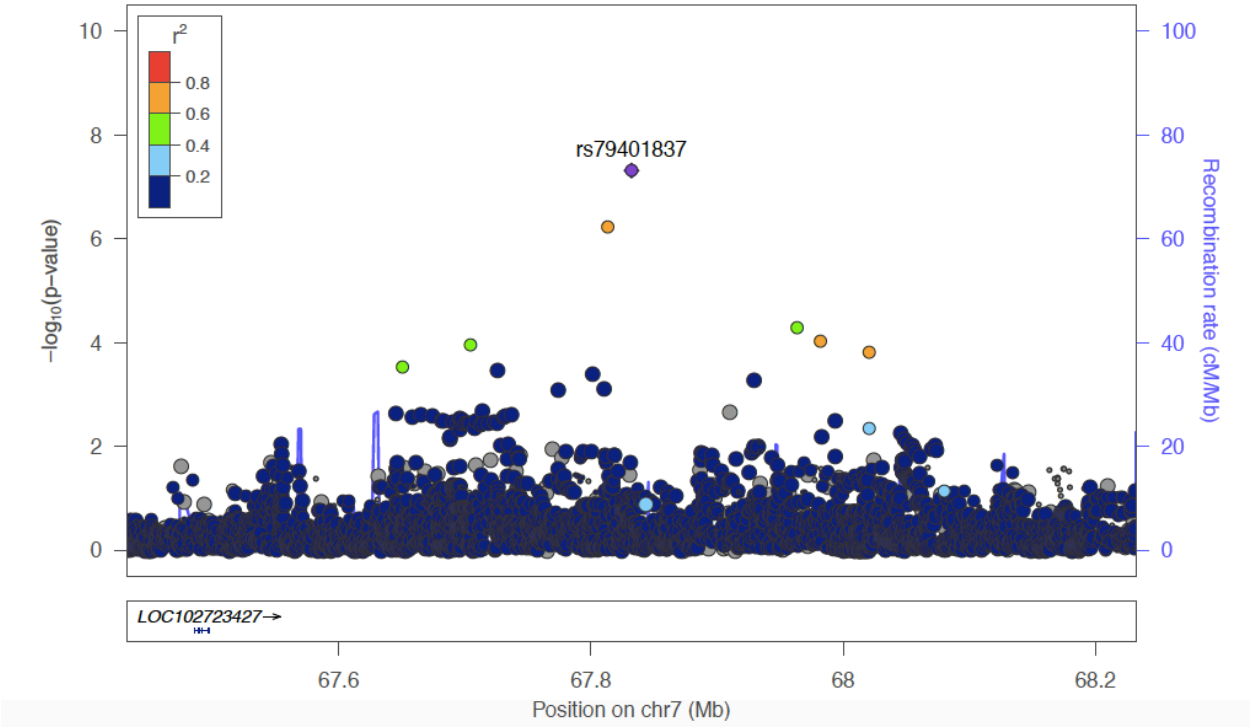
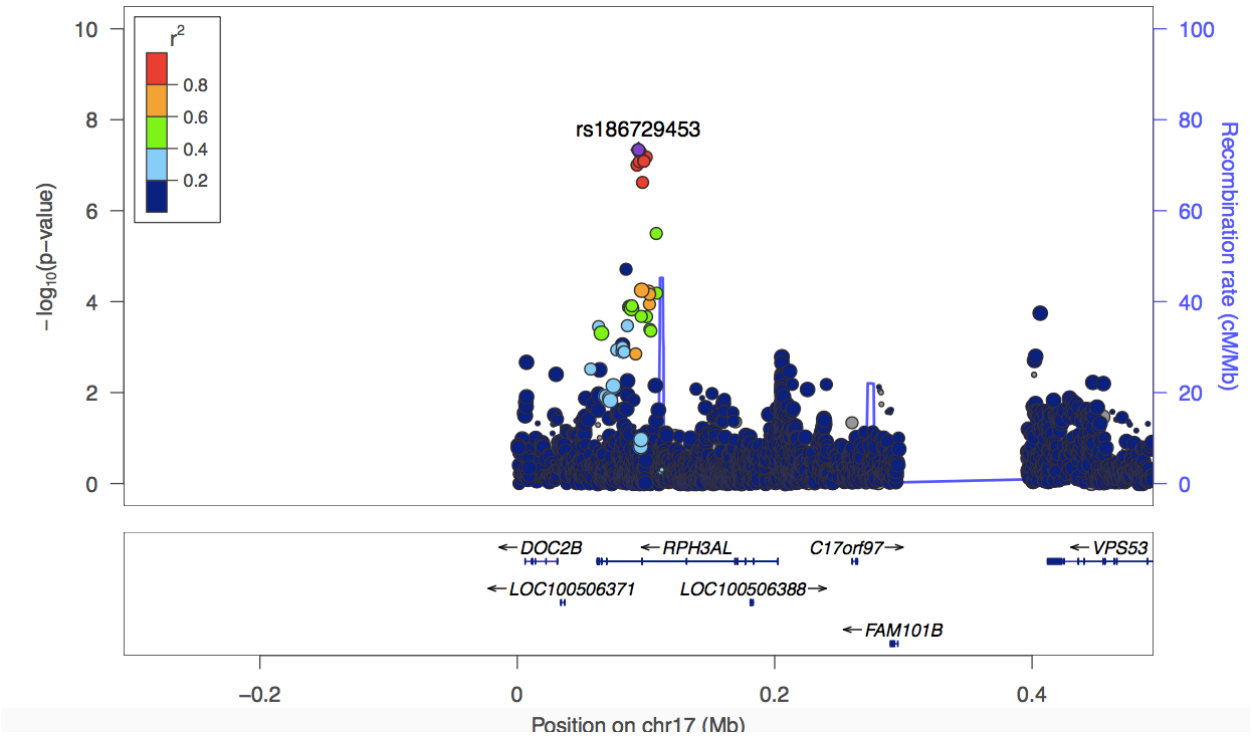


Figure 6: Regional Association Plot for rs79401837



For internalizing characteristics five markers reached genome wide significance (p = $4.57x10^{-8}$ – $4.99x10^{-8}$). All five of these markers are located on chromosome 17 in the gene rabphilin 3A-like (RPH3AL) and were only tested in the EUR ancestry group. Figure 7 shows the regional association plot for these markers.

Figure 7: Regional Association Plot for significant markers in RPH3AL



FUMA

Through FUMA a genome-wide gene set analysis was done using MAGMA (de Leeuw, Mooij, Heskes, & Posthuma, 2015) on the results from the externalizing and internalizing GWASs. These tests resulted in no significant gene sets at a corrected *p*-value threshold of $p < 2.72 \times 10^{-6}$. To further inspect the genome wide significant and suggestively significant markers a set of "lead" markers from each GWAS was also entered into the FUMA program. The rows in bold in Table 7 show the markers from the internalizing GWAS that were entered as lead SNPs in a FUMA analysis to explore the function. The three markers in Table 6 as well as rs10165682 (chr 2, $p = 1.42 \times 10^{-6}$), rs4643870 (chr 4, $p = 1.06 \times 10^{-6}$), rs35078547 (chr 14, $p = 1.55 \times 10^{-6}$) from the externalizing GWAS were used as lead SNPs in a FUMA analysis to explore their function.

There were 182 SNPs from the externalizing GWA analyses that were in LD ($r^2 > 0.5$) with the pre-selected lead SNPs. None of these SNPs mapped to genes and most were in intergenic

regions. The exception was rs35078547, the lead SNP on chromosome 14, which of the 14 SNPs in LD with the marker 4 were located downstream of LINC00226 and 5 were located upstream of LINC00221.

There were 97 SNPs from the internalizing GWA analyses that were in LD (r^2>0.5) with the pre-selected lead SNPs. The 14 SNPs in LD with rs186729453 were all located in an intron of the RPH3AL gene. On chromosome 2, 6 SNPs were located in an intron of the RMDN2 gene and 40 SNPs located on in an intron of a ncRNA – RMDN2-AS1. Additionally, 5 SNPs on chromosome 6 are located in an intron of a ncRNA and 3 SNPs located upstream of or in an intron of a ncRNA.

Discussion

This chapter applied a variety of analytic methods to examine the genetic influences on externalizing and internalizing characteristics within binge drinkers. We estimated the overall heritability due to common variants, performed agnostic tests to discover associated individual variants, and followed up on the function of significant and suggestively significant markers. The results reveal some promising avenues for follow up in future analyses, but suggest that phenotypic refinement alone cannot overcome the limitations of modest sample sizes. The primary findings are discussed below.

For SNP-based heritability, the estimate for externalizing characteristics was not significantly different from zero. This is surprising due to the well-established significant heritability of alcohol use and externalizing characteristics separately as well as the known shared genetic influences between externalizing and alcohol. There are several plausible explanations

why in our sample externalizing characteristics within binge drinkers did not show significant SNP heritability. First, it is possible that since the sample was reduced to a heavier drinking sample (reducing the phenotypic variance regarding alcohol use) and externalizing behaviors have been shown to have minimal genetic influences that are not shared with problem substance use (Kendler et al., 2003). There may not be minimal genetic variance within this sample that relates to externalizing characteristics only. Relatedly, we are underpowered in this sample to detect such potentially small effects. Table 8 shows the power calculations for SNP-based heritability given a range of samples sizes including those in the present analyses. As seen in the table, using the full sample we are well powered to detect heritabilities of 0.4 or greater, with only the EUR ancestry group being well powered to detect any heritability estimates before meta-analysis. This means if there was a reduction in heritability of externalizing characteristics in the binge drinking sample due to a lack of variation in alcohol use we would be unable to detect it.

Table 8: Power calculations for SNP-based heritability estimates

	SNP –based Heritability							
	N	0.05	0.1	0.2	0.4	0.5	0.6	0.8
Sample Size	150	0.0501	0.0503	0.051	0.0541	0.0565	0.0593	0.0667
	250	0.0502	0.0507	0.0529	0.0615	0.0681	0.0761	0.0969
	500	0.0507	0.0529	0.0615	0.0969	0.1241	0.1578	0.2441
	1000	0.0529	0.0615	0.0969	0.2441	0.3526	0.4751	0.7156
	1430	0.0559	0.0737	0.1477	0.44	0.6183	0.7744	0.9513
	1879	0.0602	0.0913	0.221	0.6616	0.844	0.9458	0.9974
	2454	0.0674	0.1213	0.3419	0.8737	0.9726	0.9965	1.0000

Note: Calculated using GCTA-GREML Power Calculator (http://cnsgenomics.com/shiny/gctaPower/)

With regard to internalizing characteristics in binge drinkers, we observed a modest SNP heritability estimate ($h^2 = 0.25$). Although the standard errors on this estimate are large, the estimate is in line with other estimates of SNP heritability of major depressive disorder (Lubke et al., 2012). This may indicate that unlike externalizing characteristics, internalizing characteristics are no more or less heritable in a binge drinking sample as compared to the general population.

Although it is important to note that even in the meta-analyzed sample we are underpowered to detect SNP heritability estimates below 0.4.

There were two genome-wide significant (GWS) markers for externalizing characteristics. The first (rs71569819) was located on chromosome 1 in the endothelin converting enzyme 1 (*ECE1*) gene. SNPs in this gene (rs212524, rs213032) have been previously identified in a meta-analysis of height (Wood et al., 2014) and associated with sleep duration in a small study of childhood obesity in Hispanic children (Comuzzie et al., 2012). There has been some previous evidence suggesting a relationship between sleep duration and impulsivity, especially in children (Gruber, Cassoff, Frenette, Wiebe, & Carrier, 2012; Scharf, Demmer, Silver, & Stein, 2013). One study estimated 81% of the relationship between sleep quality and externalizing behavior is due to genetic influences (Barclay, Eley, Maughan, Rowe, & Gregory, 2011). This suggests a potential relationship by which markers in the *ECE1* gene are associated with externalizing characteristics in this sample. The second GWS marker (rs79401837) is located in an intergenic region on chromosome 7 with no previous associations.

The five GWS markers for internalizing characteristics were located in an intron of the rabphilin 3A-like (*RPH3AL*) gene. An additional 9 suggestively significant makers in high LD with were also located in this intron of *RPH3AL*. In a study of women with borderline personality disorder or major depressive disorder, Prados et al. (2015) found an association with methylation sites within the *RPH3AL* gene and childhood maltreatment scores, such that these sites were associated with lower childhood maltreatment in depressed individuals (a majority of the borderline personality disorder patients were also diagnosed with major depressive disorder). While this study supports the potential role of *RPH3AL* in relation to internalizing characteristics, it is not a direct replication of our findings.

Therefore, we conducted a series of follow-up analyses in an attempt to replicate the association of variants within the *RPH3AL* gene and internalizing in binge drinkers. We had access to three samples containing participants measures of drinking and internalizing behavior to at similar ages to the S4S sample; the Avon Longitudinal Study of Parents and Children (ALSPAC), the Finnish Twin Cohort Study (FinnTwin), and the prospective subsample of the Collaborative Studies on the Genetics of Alcoholism (COGA). In each sample, we tested the association between the top markers on chromosome 17 (listed in Table 7) and a measure of internalizing characteristics among past month heavy drinkers. As shown in Table 9, none of the variants that were GWS or borderline significant in the S4S discovery sample were significant in any of the three replication samples. Since the associated variants in a GWAS are often not the causal variant but in LD with the causal variant, we also conducted a test of the whole *RPH3AL* gene using MAGMA (de Leeuw et al., 2015). As shown in Table 10, *RPH3AL* was not significantly associated with internalizing behavior in any of the three heavy drinking replication samples. While the results of both the individual variant tests and gene tests suggest the initial results be interpreted cautiously, some limitations to the replication analyses are also applicable. First, the minor allele frequencies (MAF) of these variants are between 0.023 - 0.026 in COGA and FinnTwin and 0.042-0.044 in ALSPAC. These MAFs are similar to S4S but combined with particularly small sample sizes of COGA (N=534) and FinnTwin (N=321) means there is likely very little variation in these samples across these markers. Additionally, data from ALSPAC was only available for age 18 which is just the beginning of the young adult period that is captured by the S4S sample. Therefore, the influence of variation in the *RPH3AL* gene on internalizing characteristics in heavy drinkers still warrants further exploration.

Table 9: Attempted replication of individual variants in *RPH3AL*

	COGA		FinnTwin		ALSPAC	
	Beta	P-Value	Beta	P-value	Beta	P-value
rs375077981	0.1891	0.5756	0.1667	0.7406	N/A	N/A
rs145520862	0.1871	0.5799	0.0758	0.8824	-0.0341	0.9459
rs186729453	0.1871	0.5799	0.0758	0.8824	0.0000	1.0000
rs75336375	0.1871	0.5799	0.0758	0.8824	0.0111	0.9825
rs76409107	0.1871	0.5799	0.0758	0.8824	0.1621	0.7482
rs11651220	0.1459	0.6556	-0.0621	0.9053	0.0652	0.8979
rs11651297	0.1459	0.6556	0.1148	0.8227	0.0637	0.9003
rs143909369	0.1193	0.7066	0.0592	0.9096	0.8560	0.8698

Table 10: Results of *RPH3AL* gene-based association test

	SNPs	N	Z statistic	P-value
COGA	168	321	-0.6907	0.7551
FinnTwin	55	534	-0.8692	0.8076
ALSPAC	202	1665	1.0681	0.1427

Most of the variants implicated in the current analyses (including those in *RPH3AL*) do not have a clear mechanism of action by which they might affect the phenotype. This is a common finding when examining the genetics of complex psychiatric traits or disorders. Previous research has shown that variants associated in complex psychiatric traits are often found in non-coding regions (Finucane et al., 2015; Peterson et al., 2017). These variants may play a role in regulating gene expression which future studies could explore but is currently outside the scope of the present study.

The results of the current study should be considered in the context of several limitations. First, as discussed above, the sample size was small for these types of genetic analyses. We hypothesized that examining externalizing and internalizing characteristics in the context of heavy drinking individuals would eliminate some of the "noise" created by heterogeneity not previously accounted for in other studies and thus increase power; however, it is clear that a sample size

greater than the current sample (N=2,454) is needed to confidently explore these subtypes. Second, our measure of internalizing or externalizing encompassed a range of those behaviors (depression and anxiety, impulsivity and antisocial behavior). This allows for internalizing or externalizing to manifest in multiple ways and still be a considered in relation to problem alcohol use. However, this assumes that each of these sub facets of internalizing or externalizing share broader genetic influences. While there is some evidence for this (Kendler, Aggen, et al., 2011; Kendler et al., 2003), it is possible that a focus on more specific facets of internalizing and externalizing better characterize genetic subtypes of problem alcohol use.

Despite these limitation, there are many potential future directions for this work. First, these same questions should be explored in a larger sample to confirm the SNP heritability estimates and potentially replicate the GWA findings. Similarly, larger samples that are not limited to individuals of European ancestry will be needed to understand whether current findings generalize across all individuals or are specific to those of African and/or European descent. Additionally, conducting similar analyses in a sample with diagnostic level measures of both internalizing and externalizing disorders may yield more convincing results. It may be that those most severely affected by internalizing or externalizing disorders represent those with the highest genetic predisposition towards either trait and alcohol problems. These most severely affected individuals may be unlikely to have enrolled in the S4S project and continued through university. In conclusion, the analyses presented here represent an initial exploration into differential genetic influences on subtypes of problem alcohol users, a topic that is currently understudied.

Chapter 4: Underlying Neurobiological Differences between Externalizing and Internalizing Binge Drinkers

Neurobiology of Addiction

In addition to genetic influences on alcohol use, there are many intermediate factors that play a role in the development of alcohol use. Extensive research has been conducted examining differences in neurobiological differences that set those addicted to alcohol apart from others. Several theories have been put forward to help understand the underlying neurobiology among individuals who transition from healthy alcohol use to alcohol misuse. Some theories suggest this transition is the result of a change from using alcohol for the positive, rewarding effects to using a substance to eliminate the negative symptoms or withdrawal (Koob et al., 2004; Koob & Le Moal, 2008; Solomon & Corbit, 1978). Others argue that problem use is caused by a loss of control as brain regions involved in decision making become less engaged, shifting use from voluntary to compulsive (Everitt & Robbins, 2005). Still others suggest that over time cues associated with use (such as a drinking location, a certain time of day, a wine glass) become reinforcing themselves and cause craving, thus leading to compulsive use (Robinson & Berridge, 1993). While these theories differ with regard to the specific mechanism driving the shift from use to dependence, they all point to neurobiological changes as essential in the progression from use to dependence.

The majority of these theories propose changes with regard to neurological systems that govern reward motivation and behavioral control. In healthy controls, the ventral striatum (VS) and ventromedial prefrontal cortex (vmPFC; Bartra, McGuire, & Kable, 2013; Haber & Knutson,

2010) are involved in reward motivation. More specifically these brain regions work together in healthy individuals to determine the value (reward) of a stimulus/choice. The VS has also been associated with a preference for immediate rewards and therefore reward seeking/sensitivity more generally (McClure, Laibson, Loewenstein, & Cohen, 2004). With regard to behavioral control, the dorsolateral prefrontal cortex (DLPFC) and right inferior frontal gyrus (RIFG) show activation in healthy controls on tasks involving impulsive choice and impulsive response, respectively (Kable & Glimcher, 2007; Simmonds, Pekar, & Mostofsky, 2008). Research using functional magnetic resonance imaging (fMRI) has demonstrated that these systems are dysregulated in individuals with problem alcohol use. These findings have given support to the theories outlined above and further detailed below.

Incentive Salience. Robinson and Berridge (1993) proposed a theory of incentive sensitization (also called incentive salience) to explain the transition from normative substance use to problem substance use or a transition from "liking" a substance to "wanting" a substance. This theory posits that over repeated use individuals come to find stimuli that have been repeatedly paired with use of a drug to be rewarding in and of themselves. As an example, Due, Huettel, Hall, and Rubin (2002) found increased activation in reward circuitry in response to viewing smoking cues (pictures of cigarettes, people smoking and holding cigarettes) in smokers compared to nonsmokers. Similarly, Kareken et al. (2004) showed increased activation in the nucleus accumbens when high risk drinkers inhaled alcohol vapors compared to low risk drinkers demonstrating an increased salience to alcohol cues. A meta-analysis of cue reactivity in alcohol dependent individuals showed reliable activation in bilateral ventral striatum (VS), left pallidum, right amygdala, left thalmus, right inferior frontal gyrus, and left middle frontal gyrus (Kuhn & Gallinat, 2011). This meta- analysis found further support for activation in the VS and amygdala

in response to cues in nicotine and cocaine dependent individuals. However, Wilson, Sayette, and Fiez (2004) argue that especially in non-treatment seeking dependent individuals, the dorsolateral prefrontal cortex (DLPFC) and orbitofrontal cortex (OFC) are additionally important brain areas involved in cue-reactivity. It is thought that since these areas are involved in goal-directed behavior and decision making, when shown a cue non treatment seeking individuals are recruiting brain areas involved in deciding to use.

Hedonic Allostasis. Solomon and Corbit (1974) proposed an Opponent Process model theorizing that hedonic states were automatically modulated by the central nervous system to reduce their intensity. The Opponent Process theory posits that there is a primary process associated with the pleasurable effects of drug use and an opponent process associated with the negative effects of drug use or withdrawal. Over repeated drug use the opponent process grows larger and individuals feel less of the pleasurable effects and more of the withdrawal effects. Similarly, Koob et al. (2004) proposed a theory of Hedonic Allostatis which suggests that continued drug use is a type of negative reinforcement where individuals use the drug to remove the negative effects/withdrawal symptoms of a drug. Unlike the Opponent Process Theory, the Hedonic Allostatis Theory posits that a driving force of addiction is due to a change in the individual's baseline state or homeostatic point. Therefore, eventually an individual no longer finds smaller healthy rewards to release enough dopamine to be rewarding and instead needs the larger dopamine release from drug use in order to return to baseline. This altered set point has been consistently demonstrated in the animal literature (Schulteis & Liu, 2006; Schulteis, Markou, Cole, & Koob, 1995). Perhaps the most convincing evidence for an altered base line state due to substance use is the work by Volkow et al. (2007) using PET scanning, which showed that chronic alcoholics felt less high and enjoyed the drug less than controls despite releasing similar levels of

dopamine in the VS. There have been similar findings using fMRI, Gilman, Ramchandani, Crouss, and Hommer (2012) demonstrated that intoxicated heavy drinkers show blunted activation in the nucleus accumbens while intoxicated compared to light drinkers.

Compulsivity. Everitt and Robbins (2005) suggest that the shift from voluntary to compulsive substance use is mirrored by a similar shift in activation in related brain regions. More specifically, a shift from engagement of the prefrontal cortex, focused on decision making, to the striatum, driven by reward. This shift is supported by decreased brain volume in the frontal lobes in chronic alcoholics (Pfefferbaum, Sullivan, Mathalon, & Lim, 1997). With the decision making ability of the prefrontal cortex diminished, compulsive use is then driven by the striatum, specifically the dorsal striatum (Doherty et al., 2004).

It is important to note that the three theories described above are not mutually exclusive. Within one individual each theory may explain a different stage on their path to addiction. For example, early on a person may find it is harder to stop themselves from having another drink, indicating a shift from prefrontal control to striatal control. This continued drinking leads to tolerance and a change in the person's homeostatic point. Therefore, they continue to drink to reduce negative affect instead of to experience the pleasant effects of alcohol. This further reinforces alcohol related cues causing craving when the person abstains from using. This cycle of addiction has been described by Koob and Volkow (2010), who classify these three stages as; binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation. The subsequent analyses focus on three neurobiological constructs that index these three stages; behavioral inhibition, emotion reactivity, and reward sensitivity. Besides these theories, much additional work has examined each construct individually.

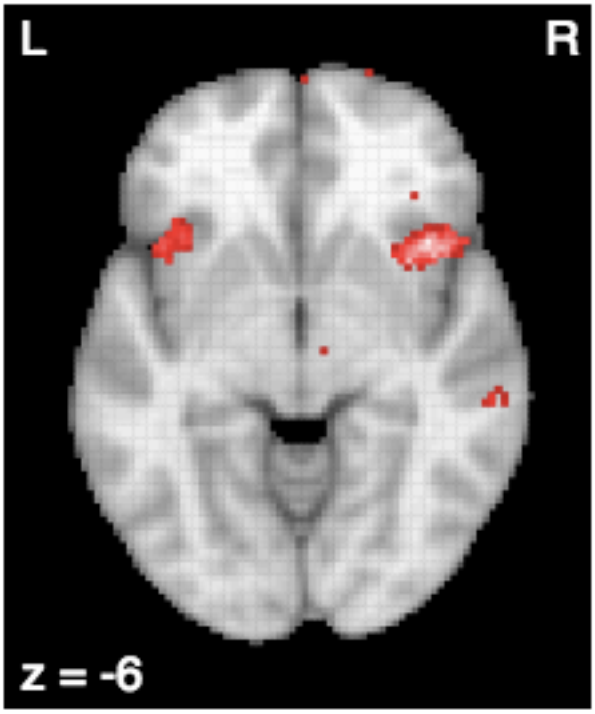
Behavioral Inhibition

Whereas the previous theories center on altered motivation toward drugs relative to natural rewards, atop both frameworks is the critical role of self-control neurocircuitry to restrain drug-seeking behavior. The ability to control or inhibit one's actions is a key part of human behavior, necessary for adapting to new environments/stimuli. Lack of behavioral inhibition or a difficulty controlling one's actions is a hallmark of alcohol abuse and binge substance use, for example the inability to stop consuming alcohol once an individual has started. The phenotypic and personality manifestations of this kind of impulsivity have been reviewed in Chapters 1 and 2. Therefore, in this chapter we focus on the behavioral measurements of impulsivity. The two main forms of behavioral impulsivity are choice impulsivity and rapid response impulsivity. Choice impulsivity encompasses both the preference for immediate rewards, even if they are smaller, over delayed rewards, even if they are larger and the diminished ability to tolerate delays (Hamilton, Mitchell, et al., 2015). Not surprisingly, this type of impulsivity is related to neural activity in brain areas responsible for both goal directed behavior and reward sensitivity (Kable & Glimcher, 2007) with areas in the prefrontal cortex (i.e. DLPFC) associated with choosing to defer gratification and the VS associated with choosing immediate rewards (McClure et al., 2004).

The other form of impulsivity, which is relevant to the present set of analyses, is rapid response impulsivity. Rapid response impulsivity is a diminished ability to inhibit a primed response (Hamilton, Littlefield, et al., 2015). Behavioral inhibition (or response inhibition) is most often measured using behavioral tasks; one of the most common being Go/No-go tasks which require the participant to suddenly inhibit a response when a specific stimulus is presented. This task has been shown to activate the prefrontal cortex, specifically the right inferior frontal gyrus (see Figure 8)(Simmonds et al., 2008). Group differences in brain activation in response to the

Go/No-go task have been established in heavy drinkers compared to light drinkers (Ahmadi et al., 2013; Ames et al., 2014; Campanella et al., 2016). With some studies demonstrating increased activation in heavy drinkers (Ames et al., 2014) which is often interpreted as this group needing to expend more energy to complete the task. While other studies show decreased activation in heavy drinkers (Ahmadi et al., 2013) thought to represent a deficit in cognitive resources. These differences are likely a factor in driving substance abuse rather than being a result of heavy use, as differences are seen prior to the initiation of heavy drinking (Norman et al., 2011; Wetherill, Squeglia, Yang, & Tapert, 2013) and in individuals with a family history of (i.e., predisposition toward) alcoholism (Schweinsburg et al., 2004).

Figure 8: Brain activation in the right inferior frontal gyrus corresponding to "response inhibition"



Note: The graphic is from a meta-analysis of studies generated from neurosynth.org

Rapid response impulsivity (specifically using Go/Nogo tasks) have been associated with long term substance use outcomes. Although the direction of effect has not been consistent. Berkman, Falk, and Lieberman (2011) found that increased activation in the right inferior frontal gyrus, supplementary motor area, and basal ganglia attenuated the otherwise positive relationship between cravings and subsequent smoking in smokers attempting to quit. Conversely, Prisciandaro, Myrick, Henderson, McRae-Clark, and Brady (2013) found that increased activation in the left postcentral gyrus was associated with a positive cocaine urine drug screen in cocaine dependent individuals at a one week follow up visit.

Behavioral inhibition has also been studied as a function of externalizing and internalizing characteristics. Similar to the findings in alcohol abusers, deficits in behavioral inhibition have been seen in a range of externalizing disorders (Albrecht, Banaschewski, Brandeis, Heinrich, & Rothenberger, 2005). Impulsivity more broadly has also been found to correlate with activation in a Go/No-go task (Asahi, Okamoto, Okada, Yamawaki, & Yokota, 2004; Horn, Dolan, Elliott, Deakin, & Woodruff, 2003) but with mixed results. Asahi et al. (2004) showed a negative correlation between activation in the right dorsolateral prefrontal cortex (DLPFC) and motor impulsivity while Horn et al. (2003) showed a positive correlation between activation in the right inferior frontal gyrus and Eysenck's impulsivity scale.

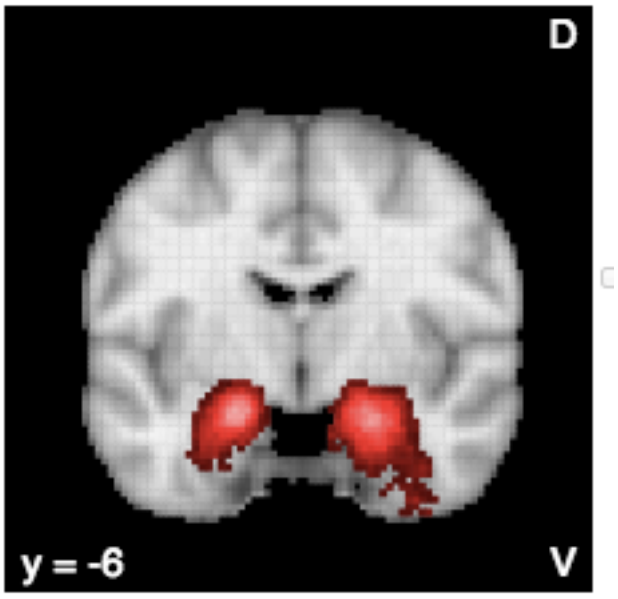
There have been few studies of response inhibition in individuals with internalizing disorders. But there is some evidence that individuals with internalizing characteristics are better able to maintain cognitive control (Sehlmeyer et al., 2010). In one study, those with comorbid externalizing and internalizing disorders performed better on a behavioral inhibition task than those individuals with externalizing disorders alone (Lipszyc & Schachar, 2010). However, this protective effect may not hold true for alcohol use disorders. In a study of in comorbid alcohol

dependence and anxiety/depression, Sjoerds, van den Brink, Beekman, Penninx, and Veltman (2014) found that alcohol dependence severity was more associated with response inhibition than depression or anxiety symptoms.

Emotion Reactivity

The ability to process and regulate one's emotions is important for navigating interpersonal interactions. Dysregulation of this system is a hallmark of some psychiatric disorders, especially internalizing disorders (Leppänen, 2006; Rauch, Shin, & Wright, 2003). In neuroimaging studies, activation in the amygdala is often measured in tasks where participants view faces expressing a variety of emotions (See figure 9). In general, greater amygdala activation is seen in response to emotional stimuli, like faces, with faces expressing negative emotion eliciting the greatest activation. This is especially true for individuals with depression who consistently show increased activation to sad faces compared to happy faces (Fu et al., 2004; Surguladze et al., 2005). Similarly, increased amygdala activation to faces has been seen in anxiety prone individuals (Etkin & Wager, 2007; Stein, Simmons, Feinstein, & Paulus, 2007). Additionally, individuals with anxiety or depression show increased amygdala activation to neutral faces compared to healthy controls (Filkowski & Haas, 2017) indicating a potentially increased sensitivity to hostility and/or threat.

Figure 9: Brain activation in the bilateral amygdala corresponding to “faces”



Note: The graphic is from a meta-analysis of studies generated from neurosynth.org

Fewer studies have examined the relationship between externalizing characteristics and amygdala activation in response to emotional faces. There is some evidence for reduced amygdala activation to fearful faces in individuals with antisocial behavior (Hyde, Shaw, et al., 2016; Jones et al., 2009), although this association may be driven by the psychopathic and callous-unemotional traits often associated with antisocial behavior (White et al., 2012).

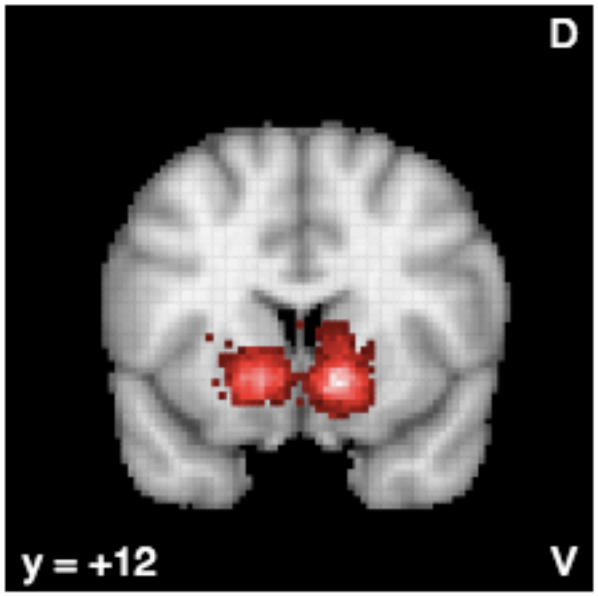
The relationship between alcohol and negative emotion is complicated and dynamic. At first alcohol can have an anxiolytic effect and some people may drink to cope with anxiety or depression symptoms. However, with prolonged use alcohol can have an anxiogenic effect leading to increased incidence of internalizing disorders. Gilman et al. (2008) demonstrated this anxiolytic effect in the brain by showing a decrease in amygdala activity to fearful faces when individuals were intoxicated compared to when they were sober. In addition to seeing this blunted response as an acute effect of alcohol, alcohol dependent individuals show blunted amygdala activation

across all types of emotional faces compared to controls (Marinkovic et al., 2009). While this blunted response may be the result of chronic alcohol consumption, there is also evidence that those with a family history of alcohol dependence show decreased activation in the amygdala in response to faces (Glahn, Lovallo, & Fox, 2007) demonstrating that this difference may be a risk factor for substance abuse and not an effect of use.

Reward Sensitivity

An increased sensitivity to reward is a hallmark of substance abuse, including alcohol abuse. The Monetary Incentive Delay task (MID) is designed to measure response to reward and punishment indicated by activation in the nucleus accumbens (NAcc, see figure 10 (Knutson, Adams, Fong, & Hommer, 2001). The MID task is able to separate neural activity associated with anticipation of receiving a reward/punishment and actually receiving the reward/punishment. Substance dependent individuals, specifically alcohol dependent individuals, have shown increased activation in the NAcc in response to the prospect of receiving a reward (Bjork, Smith, & Hommer, 2008), indicating an increased sensitivity to reward. But other studies in alcohol dependent individuals have showed decreased activation in the VS (Beck et al., 2009; Wrase et al., 2007). However, this activation in this region may differ across substances (Karoly et al., 2015). When examining individuals with a family history of alcoholism some studies have found no difference (Bjork, Knutson, & Hommer, 2008; Muller et al., 2015), while others have found decreased NAcc activation compared to those without a family history (Andrews et al., 2011; Yau et al., 2012).

Figure 10: Brain activation in the bilateral amygdala corresponding to "reward anticipation"



Note: The graphic is from a meta-analysis of studies generated from neurosynth.org

In several of these studies a strong relationship was seen between reward sensitivity/ NAcc activation and measures of impulsivity (Beck et al., 2009; Bjork, Knutson, et al., 2008). Additional studies have directly measured reward sensitivity in the presence of externalizing characteristics solely, such as risk taking adolescents and impulsive-antisocial traits (Bjork, Smith, Chen, & Hommer, 2010; Buckholtz et al., 2010), indicating that the increased reward sensitivity seen in alcohol abusing individuals is the result of a broader predisposition to externalizing. Weiland et al. (2013) tested this theory and found that NAcc activation to reward sensitivity mediated the relationship between sensation seeking and alcohol use.

Far less research has been conducted to examine the role of reward sensitivity in individuals with internalizing characteristics or disorders. However, a few studies have found decreased activation in the NAcc in response to reward among individuals with depression or depressive symptoms (Admon et al., 2017; Hägele et al., 2014). The decreased activation to

reward may be more specifically related to the anhedonia experienced by individuals with depression (Wacker, Dillon, & Pizzagalli, 2009).

Some of these three constructs (behavioral inhibition, emotion reactivity, and reward sensitivity) have been studied together. Specifically, Nikolova and Hariri (2012) examined emotion reactivity and reward sensitivity in college students and were able to predict stress-related drinking at a later time point. For those who were low in reward sensitivity (low VS reactivity), recent life stress has no effect on their level of drinking. However, for individuals who were highly reward sensitive (high VS reactivity) and had low levels of amygdala reactivity (less reactive to emotions) recent stress had a significant positive association with their alcohol use. Therefore, demonstrating a unique neural profile that could predict stress – related drinking.

Stemming from the work of Nikolova and Hariri (2012), the current study seeks to integrate previous research and further clarify the relationship among neurobiological processes, alcohol misuse, and psychopathology by testing for potential brain activation differences between internalizing and externalizing binge drinkers. We focused on the three constructs reviewed above (behavioral inhibition, emotion reactivity, and reward sensitivity) since they are known to be associated with problem alcohol use. With regard to behavioral inhibition, we hypothesize that there will be increased activation in the right inferior frontal gyrus (RIFG) in externalizers compared to internalizers, due to internalizers having increase behavioral control. With regard to emotion reactivity, we hypothesize there will be increased activation in the amygdala to faces and negative emotions in internalizers compared to externalizers, due to internalizers heightened sensitivity to negative emotion. Finally, with regard to reward sensitivity, we hypothesize there will be increased activation in the nucleus accumbens in response to reward in the externalizers compared to interalizers, due to their higher levels of impulsivity.

Table 11: Primary Analyses and Hypotheses

Cognitive Construct	fMRI Task	Primary Analysis	Primary Region of Interest	Hypothesis
Reward Sensitivity	Monetary Incentive Delay Task (MID)	Anticipation during high +low reward trials vs neutral trials	Bilateral Nucleus Accumbens	Extern > Intern
Behavioral Inhibition	XY Go – Nogo Task	Lures (XX or YY)	Right Inferior Frontal Gyrus	Intern > Extern
Emotion Processing	Face Matching Task	Faces vs Shapes	Bilateral Amygdala	Intern > Extern

Methods

Subjects

Participants were recruited from the S4S binge drinking subsample. Individuals who had a factor score one standard deviation above the mean on the externalizing factor and one standard deviation below the mean on the internalizing factor were classified as Externalizers; those who had a factor score one standard deviation below the mean on the externalizing factor and one standard deviation above the mean on the internalizing factor were classified as Internalizers. 350 individuals met these criteria and were sent an email by the S4S project coordinator inviting them to participate in the current study. Individuals who were interested in participating (N=91) completed a brief screening questionnaire on the phone that asked about their physical health, mental health, and alcohol use. Participants who were healthy (measured by blood pressure, heart rate, body temperature), regular binge drinkers (drinking 4 or more drinks for women and 5 or more drinks for men at least once a month), and right-handed (N = 44) completed a screening visit. During the screening visit participants filled out a series of questionnaires, including the full

versions of the SCL-90, SSAGA antisocial behavior questions, and UPPS. They also were checked for MRI safety and completed a battery of computer tasks.

XY Go-NoGo (XYGNG) Task

Stimuli consisted of alternating "X"s and "Y"s. The participant was asked to press a button each time the "X" or "Y" was on the screen (Go trials). Sometimes the X or Y was preceded by the same letter (Nogo trial). In this case participants were instructed not to press the button and therefore inhibit their response to the stimuli (Garavan, Ross, & Stein, 1999; Kaufman, Ross, Stein, & Garavan, 2003). For example, in the pattern "X Y X Y X X Y X", the sixth letter would be a Nogo trial and the participant should not press the button. There were 250 trials: 225 Go trials and 25 Nogo trials. Each trial lasted 1 second. Participants completed this task once for a run time of 4 minutes and 45 seconds.

Emotional Face (EF) Assessment Task

In each trial, participants viewed a trio of faces in face blocks or a trio of shapes in shapes blocks, one on the top of the screen and two on the bottom (Phan et al., 2008). For each block, they were asked to select which stimulus on the bottom row matches the stimulus on the top row. Participants made a response by pressing either the left or right response button with their dominant hand. The identity of the three faces were different within a block and an equal number of male and female faces were presented. The target and matching face displayed sad, fearful, angry or happy emotions while the nonmatching face displayed a neutral emotion. Faces and shapes were presented in blocks of 4 trios of the same target shape or face. Each trio of faces or shapes is presented for 5s. There was a variable inter-stimulus interval for the faces of 2-6s and a fixed interval for the shapes of 4s. There were 24 trios presented in each run. Participants completed two runs of this task (8 min 22s each) for a total task run time of about 16 minutes.

Monetary Incentive Delay (MID) Task

In this task participants are first shown a cue which alerts them to the type of trial where they will either have the opportunity to win $5, win $0.50, lose $0.50, lose $5 or a neutral trial, to perform for no incentive (Knutson et al., 2001). Cues were displayed for ~500 ms. The participants then focused on a crosshair until a target symbol appears. They must push a button while the target symbol is on the screen (160-260 ms) in order to either win money or avoid losing money. Following the disappearance of the target, participants are given feedback on whether they lost or won money during the trial and cumulative winnings. Participants completed a practice round of this game outside the scanner to calibrate the difficulty of the task. The difficulty of task was set for each participant so that they would successfully hit the target on two thirds of the trials. Participants completed two, 8.5 minute runs of this task for a total of 100 trials, 20 trials of each cue type.

fMRI Acquisition

Imaging was performed using a 3T Philips Ingenia MRI scanner. The single run of the XYGNG task lasted 286s with a repetition time (TR) of 1500ms, an echo time (TE) of 30ms, a flip angle of 68°. The initial 12 volumes of the run were discarded. In each volume a 3.75 mm slice was collected. Each run of the EF task lasted 502s with a TR of 2000ms, a TE of 30 ms, and a flip angle of 68°. The initial 4 volumes of each run were discarded. In each volume a 3mm slice was collected. Each run of the MID task lasted 515s with a TR of 1580ms, a TE of 75ms, and a flip angle of 90°. The initial 13 volumes of each run were discarded. In each volume a 3.75mm slice was collected. Structural scans were collected using a T1 weighted sequence with a TR of 8.1ms, a TE of 3.7ms, and a flip angle of 6°.

fMRI Analysis

Preprocessing. Blood Oxygen-Level Dependent (BOLD) signal was analyzed using Analysis of Functional NeuroImages (AFNI) software (Cox, 1996). The two runs of the EF task and the MID task were concatenated and analyzed as a single time series for each task moving forward. Any time series with more than one voxel (3.75 mm) translocation across the time-course of the scan were excluded. Each functional time series was corrected for motion, spatially smoothed to 8mm full-width half maximum, aligned to the structural scan, and warped out to Talairach space.

Individual first-level statistical mapping. Processed time series were normalized to represent a percent signal change in each voxel at each timepoint, relative to that voxel's intensity mean. For each volume of the time series, at each voxel the intensity difference from the mean of the whole time-series was divided by the mean and multiplied by 100. The normalized time series were then analyzed by multiple regression (AFNI 3dREMLfit) which included six regressors describing residual motion used to correct for head motion and the temporal auto-correlation of voxel-wise noise. Activations were detected primarily as linear contrasts of event related signal change, as follows: 1) for the XYGNG task, a) activation during lures (Nogo trials) compared to the implicit background of successful go trial, b) activation to successfully stopping a response on Nogo trials compared to failing to stop the response; 2) for the EF task, a) all faces trials compared to shape trials, b) all sad face trials compared to happy faces trials; 3) for the MID task, a) reward cues (high and low combined) compared to neutral cues, b) loss cues (high and low combined) compared to neutral cues, c) reward compared to nonreward in reward trials, d) loss compared to nonloss in loss trials.

Groupwise statistical mapping. To accommodate the within and cross subject variability within group and group difference maps were calculated using AFNI's 3dMEMA program (Chen, Saad, Nath, Beauchamp, & Cox, 2012). A mask was generated that was composed of voxels with non-zero values from at least 24 subjects (20 subjects for XYGNG). This mask was used in the group map analyses to restrict the number of voxels analyzed to those consistently activated throughout the sample. A beta value, *t*-statistic, and *p*-value are calculated for each analyzed voxel. Due to the normalization of the data, described above, beta values represent the percent signal change. All results are presented at minimum voxel-wise significance threshold of $p<0.001$.

Family-wise error (FWE) correction. Due to large number of voxels that are collected during a whole brain scan, corrections for multiple testing are necessary in order to evaluate true signals. Whole brain FWE corrections were conducted on the second-level group maps. The first step is to calculate the full-width at half maximum along with the autocorrelation function for each sub-brik. Using this input, Monte Carlo simulations were then run in AFNI 3dClustSim to determine a cluster size threshold for each contrast of interest using only voxels where $p<0.001$. This threshold has been demonstrated to have a family-wise error rate of about 8% in a validation study of resting state fMRI data (Eklund, Nichols, & Knutsson, 2016). Finally, group maps were constructed with only those clusters that survived both the cluster size threshold (see results below for contrast-specific minimum cluster sizes) and a t-statistic corresponding to $p<0.001$.

Volume of Interest (VOI) Analysis. Since we hypothesized changes in specific brain regions selected *a priori*, we conducted a set of VOI analyses to test if 1) activation in these areas statistically differed between the two groups and/or 2) if activation in these regions correlated with externalizing or internalizing characteristics. As described above, the activation was normalized so that the beta weights represent the percent signal change averaged over all the time-series. To

avoid circularity and potentially exaggerated brain-behavior correlations (Vul, Harris, Winkielman, & Pashler, 2009), regions of interest were not determined from activation in the group maps but rather from predetermined anatomical locations. Task-contrast beta weights were averaged across three-dimensional masks using AFNI 3dMaskAve. Each mask was a three-dimensional cluster, composed of the central voxel surrounded by all six shared-face voxels (in 3.75 mm isotropic acquisition space). For XYGNG, the regions of interest were based on coordinates from neurosynth.org for "response inhibition", the mask coordinates in Talairach space are as follows: left inferior frontal gyrus (IFG) – 34, -18, -8 and right IFG - -42, -18, -6For MID, the nucleus accumbens locations are based on (Bjork et al., 2010), the coordinates in Talairach are as follows: left nucleus accumbens (NAcc) – 8, -11, 0 and right Nacc - -8, -11, 0. . For EF, the masks for the left and right amygdala were derived from downsampling (to 3.75 mm isotropic) the amygdala masks of the Talairach atlas provided in the AFNI package.

Results

The final scanned sample included 39 participants; 20 participants classified as externalizers (10 females) and 19 participants classified as internalizers (10 females). No participants were excluded due to head motion and there were no group differences with regard to head motion. Table 12 shows the differences between the two groups on key variables of interest and alcohol use and problems. Overall there was no difference between the two groups in terms of days drinking per month, typical drinks per day, binges per month, or alcohol use disorder symptoms. By design the groups are significantly different on sensation seeking and symptoms of depression and anxiety. The two groups did not significantly differ on past antisocial behavior.

Table 12: Comparison of Group Differences

	Internalizers			Externalizers			t-statistic	p-value
	Mean	SD	Range	Mean	SD	Range		
Age	21.76	0.97	20-24	21.5	0.76	21-24	0.912	0.369
Depression	10.26	9.18	0-31	2.6	2.66	0-11	3.501	0.002
Anxiety	5.26	6.21	0-18	0.8	1.28	0-4	3.072	0.006
Sensation Seeking	30.84	5.95	18-43	39.85	4.69	29-47	-5.233	<0.001
Antisocial Behavior	2.32	2.24	0-7	3.1	3.19	0-11	-0.892	0.379
Drinking Days	10.882	8.73	2-30	9.842	5.99	2-25	0.412	0.684
Drinks per Day	4.632	1.53	1-7.5	4.175	1.57	1.5-8	0.897	0.376
Binges per Month	5.368	4.83	0-16	4.875	4.06	1-18	0.344	0.733
AUD Symptoms	2.42	2.06	0-7	2.2	1.44	0-5	0.386	0.701

Task Behavior

XYGNG. Performance on the XYGNG task varied across participants. The average reaction time to Go Trials was 314.10 msec (SD = 45.89, range = 240.54 – 384.83). Participants on average made 9.74 (SD = 27.03, range = 0-133) omission errors, i.e. missed responding to a Go trial, which corresponds to missing 4.33% of the Go trials. Three participants missed responding on greater than 10% of the Go trial and were considered outliers. There was no difference in the subsequently reported findings when these individuals were removed from the analysis. On average, participants committed 12.38 (SD = 4.37, range = 3 - 21) commission, i.e. responded to a Nogo trial, which corresponds to incorrectly responding to 49.54% of Nogo trials. Mean reaction time was negatively correlated with commission errors (r = -0.504, p = 0.001), such that slower reaction times were associated with fewer commission errors. Mean reaction time was unrelated to omission errors (r = 0.072, p = 0.66). There were no differences between internalizers and externalizers on commission errors (p = 0.416), omission errors (p = 0.201), or mean reaction time (p = 0.943).

EF. All participants successfully completed both runs of the EF task with very few missed or incorrect trials.

MID. Reaction times were fastest on high reward trials (mean = 231.693, SD = 20.231, range = 200.513 – 290.617) and slowest on low loss trials (mean = 236.629, SD = 22.511, range = 189.331 – 305.773). Figure 11 shows reaction time to each trial type by binge drinking subtype. Participants were less likely to hit the target on neutral trials (mean = 5.21, SD = 2.32, range = 1.00 - 9.00) compared to the other trial types (mean = 12.44 - 13.74). Figure 12 shows hit rate for each trial type by binge drinking subtype. Generally, there were no group differences on reaction time (p = 0.137 - 0.868) or hits across trial types (p = 0.265 - 0.890). The exception being neutral trials where internalizers hit the target significantly more (mean = 6.00) than externalizers (mean = 4.45, $t(37) = 2.19$, $p = 0.035$).

Figure 11: Mean reaction time by MID task trial type

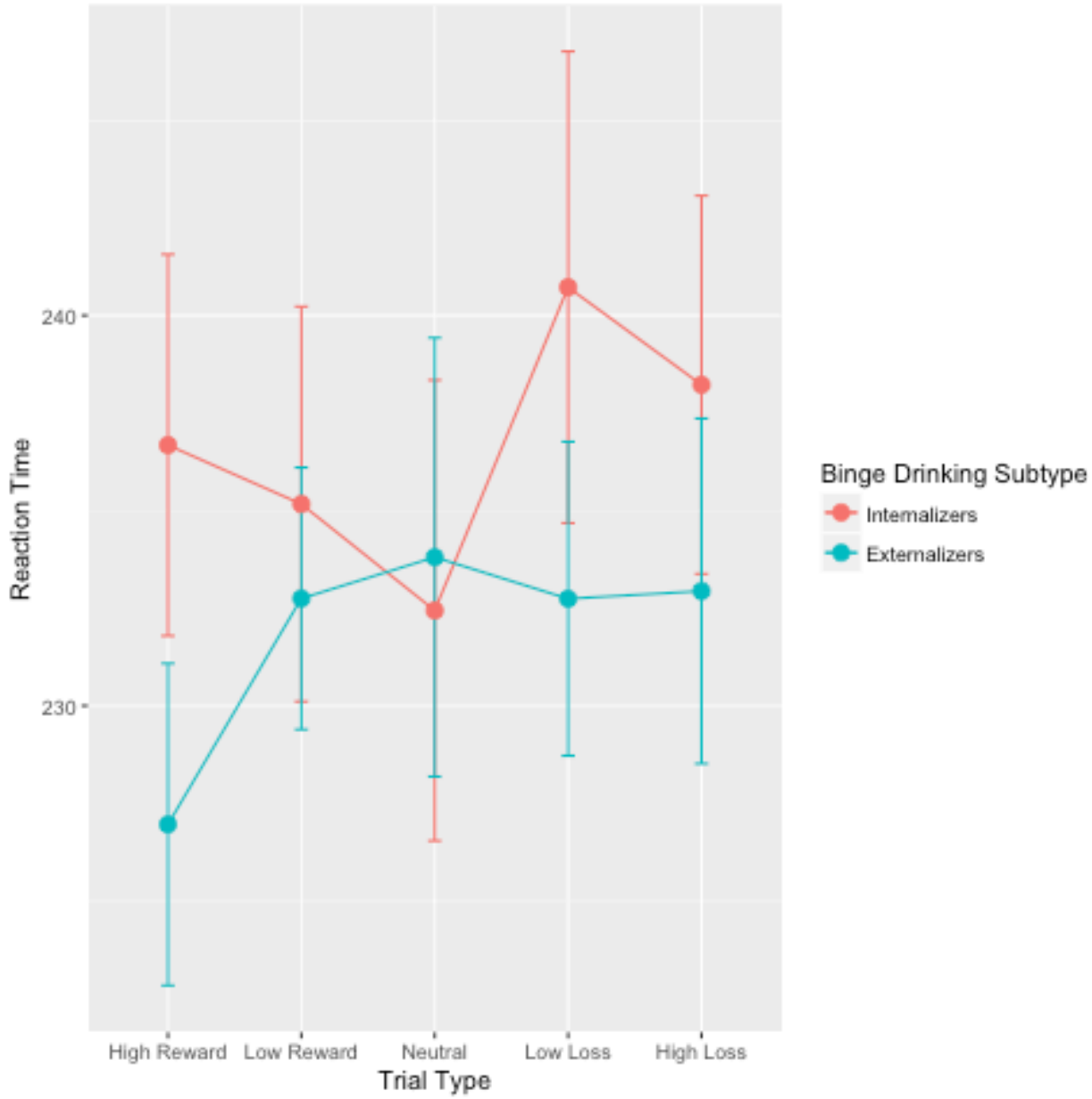
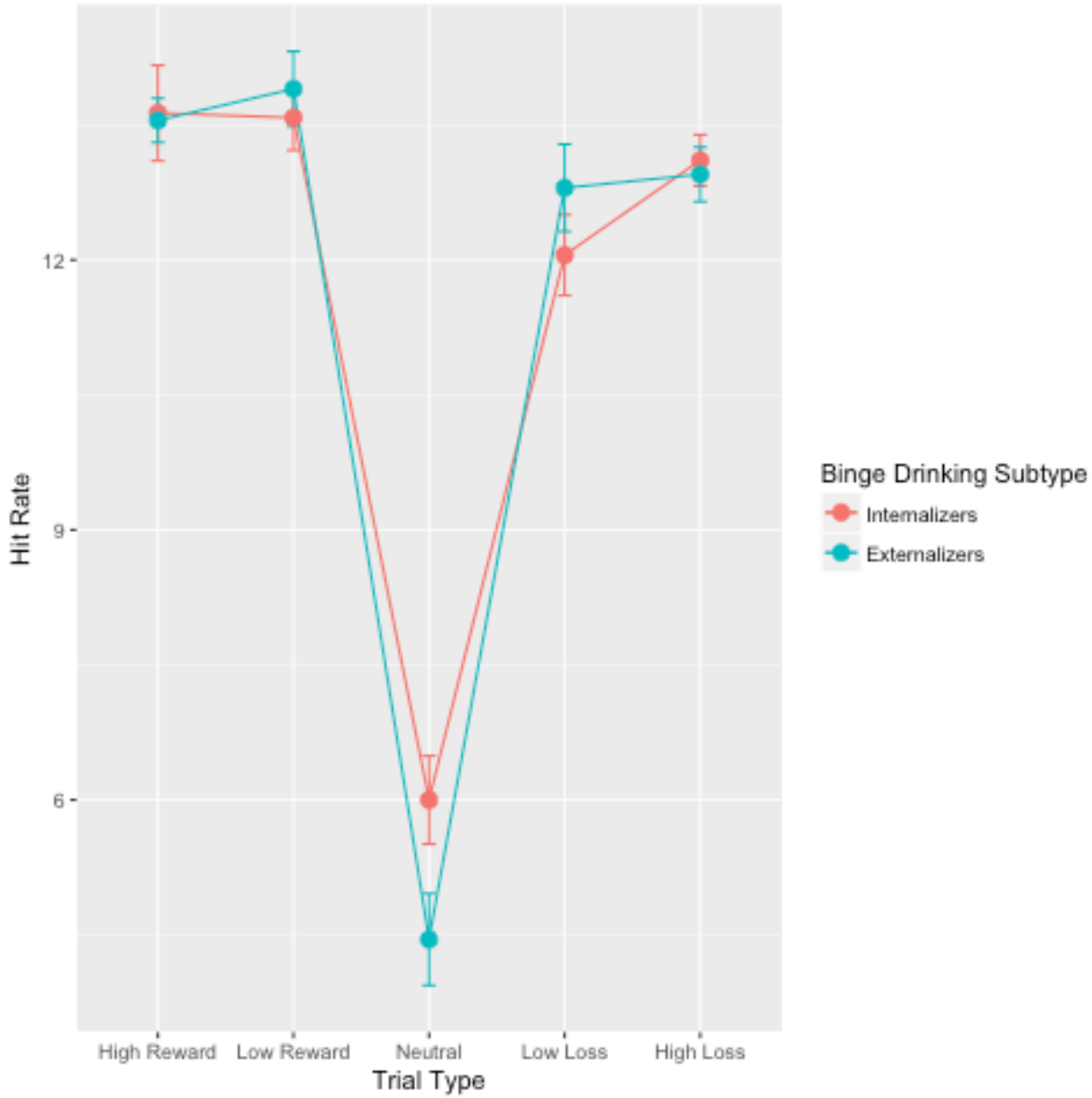


Figure 12: Mean number of hits by MID task trial type



Family – Wise Error Corrected Maps

Threshold voxelwise *t*-statistics, cluster size, and minimum number of voxels in a cluster are as follows: 1) for XYGNG, a) $t = 3.558 – 3.618$, b) 1308 – 1497 ul, c) 25 – 28 voxels; 2) for EF, a) $t = 3.559$, b) 2805 – 5200 ul, c) 53-98 voxels; 3) for MID, a) $t = 3.565$, b) 2094 – 3117 ul,

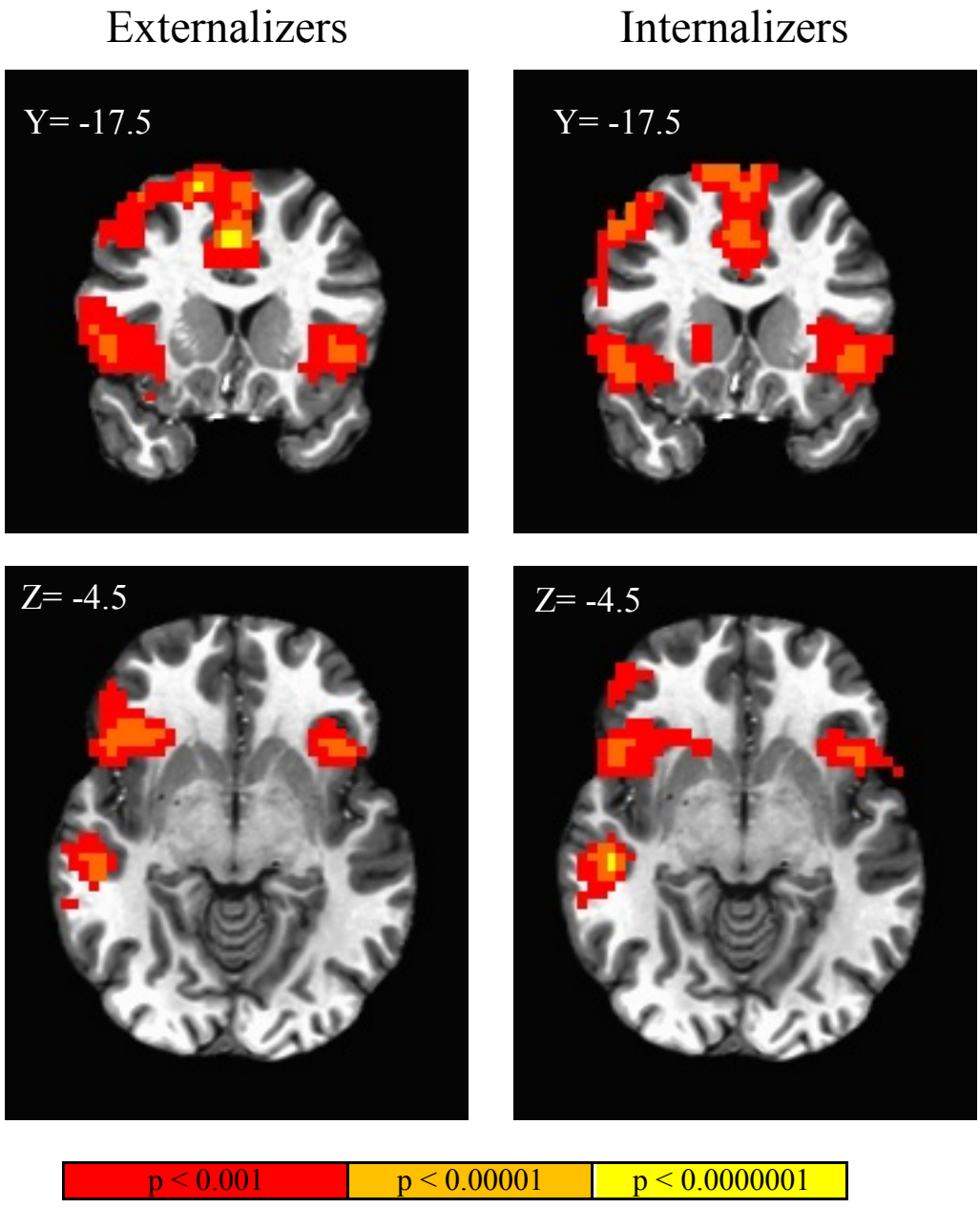
c) 39 -59 voxels. Tables 13-15 display the maximum t-statistic, related p-value and coordinates for clusters surviving the FWER. Figures 13-15 display the surviving clusters graphically with *p*-value cut off for significance. All illuminated voxels meet a minimum threshold of $p<0.001$.

Table 13: Activation maxima for Lures in the XYGNG task

	Talaraich Coordinates			*t*-statistic	Uncorrected *p*-value*
Internalizers	X	Y	Z		
Right Inferior Parietal Lobe	48	-43	50	8.4076	<1.0 x10-9
Right Middle Temporal Gyrus	59	-45	9	5.7606	<0.00001
Right Superior Temporal Gyrus	54	-28	3	6.8652	<1.0x10-7
Left Inferior Frontal Gyrus	-40	17	-7	5.9318	<1.0x10-6
Right Inferior Frontal Gyrus	50	17	-5	5.6321	<0.00001
Right Medial Frontal Gyrus	4	27	36	6.4424	<1.0x10-6
Right Posterior Cingulate	19	-50	26	-5.2788	<0.00001
Right Insula	29	-31	21	-5.1843	<0.00001
Externalizers					
Right Superior Frontal Gyrus	14	24	57	8.0899	<1.0x10-8
Right Inferior Frontal Gyrus	48	22	-2	6.4047	<1.0x10-6
Left Inferior Frontal Gyrus	-41	19	-1	5.9027	<1.0x10-6
Right Superior Temporal Gyrus	52	-26	0	5.9097	<1.0x10-6
Right Medial Frontal Gyrus	4	19	38	6.8891	<1.0x10-7

* all activated clusters survive Family-Wise Error (FWE) correction to adjusted $p < .05$

Figure 13: Second-level Group Maps - XYGNG Task – Lures

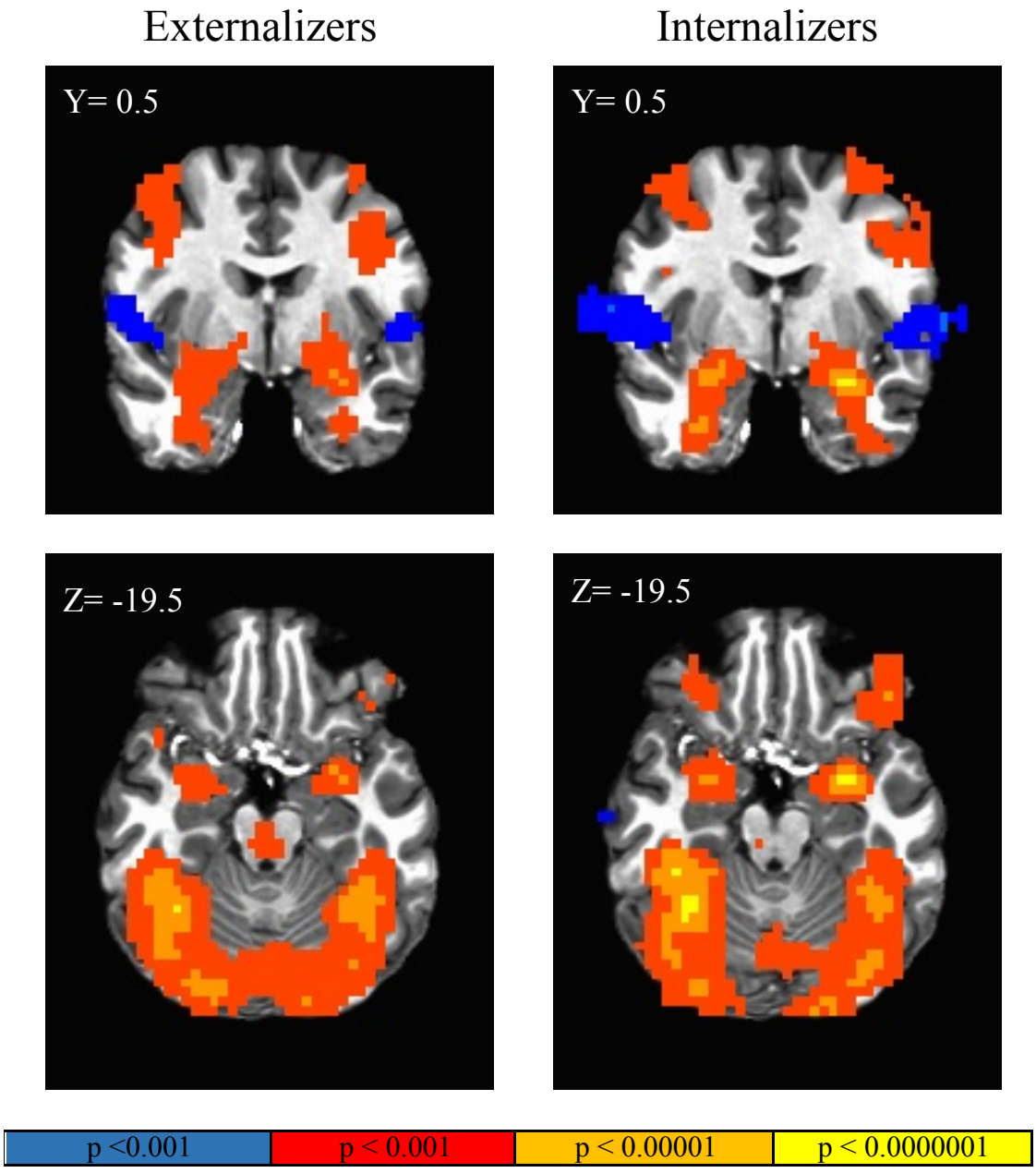


Note: Regions are colored by their unadjusted *p*-values. All illuminated voxels are part of clusters that survive FWER correction and have an adjusted *p*-value of $p<0.05$. The underlay image is a structural T1 scan from a representative participant.

Table 14: Activation maxima for faces vs shapes contrast on the Emotional Faces task

	Talaraich Coordinates			*t*-statistic	Uncorrected *p*-value
Internalizers	X	Y	Z		
Right Anterior Cingulate/Left Medial Frontal Gyrus	0	42	0	-8.7259	<1.0x10-9
Right Cingulate Gyrus/Right Medial Frontal Gyrus	2	-25	44	-6.0458	<1.0x10-6
Right Travers Temporal Gyrus	64	-15	13	-6.9271	<1.0x10-7
Left Superior Temporal Gyrus	-69	-16	3	-7.9783	<1.0x10-8
Right Cuneus	23	-93	-2	12.5437	<1.0x10-9
Left Lingual Gyrus/Left Cuneus	-22	-93	-2	11.3972	<1.0x10-9
Left Amygdala	-29	-2	-16	10.1044	<1.0x10-9
Right Amygdala	25	-2	-14	7.4645	<1.0x10-8
Externalizers					
Right Anterior Cingulate	3	48	0	-8.2559	<1.0x10-9
Right Fusiform Gyrus	37	-53	-14	10.9481	<1.0x10-9
Left Fusiform Gyrus	-38	-53	-14	8.8838	<1.0x10-9
Left Cuneus	-22	-93	-2	11.8714	<1.0x10-9
Right Cuneus	26	-93	-2	12.0442	<1.0x10-9
Left Amygdala	-29	-3	-16	7.0919	<1.0x10-7
Right Amygdala	27	-3	-14	6.5931	<1.0x10-7

Figure 14: Second-level Group Maps - EF Task – Faces vs. Shapes

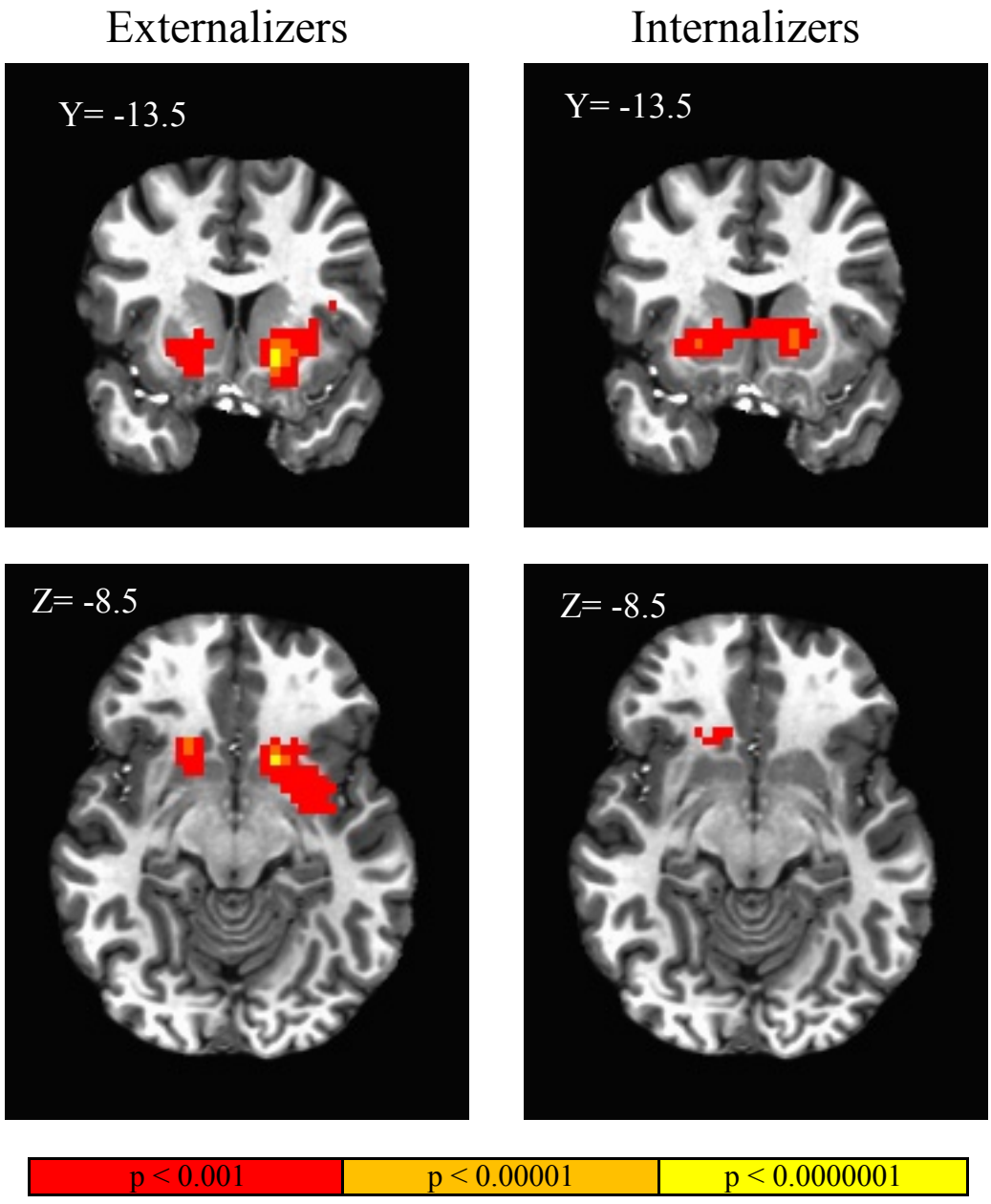


Note: Regions are colored by their unadjusted *p*-values. Warm colors indicate increased activation to faces compared to shapes. Cool colors indicate decreased activation to faces compared to shapes. All illuminated voxels are part of clusters that survive FWER correction and have an adjusted *p*-value of $p<0.05$. The underlay image is a structural T1 scan from a representative participant.

Table 15: Activation maxima of (high + low) reward vs neutral anticipation on MID task

	Talaraich Coordinates			*t*-statistic	Uncorrected *p*-value
Internalizers	X	Y	Z		
Left Ventral Striatum	-14	12	2	6.0364	<1.0x10-6
Right Insula	22	15	-2	5.1229	<1.0x10-5
Right Middle Frontal Gyrus	30	-2	41	6.0594	<1.0x10-6
Left Medial Frontal Gyrus/Right Cingulate Gyrus	-3	-2	48	5.9319	<1.0x10-6
Left Precentral Gyrus/Left Middle Frontal Gyrus	-36	-14	48	6.67	<1.0x10-7
Externalizers					
Left Precentral Gyrus	-30	-20	59	7.9825	<1.0x10-8
Left Ventral Striatum	-14	13	-7	6.7601	<1.0x10-7
Right Lentiform Nucleus/Right Claustrum	18	18	-8	5.9379	<1.0x10-6

Figure 15: Second-level Group Maps - MID Task – Reward Cues vs Neutral Cues



Note: Regions are colored by their unadjusted *p*-values. All illuminated voxels are part of clusters that survive FWER correction and have an adjusted *p*-value of $p<0.05$. The underlay image is a structural T1 scan from a representative participant.

VOI Analyses

XYGNG. There were no significant differences between groups in terms of activation in the RIFG on lures or stops vs fails (see Table 16).

Table 16: Group Means and Statistics for XYGNG Contrasts

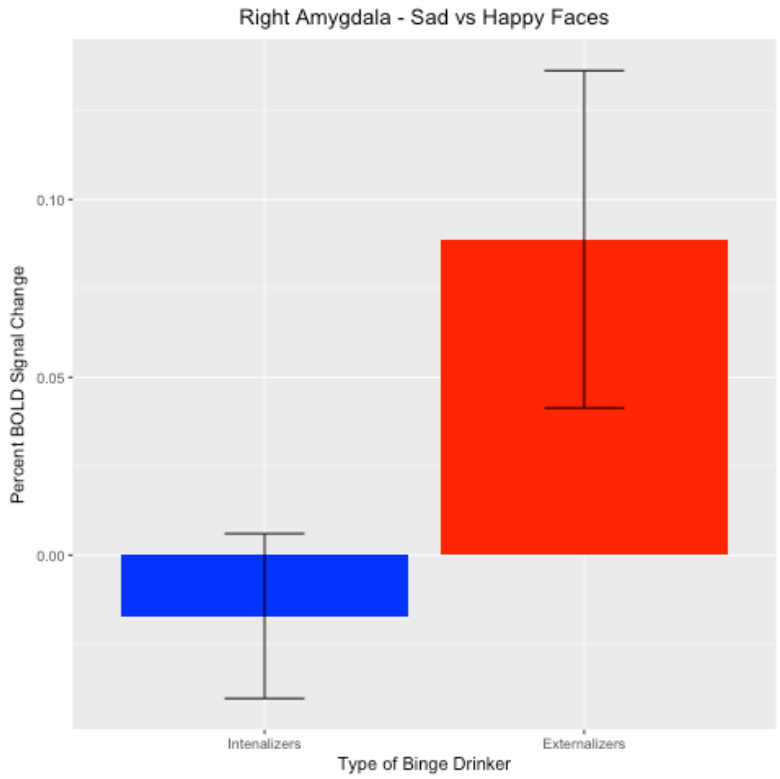
	Internalizers		Externalizers		*t*-statistic	*p*-value
	Mean	SD	Mean	SD		
Lures	0.177	0.17	0.137	0.10	0.895	0.378
Stops vs Fails	-0.063	0.14	-0.085	0.21	0.36247	0.720

EF. Table 17 shows the statistics for the EF contrasts. There was no evidence for group differences in the left or right amygdala on faces vs shapes trials. There was no significant difference between groups in the left amygdala comparing sad face trials to happy face trials. However, there was a borderline significant difference between sad face trials and happy face trials in the right amygdala such that externalizers showed increased activation to sad compared to happy faces while internalizers showed no difference in activation to sad vs happy faces (Figure 16).

Table 17: Group Means and Statistics for EF Contrasts

	Internalizers		Externalizers		*t*-statistic	*p*-value
	Mean	SD	Mean	SD		
Left Amygdala						
Faces vs Shapes	0.112	0.08	0.086	0.08	0.964	0.341
Sad vs Happy	0.013	0.10	0.097	0.20	-1.681	0.104
Right Amygdala						
Faces vs Shapes	0.127	0.09	0.103	0.11	0.744	0.461
Sad vs Happy	-0.017	0.10	0.089	0.21	-2.007	0.055

Figure 16: Percent BOLD signal change to sad faces compared to happy faces in externalizers and internalizers.



MID. Table 8 shows the statistics for the MID contrasts. The primary contrast for the MID task was comparing anticipation on high and low reward trials to neutral trials. There was no evidence of group differences on this contrast in the left NAcc or right NAcc. Secondary contrasts were anticipation on high and low loss trials to neutral trials, feedback on reward trials comparing reward to nonreward outcomes, and feedback on loss trials comparing loss to nonloss outcomes. There were no significant group differences in the left NAcc or right NAcc across all three of these contrasts.

Table 18: Group Means and Statistics for MID Contrasts

	Internalizers		Externalizers		*t*-statistic	*p*-value
	Mean	SD	Mean	SD		
Left NAcc						
Reward Anticipation	0.158	0.26	0.104	0.12	0.836	0.411
Loss Anticipation	0.161	0.24	0.095	0.09	1.151	0.262
Reward Feedback	0.054	0.16	0.056	0.17	-0.047	0.963
Loss Feedback	0.022	0.12	-0.054	0.21	1.412	0.168
Right NAcc						
Reward Anticipation	0.102	0.13	0.081	0.13	0.502	0.619
Loss Anticipation	0.082	0.14	0.057	0.13	0.592	0.558
Reward Feedback	0.081	0.13	0.051	0.19	0.571	0.572
Loss Feedback	-0.0001	0.15	-0.052	0.21	0.905	0.372

Post-hoc exploratory correlation analysis. In order to test whether activation on the contrasts of interest could be related to overall internalizing or externalizing, post-hoc correlation analyses were conducted. Similar to the test between groups, we tested whether activation in the each of the VOI regions correlated with externalizing or internalizing measured by the factor scores (see Chapter 2 for details on how these scores were created) or scores from the participants' screening day (see Table 19). For the XYGNG task, internalizing or externalizing characteristics did not significantly correlate with activation in the RIFG in response to lures or stop vs fail trials. For the EF task, screening visit sensation seeking scores were significantly negatively correlated with left amygdala activation ($r = -0.341$, $p = 0.034$) and borderline significant with right amygdala activation ($r = -0.311$, $p = 0.054$) on all faces vs shapes trials. Internalizing was significantly negatively correlated with activation to sad faces vs happy faces ($r = -0.353$, $p = 0.027$). For the MID task, neither externalizing nor internalizing characteristics were significantly correlated with left or right NAcc activation to anticipation on high and low reward trials to neutral trials. Additionally, none of the externalizing or internalizing characteristics significantly correlated with left NAcc or right NAcc with regard to anticipation on high and low loss trials to neutral trials,

feedback on reward trials comparing reward to nonreward outcomes, and feedback on loss trials comparing loss to nonloss outcomes.

Table 19: Correlations between fMRI Task Contrasts and Externalizing/Internalizing Characteristics

	Externalizing Factor Score	Internalizing Factor Score	Sensation Seeking	Depression / Anxiety Sx
Lures	-0.224	0.074	-0.088	-0.092
Stops vs Fails	-0.126	0.074	-0.075	0.024
Left Amygdala				
Faces vs Shapes	-0.250	0.187	**-0.341**	0.116
Sad vs Happy	0.249	*-0.294*	0.081	-0.027
Right Amygdala				
Faces vs Shapes	-0.214	0.177	*-0.311*	0.111
Sad vs Happy	*0.290*	**-0.353**	0.119	-0.142
Left NAcc				
Reward Anticipation	-0.087	0.105	-0.026	0.013
Loss Anticipation	-0.077	0.084	-0.036	0.033
Reward Feedback	-0.073	0.089	-0.099	0.119
Loss Feedback	-0.113	0.191	-0.200	0.086
Right NAcc				
Reward Anticipation	-0.120	0.134	-0.021	0.089
Loss Anticipation	-0.080	0.076	0.059	0.070
Reward Feedback	-0.113	0.041	-0.065	-0.172
Loss Feedback	-0.080	0.155	-0.187	0.186

Note: Bolded correlations are significant at $p<0.05$. Italicized correlation are borderline significant at $p<0.10$.

Discussion

The goal of the neuroimaging sub-study was to compare motivational and inhibitory brain activation between internalizing binge drinkers and externalizing binge drinkers. Specifically, we tested for differences between the two groups on tasks hypothesized to represent behavioral inhibition, emotion reactivity, and reward sensitivity, three constructs associated with problem substance use. We hypothesized that 1) externalizers would show greater activation in the inferior frontal gyrus on a task measuring behavioral inhibition, 2) internalizers would show greater

activation in the amygdala to faces on a task measuring emotion reactivity, and 3) externalizers would show greater activation in the nucleus accumbens to reward on a task measuring reward sensitivity. Overall, these hypotheses were not directly supported since there were no significant differences between the two groups in any of the areas of interest of contrasts of interest. However, there were some trend level findings and significant correlations in the post-hoc analyses that are worth noting and further discussed below.

There was a borderline (p = 0.055) significant difference between internalizers and externalizers in the right amygdala on the EF task, such that externalizers showed increased activation to sad faces compared to happy, while internalizers show a blunted response to sad faces compared to happy faces. This decreased activation in internalizers is further supported by a significant negative correlation between internalizing and right amygdala activation to sad vs happy faces in a single group analysis. While this reduced amygdala activation in response to sad faces in those with greater internalizing characteristics is contrary to two other studies which found exaggerated amygdala activation in response to sad faces in individuals with major depression (Fu et al., 2004; Surguladze et al., 2005), it is also important to note that in a meta-analysis comparing brain activation on different emotions, the amygdala was not shown to differentiate sad vs happy emotions (Vytal & Hamann, 2009). There are several differences between these two studies and the present study. First, participants from the current study are much younger, ages 20-23, than the Surguladze et al. (2005) and Fu et al. (2004) studies (average age of 38.7 and 43). Additionally, while the internalizers in the current study endorsed significantly more symptoms of anxiety and depression compared to the externalizers, they were not assessed for a clinical diagnosis of depression or anxiety.

Another set of findings worth noting are the post-hoc correlation analyses on amygdala activation to face trials compared to shape trials. Although there were not significant group differences in either the left or right amygdala on this contrast, there was a significant (left amygdala) and borderline significant (right amygdala) correlation between sensation seeking and amygdala activation. In both the left and right amygdala individuals with higher sensation seeking showed less activation to faces compared to shapes. There is minimal previous research on the relationship between sensation seeking and amygdala activation. Mujica-Parodi, Carlson, Cha, and Rubin (2014) proposed that the relationship between amygdala activation and sensation seeking is mediated by threat perception. However, this does not explain the decrease in activation to all emotional faces seen in our study.

There were no significant findings in the RIFG on lures or comparing stop trials to failing to stop trials in the XYGNG task; both when testing group differences but also when correlating internalizing and externalizing characteristics with activation across the whole sample. This could be due to the size of the sample. While 39 individuals may be large enough to detect group differences in problem substance users compared to non-users, it is likely that the differences between subtypes of problem users may be subtler and therefore require even larger sample sizes. From the FWE corrected whole brain maps we know that this null finding is not due to a failure of the task to elicit activation. Table 13 and Figure 13 show significant activation in the RIFG for both groups in response to lures (or Nogo trials). The *t*-statistics in Table 13 indicate that there is a greater activation in the RIFG on lures trials in externalizers (t = 6.4047) compared to internalizers (t = 5.6321) which, although not significant in VOI analyses, shows a trend in the hypothesized direction.

We also found no significant associations in the NAcc on four different contrasts in the MID task. Again, this was true for testing both group differences and the post-hoc correlations. While sample size makes it hard to be confident that these are truly null findings there are other explanations worth considering besides the small sample. In addition to the magnitude of group differences being potentially smaller between subtypes, the contrasts on which they differ may be more nuanced. In order to not be over burdened by multiple testing corrections in an already small sample, we limited our VOI analyses to canonical contrasts of interest. However, since this was a sample of all binge drinkers the two groups may not differ on contrasts that have been shown to be dysregulated in problem substance users compared to nonusers. Instead, these subtypes may differ in activation on certain types of stop or reward trials and not more general contrasts. Relatedly, there were significant differences between the groups on the number of neutral targets they hit with internalizers successfully hitting significantly more than externalizers. We did not examine neural activation to neutral cues alone but this behavioral difference serves as evidence for future follow-up. Finally, it is certainly possible that the identified subtypes of binge drinkers do not differ with regard to behavioral inhibition or reward sensitivity.

The findings of this study should be viewed in light of several limitations. As discussed above, in order to detect the potentially subtler differences between subtypes of binge drinkers a larger sample may be needed. In addition, while a DSM-5 screening checklist was given to participants, a diagnosis of an anxiety disorder or major depression was not necessary for the internalizing group. With regard to externalizing severity, the two groups ultimately did not significantly differ in their antisocial behavior. This is due to the generally low levels of antisocial behavior in both groups. However, because they did not differ on this behavior it is unclear if

antisocial behavior would have been a driving factor in neurobiological differences between these subtypes. Finally, in order to test multiple constructs in the constraints of affordable scanner time only one or two runs of each task were given. This especially affected power to detect differences on the XYGNG task since there was only one run of that task which meant 6 participants had no failed stops and could not be used in analyzing that contrast.

The current study was the first to test for differences in brain activation between subtypes of binge drinkers on three well validated fMRI tasks used to index behavioral inhibition, emotion reactivity, and reward sensitivity. We found no group differences in brain activation on tasks measuring behavioral inhibition and reward sensitivity. We found suggestive evidence that internalizing binge drinkers are show more activation to faces than externalizing binge drinkers. But internalizing binge drinkers show less activation than externalizing binge drinkers when viewing sad faces compared to happy faces. Future studies of problem alcohol users will need be needed to replicate the both the null and suggestive findings presented here. Larger samples sizes are needed not only to detect potential group differences but also to better examine these factors along a continuum as that may be a truer representation of the relationship between externalizing/internalizing characteristics with alcohol use in the general population. But for now, the current study presents preliminary evidence that emotion reactivity in binge drinkers is a function of both sensation seeking and internalizing characteristics.

Chapter 5: Overall Discussion and Future Directions

The goal of this dissertation was to better understand the genetic and neurobiological influences on subtypes of binge drinkers. This was accomplished by using factor analysis to create factor scores representing an individual's general level of internalizing and externalizing over the course of their time in college. These scores were then used in a series of genetic analyses on a binge drinking sample that 1) examined the heritability, due to common genetic variation, of these characteristics, 2) identified specific genetic markers associated with these characteristics, and 3) sought to understand the function of any associated variants. The factors scores were also used to select from the binge drinking sample a subset of individuals classified as either internalizers (high on internalizing, low on externalizing) or externalizers (high on externalizing, low on internalizing) to undergo an fMRI scan which measured their brain activity in response to three tasks indexing behavioral inhibition, emotion reactivity, reward sensitivity.

The genetic analyses indicated modest heritability of internalizing characteristics in binge drinkers, consistent with previously reported estimates. For externalizing characteristics, the estimate of SNP-based heritability was not statistically different from zero, possibly indicating that there is little genetic influence on externalizing characteristics in college students once accounting for problem alcohol use. Genome-wide association (GWA) analyses indicated genome-wide significant (GWS) associations for both externalizing and internalizing characteristics. Both findings were located in genes (*ECE1* and *RPH3AL*, respectively) and had existing support in the literature although tenuous.

The neuroimaging analyses indicated that both groups showed significant brain activation on all three tasks and in the brain areas of prioritized interest. Regarding the XYGNG task, indexing behavioral inhibition, and the MID task, indexing reward sensitivity, there were no statistically significant group differences in the areas of interest (RIFG and bilateral NAcc). Additionally, when looking across groups activation in these regions did not correlated with overall externalizing or internalizing or measures of externalizing and internalizing from the participants' screening visit. While there were no significant group differences in the amygdala during the EF task indexing emotion reactivity, activation in the amygdala across the two groups was significantly correlated with out of scanner measures of internalizing and externalizing. With sensation seeking scores being negatively correlated with amygdala activation when comparing face to shape trials such that participants with higher sensation seeking scores showed lower activation to faces compared to shapes. While the internalizing factor score negatively correlated with amygdala activation when comparing sad face trials to happy face trials such that individuals higher on the internalizing factor showed decreased activation to sad faces.

These analyses represent an initial exploration into the potential genetic and neurobiological differences between subtypes of binge drinkers. Both sets of analyses would likely benefit from much larger sample sizes as the differences between subtypes of alcohol users are likely to be subtler than differences between problem users and nonusers. Currently there are large scale efforts in both the field of genetics (PGC-SUD) and neuroimaging (ABCD) to understand the genetic and neurobiological influences on problem substance use more broadly. These efforts are essential for us to begin to understand the likely small effects on problem alcohol use. That being said smaller samples that include more refined measures of the traits of interest

and analyses of subtypes will prove to be just as important in understanding how genes and neurobiology come together to produce problem behavior.

Similarly, while these analyses take a quantitative approach to the association of internalizing and externalizing with alcohol use in the genetic analyses, the neuroimaging analyses focus on two distinct groups. In reality, most people do not classify neatly into two subtypes. First, there are likely to be binge drinkers who have both externalizing and internalizing characteristics as well as those who have none. Additionally, anxious people may be impulsive in different ways that depressed people. We are not yet able to predict a person's likelihood to develop an AUD with 100% certainty but recognizing and incorporating the potential for individual differences in the outcomes of interest in genetic and neuroimaging studies is a start.

Overall, the analyses presented in this dissertation are an attempt to incorporate two factors (externalizing and internalizing) that are known to be comorbid with problem alcohol use. The association between these factors and problem alcohol use has been extensively studied on the phenotypic level but in examined far less often in genetic or neuroimaging studies. In fact, this is the first study to directly test differences in brain activation between internalizing and externalizing problem drinkers. Although larger sample sizes and replication are necessary, preliminary evidence was found for new genetic markers associated within problem drinkers. Additionally, there is support for differences among binge drinkers in emotion reactivity as measured in the amygdala associated with greater internalizing and lower sensation seeking.

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VITA

Megan Elizabeth Cooke was born on November 8th, 1985 in New Haven, Connecticut. She graduated from St. Mark's School, Southborough, Massachusetts in 2004. She received her Bachelors of Science in Psychology from Furman University in 2008. While at Furman University, Megan worked with Dr. Cinnamon Stetler, PhD on her research in Psychoneuroimmuology. After graduating from Furman University, Megan worked as an IRTA in the Psychopharmacology lab of Dr. Vijay Ramchandani at the National Institute for Alcohol Abuse and Alcoholism. In August 2011, Megan began her PhD in Clinical and Translational Sciences with a concentration in Psychiatric, Behavioral, and Statistical Genetics at Virginia Commonwealth University under the supervision of Dr. Danielle Dick.