Family History of Alcohol Use Disorder Affects Intrinsic Connectivity in Large-scale Brain Networks of Adults

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Abstract

Previous research has implicated large-scale neural networks, such as the salience network (SN), central executive network (CEN), and default mode network (DMN) in risk for a wide range of neuropsychiatric disorders. Specifically, perturbations in network interconnectivity of the SN has been theorized to reflect changes in salience processing, such as that seen in alcohol use disorders (AUDs) and other addictive disorders. The aim of this study was to investigate differences in intrinsic connectivity between adults with and without a family history of AUDs. To address this, 58 adult participants (22 family history positive (FHP), 36 family history negative (FHN)) underwent a resting state fMRI scan and functional connectivity analyses were performed. Importantly, the groups did not significantly differ in terms of their age, IQ, socioeconomic status, and alcohol consumption, and none of the recruited participants had a lifetime history of substance use disorders. Results show that FHP adults were characterized by decreased connectivity between right frontoinsular cortex (rFIC), a critical node in the SN, and frontoparietal regions of the CEN. Additionally, whole brain functional connectivity map contrasts revealed potential differences in connectivity between ventral tegmental area (VTA), another node in the SN, and DMN. These findings suggest dysfunctional interactions between SN and CEN, typically identified as “task-positive networks”, may associate with the familial transmission of AUDs. Future research should utilize effective connectivity and imaging genetic approaches to more closely examine these group differences at a systems neuroscience level of analysis.
The Importance of Alcohol Use Disorder Treatment and Prevention

Substance use disorders (SUDs) negatively influence both individuals and society as a whole. One of the most prevalent and problematic forms of substance use disorder is alcohol use disorder (AUD). Astoundingly, one in four Americans will deal with alcohol or drug problems at some point in their lifetime (“The Science of Addiction,” n.d.). At the individual level, alcohol abusers face both impairments in cognitive abilities and emotional distress because of their excessive drinking. In addition, alcohol abuse imposes a risk for domestic violence, sexual violence, driving offenses, child abuse, risky sexual behaviors, homicide, and suicide (“Alcoholism,” 2013). Family members of afflicted individuals also encounter the wide range of social, emotional, and financial consequences associated with maladaptive drinking behaviors. In particular, problematic alcohol consumption leads to physical disabilities and premature death, which place both severe emotional and financial burden on the family unit.

On a broader scale, AUDs cost the US economy 235 billion dollars every year, according to the National Institute on Drug Abuse (“Drug Facts,” n.d.). These costs are largely accumulated as a result of lost workplace productivity. During 2007, surveys showed that 9 percent of American full-time workers reported heavy alcohol use. Moreover, 30 percent reported some form of binge drinking. (“Alcohol Alert,” n.d.). Besides lost productivity, rates of death due to alcohol consumption are also staggering, especially with regard to risk populations. According to the World Health Organization, harmful alcohol consumption is ranked as the third leading cause of health problems around the globe (“WHO Global Strategy,” n.d.). Annually, alcohol abuse causes 4 percent of the world’s deaths – that is, 2.5 million people die as a result of alcohol use.
Sadly, abuse of alcohol can also lead to deaths of many young people: 320,000 young adults, people between the age of 15 and 29, die yearly of alcohol related problems.

Even though epidemiological studies indicate that alcohol problems have continually affected between 16 and 20 million people in the United States (“CASAColumbia.org: News Room: Press Releases: Califano Calls for Fundamental Shift,” 2007), the fact remains that alcohol use disorder is a preventable and treatable disease. As the biological basis of AUD become better characterized, treatment and prevention strategies will be reevaluated and redesigned with putative neurobiological mechanisms in mind. As neurobiological research on AUD progresses, identifying behavioral interventions and developing targeted pharmacotherapies will continue to lessen the global consequences of alcohol abuse. By refocusing research efforts on uncovering AUD behavioral and neurocognitive predictors, scientists and clinicians may be able to ameliorate personal and economic costs of alcohol abuse and dependence.

Populations at risk for developing alcohol use disorders

One of the most effective ways to avoid the devastating effects of AUDs is by identifying prevention strategies and strengthening knowledge about AUD risk. By identifying vulnerable populations and determining risk characteristics, potential alcohol abusers may be identified and offered support. Namely, attention to AUD risk patterns could aid in designing interventions that prevent problem users from developing some form of alcohol abuse behavior. Several groups may be at increased risk for developing AUDs: frequent drinkers, individuals with high trait impulsivity, young adults, adolescents, binge drinkers, or individuals with pre-existing psychiatric disorders. However, because many of these groups, such as young adults or individuals with high
trait impulsivity, may demonstrate differences on several psychological measures – drinking motives, binge drinking frequency, overall drinking consumption – analyses attributing neurobiological markers to specific risk factors may be confounded since these individuals already possess multiple risk characteristics. Moreover, heavy drinkers in particular are likely to have incurred some form of brain damage due to the neurotoxic effects of excessive drinking and thus brain differences in these groups could not be attributed to pre-existing risk factors. One characteristic that allows researchers to isolate a very specific, heritable form of AUD risk is AUD family history status. Notably, even if a healthy, non-impulsive adult does not currently drink heavily themselves, they still may be considered at high risk for an AUD because of inheriting certain biological or genetic dispositions. As these biologically inherited risk factors are likely still measurable in brain structure and function differences, AUD family history status may be helpful in dissociating a specific type of risk. In sum, investigation of the effects of family history status, and examination of the neurobiology of AUD family history positive (FHP) and family history negative (FHN) adults therefore allows a more focused investigation of a biologically heritable risk factor for AUD.

**AUD Family History and Inheritance of Alcohol Related Problems**

Many previous investigations into the neurobiological differences between AUD FHP and FHN adults have been initiated with the goal of determining how potential biological risk factors are transmitted from generation to generation in families. These studies have been largely fueled by findings from family, adoption, and twin studies that have explored the genetic basis of alcohol addiction (Agrawal & Lynskey, 2008). Evidence from several lines of investigation has produced an estimate that 30 to 70 % of
AUD risk is inherited (Agrawal & Lynskey, 2008). In particular, familial transmission of substance use disorder has been traditionally analyzed by using a combination of twin and adoption studies. Carefully reviewing these investigations is necessary, since the results of these individual studies are greatly influenced by inclusion/exclusion criteria and sample selection method; while some studies collect information from probands (the first affected individual in a family who seeks help or treatment in clinics) others sample communities or larger populations. While genes and environment obviously interact, twin studies and adoption studies may allow us to isolate biologically heritable influences on risk. Classic twin studies have analyzed the incidence rate in monozygotic twins and compared the incidence rate to dizygotic twins. In a similar vein, adoption studies have traditionally compared the concordance rate between behaviors of children with their adoptive and biological parents. While correlation between offspring and biological parent behavior suggests genetic or epigenetic influence, correlation between offspring and adoptive parent suggests environmental influence.

Additionally, studies investigating inheritable risk factors for AUD need to be careful to consider genotype-environment interactions (McGue, 1999). Genetics may predispose certain individuals to have traits, but stressors, such as harmful family relations, may mediate the influence of genes on ultimate alcohol behavior outcomes. Furthermore, the complex nature of examining neurobiological risk factors for alcohol abuse, alcohol dependence and other SUDs are characterized by a high degree of heterogeneity (Wong & Schumann, 2008). Underlying this phenotypic heterogeneity is genetic heterogeneity and polygenicity; that is, the phenotypic complexity arises because each individual gene contributes a component of the SUD phenotype independently, and
multiple genes also interact in a concerted fashion. Thus, a single gene or dysfunctional neurobiological mechanism will never be identified as the sole AUD risk factor. Lastly, it should be noted that particular heritable neural risk factors may be specific to certain types of SUDs, or could represent a common risk factor for developing a range of SUDs (Bierut et al., 1998). For example, a common etiological factor, such as cognitive impulsivity, could predispose individuals for many different SUDs.

**Evidence from Twin Studies**

Prior to the 2000s, influential twin studies showed that there was a higher concordance rate of alcohol abuse between monozygotic twins compared to dizygotic twins (McGue, 1999). Importantly, these studies showed that inheritance of alcoholism does not follow a simple Mendelian inheritance pattern. Earlier twin studies, using primarily males, showed that heritability of alcoholism was estimated to range from 49% to 64%. One study in particular that examined the heritability of AUDs reported that 48%-58% of alcohol addiction inheritance was biologically heritable (Prescott & Kendler, 1999). This study possessed a methodological advantage compared to previous studies, which had primarily focused on samples from clinical settings and archival data: it was the first twin study using U.S.-based population data. A more recent twin study conducted by Kendler and colleagues (2003) also used a population-based method and found results similar to earlier studies. Moreover, through multivariate twin modeling, Kendler and colleagues (2003) also found that when analyzed in the broader range of psychiatric and SUDs, alcohol dependence related to disorder-specific genetic factors, such that familial risk patterns were not shared across several different
Evidence from Adoption Studies

Goodwin, Schulsinger, Hermansen, Guze, and Winokur (1973) provided an early example of the way in which adoption studies could be used to isolate the influence of biologically heritable factors from environmental factors. Largely agreeing with later adoption and twin studies, the scientists found that offspring of presumed alcoholics were four times more likely to receive the diagnosis themselves sometime later in life. The adopted individuals in this study were separated from their biological parents within the first six weeks of life and were all raised by nonrelatives. Therefore, the biological relatives had little to no influence on the raising of the child or their environment. Additionally, the researchers found that alcoholics’ offspring had approximately twice as many alcohol problems when compared to children of non-alcoholics. While these early results were promising, this study was limited by its small sample size ($n=55$) and inclusion of only male participants. A more recent study with a much larger sample also found that adopted men who had both environmental and biologically heritable risk factors were at four times higher risk for developing severe alcoholism (Sigvardsson, Bohman, & Cloninger, 1996). These researchers utilized a sample from government registries: 577 men and 660 women’s data were analyzed.

Effects of Gender on AUD Inheritance Patterns

Despite a high degree of uniformity in results from twin and adoption studies with regard to male AUD inheritance patterns, the findings with respect to female AUD inheritance risk are not as straightforward and simple to interpret. In addition to the effects of alcoholism family history on risk behavior, there has been a great deal of
attention focused on sex differences in the risk for AUD development. As Prescott explains, the ability to examine sex differences in the etiology of AUDs is inherently limited by certain methodological problems, such as the fact that AUDs afflict relatively fewer females. Despite these limitations, research has begun to explore the ways in which male and female individuals could differ in their AUD inheritance risk and etiology. Later work by Prescott and colleagues (2005) confirmed the potential gender differences in alcoholism heritability. As (Nolen-Hoeksema, 2004) suggests, even though many early twin studies provided evidence for a stronger role of biologically heritable factors in the development of AUD in males, the results of other studies have called this claim into question (Nolen-Hokesema, 2004; Khan et al., 2013; Heath et al., 1997; Prescott & Kendler, 1999). Moreover, the study of female-specific alcoholism heritability is confounded by many factors: sociocultural barriers may inhibit women from seeking treatment for AUDs and as a result many of the twin, adoption, and family studies may be inherently biased. However, while there are indeed different sociocultural influences on male versus female drinking behavior, one particularly promising way of analyzing gender differences with twin studies is to independently select pairs based on their gender composition (i.e., analyzing the differences between male-male pairs, male-female pairs, and female-female pairs) To date, most data show that the similarity in male versus female inheritance pattern is greater than the differences (Nolen-Hoeksema, 2004). Nevertheless, it is likely that gender does affect the neural pathways mediating addictive behavior and AUD risk in some way. As no previous fMRI studies have systematically examined the neural connectivity basis of gender effects with a larger FHP sample, a supplementary goal of this study’s analyses was to compare connectivity across FHP
males and females.

Utility of focusing on healthy FHP individuals

Directing research efforts towards the study of healthy adult individuals who carry biological risk factors allows us to isolate AUD risk without needing to account for confounding effects of heavy alcohol consumption (DeVito et al., 2013). Of course, factors related to drinking behaviors could still differ significantly between groups. Variables that could influence alcohol consumption such as drinking motives, age, IQ, trait impulsivity, socioeconomic status, and lifetime history of psychiatric disorders could easily bias comparisons between healthy individuals with and without family history of alcoholism. In order to isolate the AUD risk specifically due to biologically heritable factors, the effects of these potential confounds should be controlled for when doing statistical analyses.

Examining Potential Neurocognitive Differences in AUD FHP Individuals

All cognitive neuroscience studies to date have examined the neurobiological effects of AUD family history by analyzing regional activations that correspond to certain cognitive or behavioral task conditions. The examination of these localized regions serve as a starting point for more intensive investigations into the potentially dysfunctional neural circuit basis of alcohol abuse and dependence.

Localizable Effects of Family History Status

Behavioral undercontrol has been associated with lifetime prevalence of alcohol dependence in both males and females (Nolen-Hoeksema, 2004). Thus, areas implicated in cognitive control and behavioral inhibition may be potentially disrupted in adults who
carry biologically inherited risk for AUD. Schweinsburg et al. (2004) were among the first researchers to undertake an fMRI study examining the neural correlates of AUD inheritance risk. This study showed that despite similar levels of cognitive control assessed behaviorally, FHP youth, compared to FHN youth demonstrated less activation in frontal regions implicated in response inhibition. Related to these neurobiological findings, Lovallo, Yechiam, Sorocco, Vincent, and Collins (2006) conducted a behavioral study which found between-group differences in working memory capacity, a cognitive function tightly associated with the frontal cortex. Later, Heitzeg, Nigg, Yau, Zubieta, and Zucker (2008) used fMRI to examined an adolescent sample and determined that resilient (problematic drinking/early-onset drinking) children of alcoholics engaged brain regions related to affective monitoring and behavioral regulation, such as the orbitofrontal gyrus and the left insula. On the other hand, the study’s vulnerable FHP group overactivated structures typically associated with cognitive control processing (dorsomedial prefrontal cortex, dmPFC) and underactivated key areas related to emotion processing, like the extended amygdala and the ventral striatum. In summary, the researchers argued that these differences in brain activation demonstrated the potential for two different neural pathways to mediate AUD vulnerability in FHP vs. FHN adolescents. The researchers, however, did not exclude for attention deficit disorder, conduct disorders, or other SUDs and, therefore, the results of the study should be interpreted with caution.

More recently, cognitive neuroscience research has shown differences in striatal functioning in people with biologically inherited risk for AUDs; Heitzeg, Nigg, Yau, Zucker, and Zubieta (2010) found significantly less deactivation in the ventral striatum.
and brain stem areas among participants with a family history of AUD, during a Go/No-Go response inhibition task. This study’s results were also confounded by recruitment strategy: the scientists did not exclude children of alcoholics with prior diagnosed attention deficit disorder, conduct disorder, or other SUD. Based on another fMRI study using a modified incentive delay task, Andrews et al. (2011) found the nucleus accumbens to be less activated in individuals who were alcoholism FHP. In addition to these fMRI studies, a recent positron emission tomography study measured conditioned ventral striatum dopamine release using [11C]-raclopride, finding elevated release among participants with a family history of alcoholism relative to those without (Oberlin et al., 2013). Lastly and most importantly to the fMRI study at hand: a recent study by DeVito and colleagues (2013) showed that greater activation in the inferior frontal gryus and left anterior insula distinguished FHP individuals from FHN individuals while successfully inhibiting prepotent responses during a Go/No-Go task.

Towards Connectivity Based Approaches

While cognitive neuroscience methods over the past decade have allowed scientists and clinicians to identify regional brain activation markers of AUD risk, the recent large-scale trend towards multivariate connectivity approaches for investigating neurocognitive function has begun to allow researchers to probe AUD familial transmission at a systems level of analysis. These connectivity methods allow analysis of biologically plausible circuits and networks that may be functionally disrupted in the offspring of alcoholics. Specifically, one may search for intermediate phenotypes of complex neurobehavioral disorders using connectomic techniques, such as resting state fMRI functional connectivity (Fornito & Bullmore, 2012). Intermediate phenotypes must
meet certain criteria to be considered as such; most notably, these phenotypes must (1) be heritable and quantitative; (2) distinguish healthy controls from affected individuals; and (3) be involved with the cause or etiology of the disease state (Weinberger & Meyers-Lindeberg, 2006; Fornito & Bullmore, 2012; Walters & Owen, 2007). Because healthy FHP individuals permit investigation of AUD risk without needing to account for excessive alcohol consumption, abuse, and dependence (DeVito et al, 2013), testing putative intermediate phenotypes in these types of samples is a particularly promising avenue of research.

Adopting this approach while utilizing current knowledge about disease-disrupted neural circuitry and a large-scale intrinsic neurocognitive network is valuable for forming and testing hypotheses about the neurobiological basis of familial inheritance of AUDs, and neuropsychiatric disorders more broadly defined.

**Large-scale Neurocognitive Networks and Psychopathology.**

The majority of human neuroscience investigations into AUDs to date have focused on analyzing the brain regions necessary for salience processing and cognitive control. In particular, many neuroimaging studies have identified neural correlates of impulsivity (c.f. DeVito et al, 2013; Boettiger et al, 2007). However, recent studies using resting state fMRI data have found three basic and distinct neurocognitive processes are substantiated in intrinsic brain organization. These three distinct neurocognitive networks, referred to as the default mode network (DMN), the salience network (SN), and the central executive network (CEN), have been broadly construed as falling into two categories: task negative (DMN), being disengaged during cognitively demanding tasks requiring exogenous direction of attention and activated during tasks requiring self-
referential processing, and task positive (SN, CEN), which are engaged and co-activated during tasks requiring exogenous direction of attention (Fox & Raichle, 2007; Seeley et al., 2007). Based on the large amount of research examining these intrinsic brain networks and their cognitive functioning, Vinod Menon has proposed what he calls the “triple network model of psychopathology” (Menon, 2011). As Menon suggests, potentially most relevant to the study of SUDs is the interaction between the task positive networks, SN and CEN, and their related sub-circuitry.

**Salience Network**

The salience network, which has anterior insula/frontoinsular cortex and anterior cingulate cortex as its primary nodes, has been principally implicated in attention switching and the initiation of exogenous and endogenous cognitive control signals (Menon & Uddin, 2010; Sridharan, Levitin, & Menon, 2008). The right frontoinsular cortex (rFIC) specifically has been shown to be important for switching between task positive (CEN) and task negative (DMN) networks. Sridharan and colleagues (2008) used Granger Causality analysis during a resting state and a visual attention task to show that the rFIC plays a casual role in switching between the unique neurocognitive functions of these networks. Seeley et al., 2007 also found that regions typically associated with aberrant reward processing in substance abusers, such as the ventral tegmental area (VTA; Kelley & Berridge, 2002), were also nodes of the lager salience network. Thus, investigation of these SN nodes, FIC and VTA, and their functional circuitry serves as a starting point for examination of putative connectivity intermediate phenotypes of AUD and AUD neurocognitive biomarkers.
Central Executive Network

According to Menon (2011), the Central Executive Network’s primary functions include attention, working memory, planning, and decision making. Anatomically, it is anchored by the dorsal prefrontal cortex (PFC) and posterior parietal cortex. Using standard general linear model (GLM) based neuroimaging analyses, these same areas have been broadly implicated in subjective decision making and impulsive behavior. For example, Boettiger and colleagues (2007) showed that BOLD activation in the dorsal PFC and posterior parietal areas during subjective intertemporal reward choice scaled directly with immediate reward selection bias, the preference of choosing a sooner reward of lesser value over a later reward of greater value. More broadly, the engagement of the CEN, influenced by saliency signals from the SN, allows manipulation of active information in working memory and maintenance of goal-directed cognition and behavior (Menon & Uddin, 2010).

Salience Mapping

Menon (2011) proposes that many psychiatric disorders like AUDs can be potentially explained in terms of maladaptive salience mapping and dysfunctional interconnectivity between the three intrinsic brain networks. Dysregulated salience mapping can be caused by (1) aberrant stimulus mapping; (2) aberrant reward signals; or (3) aberrant self-referential processing. In the case of AUD risk based on family history, weak salience mapping into the CEN could predispose individuals to develop the same neurocognitive dysfunction as their affected relatives. This would largely agree with data showing that FHP individuals are more impulsive because of differences in impulse
control (e.g., DeVito et al., 2013). Thus, aberrant interactions between SN connectivity and CEN connectivity should be a target for current research on the familial transmission of alcohol abuse and dependence.

**Summary and Hypotheses**

Currently, the effects of family history of AUD on the brain and cognition are poorly understood. Recent investigations into the brain’s intrinsic functional organization are able to inform current research on the heritable neural risk factors of alcohol abuse and dependence. For the current study, we hypothesized that adult individuals who report relatives with problematic drinking would exhibit relatively weaker interconnectivity between salience and central executive networks compared to those with no such history. Moreover, this connectivity difference will relate to weakened salience mapping and diminished outflow from the ACC/FIC-based salience network into the frontoparietal cortex based central executive network (CEN). In particular, we hypothesize that diminished rFIC-CEN connectivity would distinguish FHP individuals from FHN individuals. In addition to this focused goal of examining network connectivity differences between FHP and FHN adults, a supplementary aim was to determine potential gender effects within the FHP sample. As no neuroimaging research has explored this area of investigation previously, no directional hypotheses were specified beforehand. By examining this problem from a systems neuroscience perspective, we can test the possibility of specific connectivity intermediate phenotypes and better develop research into putative neurocognitive biomarkers for AUD risk. In addition, these connectivity and network approaches also allow researchers to more thoroughly disambiguate inheritance risk subgroups such as FHP males and females.
Methods

Data Collection:

Participants

Participants (ages 18-40) were recruited from the University of North Carolina (UNC) and the surrounding Chapel Hill community via advertisement. Exclusion criteria included any contraindications to MRI, current or past psychoactive drug use (prescription medication or illicit substances), aside from moderate caffeine or alcohol, any known neurologic or psychiatric conditions, left-handedness, and non-native English speakers. Individuals were also excluded if they reported using marijuana more than once a month over the past year.

Participants were assigned to one of 4 groups based on their age (adult versus emerging adult) and alcohol use (high versus low/moderate consumption). These groups were chosen to test hypotheses specific to the main task-based fMRI study of intertemporal reward choice, which will not be discussed here. Alcohol use was classified using the Alcohol Use Disorders Identification Test (AUDIT; Saunders, Aasland, Babor, De la Fuente, & Grant, 1993). Specifically, people who scored >4 on the AUDIT consumption subscale (AUDIT-c) were classified as High AUDIT-c, whereas those who scored <4 were classified as Low AUDIT-c. Within AUDIT-c classification, participants aged 18-24 were grouped as emerging adults, while those ages 26-40 were defined as adults, with gender ratios balanced within each of the four groups. Potential participants were screened for psychiatric illness with the Mini International Neuropsychiatric Interview (MINI; Sheehan, et al., 1998). Immediately prior to scanning, participants were screened
for acute alcohol use via breathalyzer, and illicit drug use through a urine drug screen. Subjects received monetary compensation for participating. Consent was obtained, as approved by the UNC Office of Human Research Ethics.

**Demographic Information**

58 participants (30 female) were included in the resting state functional connectivity MRI analysis. The groups were subdivided into Family History Positive (FHP) and Family History Negative (FHN) groups based on their responses to the Family Tree Questionnaire for assessing family history of alcohol problems (FTQ; Mann et al., 1985). Specifically, individuals were characterized as FHP if they reported any possible problem drinking biological parent, sibling, or grandparent. Participants’ ages ranged from 18 to 40 years old with the FHN group being statistically similar ($M=27.25$ years old, $SD=6.49$ years old) to the FHP group ($M=26.09$ years old, $SD=4.85$ years old).

**Psychological Questionnaires**

Prior to scanning, participants completed a battery of standard questionnaires, including: the Depression Anxiety Stress Scales (DASS; Lovibond & Lovibond, 1995), the Barratt Impulsivity Scale (BIS; Patton, Stanford, & Barratt, 1995), the Family Tree Questionnaire (FTQ) for Assessing Family History of Alcohol Problems, the Drug Use Screening inventory (DUSI; Tarter, 1990), Cooper’s Drinking Motives Questionnaire-Revised (DMQ-r; Cooper, 1994), Conners Adult ADHD Rating Scales-Self Report (CAARS-SR; Conners, Erdhart, & Sparrow, 1999), the Alcohol Use Questionnaire (AUQ; Mehraban & Russell, 1978) the Carolina Alcohol Use Pattern Questionnaire (CAUPQ), the Shipley Institute of Living Scale (SILS; Shipley, 1940), a socioeconomic
status questionnaire (BSMSS; Barratt, 2006), and the Future Time Perspective Inventory (FTPI; Wallace, 1956). These questionnaires are meant to evaluate a series of behaviors that are relevant to impulsivity, personality traits, mood states, substance use, general lifestyle choices, socioeconomic status, and family history. Question one of the CAUPQ (CAUPQ Q1) assessed the participant’s amount of alcohol consumption on a typical drinking day; this question and other drinking measures may account for variance in SN and CEN circuitry connectivity, and thus we decided to control for these effects during our statistical analyses.

**Magnetic Resonance Imaging (MRI):**

Resting-state functional MRI (fMRI) data was acquired as 243 T2*-weighted images (EPI) on a Siemens 3T Tim Trio whole body magnetic resonance imaging scanner equipped with a TEM send-receive radio frequency (RF) head coil, using a 1-shot gradient-echo EPI pulse sequence (first nine subjects: TR = 2.2 s, TE = 25 ms, flip angle = 80°, 40 coronal slices, FoV = 192×192 mm; acquisition voxel size = 3.5×3.5×3 mm with a 0.5 mm inter-slice gap; remaining subjects: TR=2 s, TE=25 ms, flip angle = 50°, 35 axial slices tilted by 30° from horizontal; FoV = 192×192 mm; voxel size=3×3×4 mm with a 0.5 mm gap) to measure localized blood oxygenation level dependent (BOLD) contrast. The fMRI acquisition was preceded by 11s of dummy gradient RF pulses to achieve steady-state tissue magnetization and minimize startle-induced motion. Duration for each run acquisition was approximately 9 minutes. Low-resolution T1-weighted co-planar images were acquired for each participant. In addition, a high-resolution magnetization prepared rapid gradient echo (MPRAGE) T1-weighted image was
obtained for each subject during the same MRI scan session. Head movement was
restricted during the scanning session, to minimize confounding effects on image quality.
An LCD projector (Avotec Inc., Stuart, FL) projected stimuli onto a rear projection
screen, which the subjects viewed via a mirror mounted within the head coil. During the
resting state fMRI scan, subjects were directed to stay awake and look at a fixation
crosshair presented on the projection screen. They were also instructed to “let their minds
wander” and to not focus on any particular thoughts. Following the scanning session,
participants were asked to fill out a form regarding their thought patterns during the
resting state scan.

Data processing / Statistical Analyses:

Preprocessing

The fMRI data were processed offline using SPM8 software (Welcome Department of
Cognitive Neurology, London, UK) and the artifact detection toolbox (ART;
http://www.nitrc.org/projects/artifact_detect). Preprocessing of the MRI data involves the
following steps: reorientation, slice time correction, realignment, coregistration,
MPRAGE segmentation, artifact detection, spatial smoothing, and normalization. First,
data for each slice were resampled and corrected within each volume, to account for the
offsets in the time of acquisition. This matches the time of acquisition of that volume’s
reference slice. Following this slice time correction, images are motion corrected across
runs and then spatially normalized to a standard reference template in Montreal
Neurological Institute (MNI) stereotactic space, using both a 12-parameter affine
transformation and a nonlinear transformation using cosine basis functions. Finally, the images were resampled into 2 mm$^3$ voxels, and spatially smoothed with an isotropic Gaussian kernel of 7 mm.

**Intrinsic Connectivity Analyses**

After preprocessing, resting state BOLD data was analyzed with the functional connectivity toolbox (CONN; Whitfield-Gabrieli & Nieto-Castanon, 2012), to compare intrinsic functional connectivity between the FHP and FHN groups. The CONN toolbox serves to analyze temporal correlation between the BOLD activity of spatially distinct areas of the brain. The CONN toolbox possesses an advantage over other methods of functional connectivity analysis: specifically, the toolbox implements the component-based noise correlation method (CompCor). This strategy reduces the amount of unwanted physiological noise in the data. For this project’s analyses, we focus on examining region of interest (ROI)-to-ROI and ROI-to-voxel connectivity using bivariate correlation analyses of the BOLD time-series. During ROI-to-ROI analyses, six predefined ROI were chosen based on their relevance to salience processing, subjective valuation, and cognitive control: 1. Bilateral ventral tegmental area (VTA)/midbrain 10mm sphere ROI mask; 2. Bilateral medial orbitofrontal cortex (mOFC) ROI mask based on automated anatomical labeling (AAL; Tzourio-Mazoyer et al., 2002); 3. Bilateral ventral striatum (VS) 5mm sphere ROI mask; 4. right frontoinsular cortex 8 mm sphere ROI based on coordinates from previous resting state fMRI studies (Uddin, Supekar, Ryali, & Menon, 2011); 5. SN mask ROI generated by combining SN node ROIs from a previous study (Uddin et al., 2011); 6. CEN mask ROI generated by
combining CEN node ROIs from a previous study (Uddin et al., 2011). Right frontoinsular cortex was chosen over left frontoinsular cortex for these ROI-to-ROI analyses, because of its putative importance in switching between endogenous and exogenous attention networks (Sridharan, Levitin, & Menon, 2008). Although, an aim of the additional voxelwise analyses was to examine connectivity with a left FIC seed.

**Intrinsic Connectivity Group Comparisons:**

We then analyzed the CONN intrinsic connectivity output values ($\beta$’s, Fischer’s $r$-to-$Z$ transformed correlation coefficients) in the context of the participants’ behavioral/questionnaire/demographics data. In particular, during the ROI-to-ROI analysis, we performed an analysis of covariance (ANCOVA) on the pre-specified ROI-based connectivity $\beta$’s with family history status (FHP vs. FHN) as a fixed factor and the following covariates: total AUDIT score, cumulative BIS score, age, and CAPUQ Q1. While these measures did not significantly differ between the groups, there were trends towards significance with these variables, and thus they could potentially confound statistical analysis.

**Correcting for Multiple Comparisons:**

We followed up our ANCOVA using the Bonferroni-Holm method to correct for multiple comparisons during the ROI-to-ROI analyses. However, given the large number of comparisons of connectivity $\beta$’s (11), this may have been a relatively conservative method to correct for this problem, which could have led to inflation in Type-2 error. Only the two comparisons, which are reported here, survived this correction.
Results

Group Comparisons of Demographic and Questionnaire Measure:

The FHP and FHN groups did not differ in terms of their socioeconomic status or age (see Table 1 for more information). The estimated IQ of the FHP individuals was higher, with a trend towards significance at the .05 level. In addition, for the FHP group, 54.5% of the total number of individuals was female, and 22.7% of the group was non-white. Furthermore, for the FHN group, 50% of the total number of individuals was female, and 50% of the group was non-white. Importantly, the sample sizes for the groups of interest were unbalanced: only 22 of the total sample of 58 were family history positive. Additional statistical information about the groups’ questionnaire measures are provided in Table 1 and Table 2. Notably, the groups do not significantly differ in overall trait impulsivity and alcohol consumption behaviors. Potential differences between the groups were assessed by examining whether the bootstrapped, bias corrected and accelerated 95% confidence intervals for mean differences contained zero.
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<td></td>
<td>Lower limit</td>
</tr>
<tr>
<td>Age</td>
<td>27.3 (6.5)</td>
<td>26.1 (4.8)</td>
<td>-2.30</td>
</tr>
<tr>
<td>Estimated IQ (WAIS)</td>
<td>104.0 (6.6)</td>
<td>107.4 (5.2)</td>
<td>-6.16</td>
</tr>
<tr>
<td>Years of Education</td>
<td>16.3 (2.2)</td>
<td>16.8 (2.7)</td>
<td>-1.90</td>
</tr>
<tr>
<td>BSMSS</td>
<td>50.7 (11.9)</td>
<td>51.1 (11.7)</td>
<td>-5.97</td>
</tr>
<tr>
<td>FTPI Mean Extension</td>
<td>6.2 (4.4)</td>
<td>8.2 (4.5)</td>
<td>-4.35</td>
</tr>
<tr>
<td>BIS</td>
<td>53.3 (8.1)</td>
<td>52.8 (15.4)</td>
<td>-6.11</td>
</tr>
</tbody>
</table>

*Note.* Bootstrapped confidence intervals based on 1000 bootstrap replications. WAIS: Wechsler Adult Intelligence Scale, estimated from Shipley Institute of Living Scale. BSMSS: Barratt Simplified Measure of Social Status. FTPI: Future Time Perspective Inventory. BIS: Barratt Impulsiveness Scale.
Table 2. Means (SD) and bootstrapped 95% confidence intervals for substance use-related questionnaire measures

<table>
<thead>
<tr>
<th></th>
<th>FHN (n=22)</th>
<th>FHP (n=36)</th>
<th>Bias Corrected and Accelerated 95% Confidence Interval for Mean Difference</th>
<th>Lower limit</th>
<th>Upper Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUDIT</td>
<td>4.2 (3.2)</td>
<td>4.7 (2.7)</td>
<td>-1.97</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td>Average Caffeine</td>
<td>1.1 (0.9)</td>
<td>1.7 (3.4)</td>
<td>-2.50</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>Consumption per Day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUQ Binge Drinking</td>
<td>10.7 (11.1)</td>
<td>11.5 (8.6)</td>
<td>-6.06</td>
<td>4.20</td>
<td></td>
</tr>
<tr>
<td>DASS (Depression)</td>
<td>1.8 (2.8)</td>
<td>3.5 (4.4)</td>
<td>-3.79</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>DASS (Anxiety)</td>
<td>2.1 (2.7)</td>
<td>1.2 (1.4)</td>
<td>-0.09</td>
<td>1.96</td>
<td></td>
</tr>
<tr>
<td>DASS (Stress/Tension)</td>
<td>3.9 (4.7)</td>
<td>5.7 (4.9)</td>
<td>-4.37</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>DUSI Problem Density</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.1)</td>
<td>-0.09</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>CAUPQ-Q1</td>
<td>2.9 (2.3)</td>
<td>2.1 (1.2)</td>
<td>-0.03</td>
<td>1.85</td>
<td></td>
</tr>
<tr>
<td>CAUPQ-Q2</td>
<td>1.2 (1.5)</td>
<td>1.7 (1.4)</td>
<td>-1.23</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>CAUPQ-Q3</td>
<td>17.6 (4.0)</td>
<td>16.6 (4.5)</td>
<td>-1.04</td>
<td>3.58</td>
<td></td>
</tr>
<tr>
<td>CAUPQ-Q4</td>
<td>0.6 (1.3)</td>
<td>0.3 (0.7)</td>
<td>-0.34</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>CAUPQ-Q5</td>
<td>1.4 (1.7)</td>
<td>2.0 (1.8)</td>
<td>-1.59</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>CAARS (Inattention)</td>
<td>7.5 (3.7)</td>
<td>9.2 (5.2)</td>
<td>-4.01</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>CAARS (Hyperactivity)</td>
<td>9.9 (4.8)</td>
<td>10.5 (5.1)</td>
<td>-3.02</td>
<td>1.92</td>
<td></td>
</tr>
<tr>
<td>CAARS (Impulsiveness)</td>
<td>5.5 (3.8)</td>
<td>7.5 (4.9)</td>
<td>-4.43</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>CAARS (Self Concept Problem)</td>
<td>3.7 (2.5)</td>
<td>4.4 (3.3)</td>
<td>-2.42</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>CAARS ADHD Index</td>
<td>7.0 (4.0)</td>
<td>8.6 (4.5)</td>
<td>-3.72</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>DMQ-r (Social)</td>
<td>13.7 (4.8)</td>
<td>13.9 (4.5)</td>
<td>-2.33</td>
<td>2.44</td>
<td></td>
</tr>
<tr>
<td>DMQ-r (Coping)</td>
<td>7.3 (3.0)</td>
<td>6.9 (2.0)</td>
<td>-0.82</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>DMQ-r (Enhance)</td>
<td>10.3 (4.3)</td>
<td>12.0 (4.2)</td>
<td>-3.96</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>DMQ-r (Conform)</td>
<td>6.8 (2.8)</td>
<td>6.6 (1.6)</td>
<td>-0.95</td>
<td>1.31</td>
<td></td>
</tr>
<tr>
<td>FTQ Number of Family</td>
<td>0.1 (0.2)</td>
<td>1.6 (0.9)</td>
<td>-2.00</td>
<td>-1.14</td>
<td></td>
</tr>
<tr>
<td>Members with Potential</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking Problems</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: Bootstrapped confidence intervals based on 1000 bootstrap replications. AUDIT: Alcohol Use Disorder Identification Test. AUQ: Alcohol Use Questionnaire. DASS: Depression Anxiety Stress Scales. DUSI: Drug Use Screening Inventory. CAUPQ: Carolina Alcohol Use Pattern Questionnaire.*
ROI-to-ROI analyses:

The six predefined ROIs were tested for connectivity differences in the two primary groups of interest: individuals who self-reported having one or more biological siblings, parents, or grandparents with potentially problematic drinking (FHP) and individuals reporting no family members with possible problematic drinking behavior (FHN). Two different neural circuits of interest were differentially connected in the FHP and FHN individuals. An ANCOVA comparing rFIC-CEN connectivity between FHP and FHN groups, controlling for AUDIT scores, BIS scores, age, and “typical day” alcohol consumption, revealed a main effect of FH status on connectivity between the rFIC ROI and the CEN ROI mask ($F_{(1,51)} = 11.053$, $p = 0.003$, $\eta^2 = 0.134$). $P$ values are reported uncorrected for multiple comparisons, although this test of significance survived the Bonferroni-Holm method correction in follow-up calculations. A visual representation of the rFIC and CEN ROIs are displayed in Figure 1. The adjusted mean difference between groups for rFIC-CEN connectivity was 0.169, reflecting higher average connectivity in the FHN group (0.229) relative to the FHP group (0.060; Fig. 2).
Figure 1. Visual representation of rFIC (top) and CEN (bottom) ROIs displayed on a MNI template brain. rFIC: right Frontoinsular Cortex. CEN: Central Executive Network.
Figure 2. Bar graph representing means of beta values for rFIC-CEN connectivity for the FHN and FHP groups. SN: Salience Network. CEN: Central Executive Network. rFIC: right Frontoinsular Cortex.
In addition, connectivity between the VTA/midbrain ROI and the mOFC ROI was different between the groups: the ANCOVA for FHP vs. FHN on VTA/midbrain-mOFC connectivity, controlling for AUDIT scores, BIS scores, age, and CAUPQ Q1 “typical day” alcohol consumption, revealed a main effect of FH status, $F_{(1, 51)} = 8.746, p = 0.005$. 

$P$ values are reported uncorrected for multiple comparisons, although this test of significance survived the Bonferroni-Holm method correction in follow-up calculations. Similarly to the rFIC-CEN connectivity comparison, the eta-squared statistic (0.134) indicated a medium to large effect size. The adjusted mean difference for the VTA/midbrain-mOFC connectivity was 0.139, with the FHP group having a higher adjusted mean of 0.245 and the FHN group having a lower adjusted mean of 0.106. A visual representation of the VTA/midbrain and mOFC ROIs are displayed in Figure 3 and a bar graph representing FHP vs FHN group mean differences is displayed in Figure 4.
Figure 3. Visual representation of VTA/midbrain (top) and mOFC (bottom) ROIs displayed on an MNI template brain. VTA: Ventral Tegmental Area. mOFC: Medial Orbitofrontal Cortex.
**Figure 4.** Bar graph representing means of beta values for rFIC-CEN connectivity for the FHN and FHP groups. VTA: Ventral Tegmental Area. mOFC: medial Orbitofrontal Cortex.

Exploratory ROI-to-voxel analyses:
rFIC Connectivity: FHP vs. FHN
Exploratory ROI-to-Voxel analyses were performed using rFIC, left FIC (lFIC), and VTA/midbrain seed regions. rFIC and VTA/midbrain connectivity were specifically examined because of group differences examined in the ROI-to-ROI connectivity results. The rFIC of FHP adults was less functionally connected with six different clusters across the whole brain (Table 2, Fig. 5). These clusters were primarily located in the following AAL atlas based regions: right and left superior temporal gyrus, left inferior parietal lobule, left medial superior frontal gyrus, the triangular part of the left inferior frontal gyrus, and left superior frontal gyrus.
Table 2

Locations and coordinates for clusters of significantly greater rFIC seed connectivity in the FHP adults relative to the FHN adults.

<table>
<thead>
<tr>
<th>Location label</th>
<th>Cluster peak coordinate (MNI)</th>
<th>Number of voxels</th>
<th>Height $p$ (uncorrected)</th>
<th>Cluster $p$ (FDR corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Superior Temporal Gyrus</td>
<td>58, -20, 6</td>
<td>775</td>
<td>0.000004</td>
<td>0.000000</td>
</tr>
<tr>
<td>Left Superior Temporal Gyrus</td>
<td>-38, -38, 18</td>
<td>393</td>
<td>0.000011</td>
<td>0.000091</td>
</tr>
<tr>
<td>Left Inferior Parietal Lobule</td>
<td>-50, -54, 48</td>
<td>197</td>
<td>0.000045</td>
<td>0.005016</td>
</tr>
<tr>
<td>Left Medial Superior Frontal Gyrus</td>
<td>-10, 46, 30</td>
<td>191</td>
<td>0.000015</td>
<td>0.005016</td>
</tr>
<tr>
<td>Left Inferior Frontal Gyrus (Triangular Part)</td>
<td>-48, 18, 12</td>
<td>191</td>
<td>0.000040</td>
<td>0.005016</td>
</tr>
<tr>
<td>Left Superior Frontal Gyrus</td>
<td>-14, 32, 40</td>
<td>131</td>
<td>0.000144</td>
<td>0.024610</td>
</tr>
</tbody>
</table>

Note. Results shown based on FDR-corrected cluster threshold of $p=.05$. and uncorrected height threshold of $p=.001$
Figure 5. Visual representation of FHP > FHN contrast based on the rFIC functional connectivity map, displayed on an MNI template brain. Contrast image based on t-statistics, where hot (red) colors are areas that are more functionally connected to the rFIC in FHP individuals and cool (blue) colors are areas that are more functionally connected to the rFIC in FHN individuals. Statistical threshold: $p = .001$ uncorrected. FHP: Family History Positive. FHN: Family History Negative. rFIC: Right Frontoinsular Cortex.
VTA/Midbrain Connectivity: FHP vs. FHN

Following up from the VTA/midbrain-mOFC (ROI-to-ROI) connectivity comparison, we explored regions differentially connected to the VTA/midbrain in the FHP and FHN groups with a ROI-to-Voxel analysis. Bilateral VTA/midbrain of FHP adults was more functionally connected with three different clusters in the brain (Table 3; Figure 6). These clusters were primarily located in right and left angular gyrus and left medial orbitofrontal gyrus. A visualization of the group differences is presented in Figure 6. Along with the rFIC connectivity voxel based analysis, these results agree with the initial pre-specified ROI-to-ROI connectivity finding that used ROIs from selected neuroimaging literature and an AAL anatomical volume of interest (medial orbitofrontal gyrus). Given that these regions differentially connected to VTA in the FHP group are typically associated with the default mode network, we decide to lower the statistical threshold to $p = .01$ for visualization purposes. Statistics reported were calculated using a threshold of $p = .001$. 

Table 3

Locations and coordinates for clusters of significantly greater VTA/midbrain seed connectivity in the FHP adults relative to the FHN adults.

<table>
<thead>
<tr>
<th>Location label</th>
<th>Cluster peak coordinate (MNI)</th>
<th>Number of voxels</th>
<th>Height $p$ (uncorrected)</th>
<th>Cluster $p$ (FDR corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Angular Gyrus</td>
<td>42, -60, 30</td>
<td>276</td>
<td>0.000003</td>
<td>0.000668</td>
</tr>
<tr>
<td>Left Angular Gyrus</td>
<td>-36, -64, 28</td>
<td>151</td>
<td>0.000065</td>
<td>0.011460</td>
</tr>
<tr>
<td>Left Medial Orbitofrontal Gyrus</td>
<td>-10,58, -6</td>
<td>117</td>
<td>0.000051</td>
<td>0.022349</td>
</tr>
</tbody>
</table>

*Note. Results shown based on FDR-corrected cluster threshold of $p=.05$ and uncorrected height threshold of $p=.001$*
Figure 6. Visual representation of FHP > FHN contrast based on the VTA/midbrain functional connectivity map, displayed on an MNI template brain. Contrast image based on t-statistics, where hot (red) colors are areas that are more functionally connected to the VTA/midbrain in FHP individuals and cool (blue) colors are areas that are more functionally connected to the VTA/midbrain in FHN individuals. Statistical (height) threshold lowered to $p = .01$ for visualization purposes. FHP: Family History Positive. FHN: Family History Negative. VTA: Ventral Tegmental Area.
lIFIC connectivity: FHP vs. FHN

In addition to our a priori rIFIC seed analyses, we conducted exploratory whole brain analyses with an lIFIC seed. Both the FHP and FHN groups had their own unique lIFIC connectivity patterns; that is, both groups revealed increased functional connectivity with specific regions of the brain (Tables 4; Figure 7). While the FHP group exhibited greater lIFIC connectivity with a cluster in the occipital lobe (right calcarine fissure, right middle and superior occipital gyrus), the analysis of the FHN group revealed increased lIFIC connectivity with a few sizable clusters covering both cortical and subcortical structures: the right superior temporal gyrus/rolandic operculum cluster was contiguous with structures such as the right putamen, insula, Heschl’s gyrus, precentral gyrus, postcentral gyrus, and supramarginal gyrus; the left insula cluster was contiguous with left superior temporal gyrus, inferior frontal operculum, precentral and postcentral gyri, rolandic operculum, and Heschl’s gyrus; the right inferior frontal operculum cluster also overlapped with the right triangular part of the inferior frontal gyrus. A visualization of the group differences based on lIFIC connectivity are presented in Figure 7.
**Table 4**

*Locations and coordinates for clusters of significantly greater VTA/midbrain seed connectivity in the FHP adults and FHN adults*

<table>
<thead>
<tr>
<th>Location Label</th>
<th>Cluster peak coordinate (MNI)</th>
<th>Number of voxels</th>
<th>Height ( p ) (uncorrected)</th>
<th>Cluster ( p ) (FDR corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FHP &gt; FHN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Occipital Cortex</td>
<td>28, -98, 6</td>
<td>210</td>
<td>0.000018</td>
<td>0.005878</td>
</tr>
<tr>
<td><strong>FHN &gt; FHP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Superior Temporal Gyrus / Rolandic Operculum</td>
<td>58, -2, 8</td>
<td>1816</td>
<td>0.000001</td>
<td>0.000000</td>
</tr>
<tr>
<td>Left insula / Superior Temporal Gyrus</td>
<td>-56, 2, 24</td>
<td>1169</td>
<td>0.000002</td>
<td>0.000000</td>
</tr>
<tr>
<td>Right Inferior Frontal Operculum</td>
<td>50, 18, 16</td>
<td>146</td>
<td>0.000016</td>
<td>0.024253</td>
</tr>
</tbody>
</table>

*Note.* Results shown based on FDR-corrected cluster threshold of *p*=.05 and uncorrected height threshold of *p*=.001
Figure 7. Visual representation of FHP > FHN contrast based on the IFIC functional connectivity map, displayed on an MNI template brain. Contrast image based on t-statistics, where hot (red) colors are areas that are more functionally connected to the IFIC in FHP individuals and cool (blue) colors are areas that are more functionally connected to the IFIC in FHN individuals. Statistical (height) threshold: \( p = .001 \) uncorrected. FHP: Family History Positive. FHN: Family History Negative. IFIC: left Frontoinsular Cortex.
FHP Male and FHP Female Group Comparison

As an additional goal of this study was to explore potential connectivity differences based on FHP subgroups, we first examined differences in demographic and questionnaire measures to assess confounding factors that would limit interpretation of specific gender effects. The sample size of the FHP males was ten, while the sample size of the FHP females was twelve. The FHP male group was composed of 7 white and 3 non-white individuals, while the FHP female group was composed of 10 white and 2 non-white individuals. The groups were statistically similar with respect to age, socioeconomic status (BSMSS), and years of education. The estimated IQ of FHP males was higher than for females, with a trend towards significance at the .05 level. Notably, the FHP Male and FHP Female groups did not differ in terms of trait impulsiveness (BIS), depression/anxiety related behaviors (DASS), ADHD related behaviors (CAARS ADHD index), average caffeine consumption, alcohol use severity (AUDIT), binge drinking patterns (AUQ Binge score, CAUPQ responses), and drinking motives (DMQ-r).
rFIC connectivity: FHP Male vs. FHP Female

In addition to the comparisons of connectivity between the FHP and FHN groups, three supplementary analyses were conducted, examining voxelwise connectivity differences between the FHP males (n=10) and FHP females (n=12). First, the rFIC ROI-to-voxel connectivity comparison identified one cluster of voxels in the left superior frontal gyrus and surrounding areas was more highly connected with the rFIC seed in the FHP male group than in the FHN female group (Fig. 8; Table 5). We identified no brain regions more highly connected with the rFIC ROI in the FHP female group relative to the male FHN group.

Table 5

*Location and coordinates for cluster of significantly greater rFIC seed connectivity in the FHP adult males*

<table>
<thead>
<tr>
<th>Cluster number</th>
<th>Cluster peak coordinate (MNI)</th>
<th>Number of voxels</th>
<th>Height p (uncorrected)</th>
<th>Cluster p (FDR corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Superior Frontal Gyrus</td>
<td>-18, 2, 48</td>
<td>123</td>
<td>0.000001</td>
<td>0.038592</td>
</tr>
</tbody>
</table>

*Note. Results shown based on FDR-corrected cluster threshold of p=.05. and uncorrected height threshold of p=.001*
Figure 8. Visual representation of FHP males > FHP females contrast based on the rFIC functional connectivity map, displayed on an MNI template brain. Contrast image based on t-statistics, where hot (red) colors are areas that are more functionally connected to the rFIC in FHP male individuals and cool (blue) colors are areas that are more functionally connected to the rFIC in FHP female individuals. Statistical (height) threshold: $p = .001$ uncorrected. FHP: Family History Positive. rFIC: right Frontoinsular Cortex.
VTA/midbrain connectivity: FHP Male vs FHP Female

Using an FDR-corrected cluster threshold of $p = .05$ and an uncorrected height threshold of $p = .001$, a contrast for the VTA/midbrain functional maps of FHP males versus females revealed no areas of the brain differentially connected between the FHP subgroups.

lFIC connectivity: FHP Male vs FHP Female

While no regions of the brain were found to be differentially connected with the VTA in the FHP male versus FHP female groups, a comparison of left FIC connectivity produced two sets of intriguing results. For the FHP male group, there were seven different clusters found to be more highly connected with the lFIC (Table 6; Figure 9): a cluster based primarily in the left and right precuneus; a cluster centered on the right insula; A right and left middle cingulum cluster; a cluster in the sixth lobule of the cerebellar vermis; a smaller cluster based in the right/left precuneus; a right precentral gyrus cluster; a right superior frontal gyrus / supplementary motor area cluster. Interestingly, the lFIC connectivity pattern for the FHP females was constrained to only two statistically significant clusters: A cluster in the superior frontal gyrus/medial superior frontal gyrus area and a cluster in right Crus I-II of the cerebellum. A visualization of the group differences based on lFIC connectivity is presented in Figure 9.
Table 6

Location and coordinates for cluster of significantly greater IFIC seed connectivity in the FHP adult males and FHP adult females

<table>
<thead>
<tr>
<th>Location Label</th>
<th>Cluster peak coordinate</th>
<th>Number of voxels</th>
<th>Height p (uncorrected)</th>
<th>Cluster p (FDR corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FHP Males &gt; FHP Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Precuneus</td>
<td>6, -66, 54</td>
<td>982</td>
<td>0.000000</td>
<td>0.000000</td>
</tr>
<tr>
<td>Right Insula</td>
<td>34, 20, 8</td>
<td>224</td>
<td>0.000059</td>
<td>0.000635</td>
</tr>
<tr>
<td>Left middle cingulum</td>
<td>-8, 18, 28</td>
<td>147</td>
<td>0.000105</td>
<td>0.006022</td>
</tr>
<tr>
<td>Cerebellum Vermis (Lobule 6)</td>
<td>-30, -60, -22</td>
<td>128</td>
<td>0.000027</td>
<td>0.009295</td>
</tr>
<tr>
<td>Right Precuneus</td>
<td>2, -48, 52</td>
<td>111</td>
<td>0.000070</td>
<td>0.014629</td>
</tr>
<tr>
<td>Right Precentral Gyrus</td>
<td>50, 0, 28</td>
<td>100</td>
<td>0.000004</td>
<td>0.017204</td>
</tr>
<tr>
<td>Right Superior Frontal Gyrus</td>
<td>16, 14, 66</td>
<td>99</td>
<td>0.000095</td>
<td>0.017204</td>
</tr>
<tr>
<td><strong>FHP Females &gt; FHP Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Superior/Medial Superior Frontal Gyrus</td>
<td>-2, 48, 46</td>
<td>384</td>
<td>0.000017</td>
<td>0.000006</td>
</tr>
<tr>
<td>Right Cerebellum Crus I-II</td>
<td>38, -80, -38</td>
<td>186</td>
<td>0.000008</td>
<td>0.001072</td>
</tr>
</tbody>
</table>

*Note. Results shown based on FDR-corrected cluster threshold of \( p = .05 \) and uncorrected height threshold of \( p = .001 \)
Figure 9. Visual representation of FHP males > FHP females contrast based on the lFIC functional connectivity map, displayed on an MNI template brain. Contrast image based on t-statistics, where hot (red) colors are areas that are more functionally connected to the lFIC in FHP male individuals and cool (blue) colors are areas that are more functionally connected to the lFIC in FHP female individuals. Statistical (height) threshold: $p = .001$ uncorrected. FHP: Family History Positive. lFIC: left Frontoinsular Cortex.
Discussion

The goal of the current investigation was to examine neural connectivity differences associated with AUD family history status in adults. To date, only three studies (Herting et al., 2010; Wetherill et al., 2012; Cservenka et al., 2014) have used functional connectivity methods to examine biologically inherited AUD risk factors based on family history status. However, all of these studies examined the effects of AUD family history in adolescents. Therefore, the study at hand is the first to utilize functional connectivity methods while examining the neurocognitive effects of family history of alcohol abuse in adults.

Nevertheless, the large amount of literature on intrinsic connectivity networks can help guide interpretation. Specifically, drawing inspiration from Vinod Menon’s “triple network model of psychopathology” will be particularly valuable. As with other neuropsychiatric disorders, the triple network model predicts that AUD and AUD risk relate to dysfunctional connectivity within and between the two “task-positive” networks (salience network and central executive network) and the “task-negative network” (default mode network). Dysregulated interconnectivity, Menon argues, possibly relate to aberrant salience mapping in and out of the salience network, such that there is dysregulated reward signaling or self-referential processing, for example (Menon, 2011). Specifically, AUD, and more broadly SUD, could be potentially associated with weakened outflow from the SN into the CEN, potentially represented by diminished FIC connectivity. A supplementary hypothesis is that there would be strengthened outflow from the SN into the DMN, representing interference of self-referential processing in normal externally driven, goal directed behavior. The results of a recent resting state
fMRI study has indeed found that increased coupling between “task positive” and “task negative” networks in alcohol dependent patients results in decreased cognitive control (Schmaal et al., 2013). Taken together, support for these hypotheses would imply a functional disintegration of the typically coactive “task-positive networks”.

Both results from the ROI-to-ROI (Figures 2,4) and ROI-to-voxel analyses (Figures 5, 6, 7) potentially provide support for these ideas. The finding that FHP adults have decreased rFIC-CEN connectivity (Figure 1) gives reason to believe that weakened interconnectivity between SN and CEN underlies the disintegration of cognitive control and working memory processes which is sometimes revealed in behavioral and neuroimaging studies of adolescents and adults with AUDs or AUD risk? (Lovallo et al., 2006; Heitzeg et al., 2008). While the VTA connectivity findings are by no mean conclusive, the increased VTA-mOFC and VTA-angular gyrus connectivity pattern of the FHP adults warrant future investigation into the interconnectivity between VTA and the DMN. Given the meaningful pattern of SN-DMN interconnectivity revealed by lowering the statistical threshold of the family history status whole-brain contrast (Figure 6) and the fact that VTA is known to be intrinsically connected with SN nodes like the FIC and ACC (Seeley et al., 2007), there could be reason to speculate that VTA connectivity with the DMN is somehow biasing information processing in the brain, and weakening the normal transmission of cognitive control signals from the SN to the CEN. This hypothesis agrees with the aforementioned results of a recent connectivity study showing that cognitive control deficits positively correlated with DMN-SN and DMN-CEN connectivity (Schmaal et al., 2013). To test these more specific hypotheses, analyses involving effective connectivity methods, such as Granger Causality modeling, would
need to be utilized. As functional connectivity investigations typically defined only measure temporal coherence of the BOLD activation time series, researchers using these methods are inherently restricted in their data interpretation.

Broadly speaking, the FIC connectivity maps support the hypothesis that AUD risk, as well risk for other behavioral disorders, relates to insula underconnectivity (Uddin & Menon, 2009). As the anterior insula regions are known to be hubs for the integration of cognitive, affective, and homeostatic information (Menon and Uddin, 2011; Eckert et al., 2009), aberrant connectivity with these regions could possibly reflect several different functional consequences, which have yet to be fully investigated: dysregulated interoceptive awareness (Craig, 2009), deficits in attention processing (Touroutoglou, Hollenbeck, Dickerson, & Barrett, 2012), or maladaptive economic decision making (Kuhnen & Knutson, 2005). In addition, many of the regions found to be less connected in the FHP group, such as inferior frontal gyrus and dorsomedial PFC have been implicated in cognitive control and response inhibition (Aron, Robbins, & Poldrack, 2004); dysfunctional connectivity of these cognitive control regions with insula and other SN regions may impair executive functions and may account for the effects of family history status on regional brain activations using standard GLM based contrasts. Moreover, these other results provided more evidence for the proposal that FHP adults may have weakened transmission of cognitive control signals.

Comparing the results of this current study’s fMRI connectivity analysis of FHP and FHN adults to previous connectivity studies investigating AUD familial risk, there seems to be a shared pattern of decreased frontal connectivity within both FHP adolescents and adults. Herting et al., 2010 found that alcohol-naïve FHP adolescents
possessed decreased frontal connectivity with multiple regions of the cerebellum. However, as the cerebellum was chosen as the seed region for their analyses, other frontal/frontoparietal based connectivity differences were not fully assessed. More informatively, Wetherill et al., 2012 found that there was decreased within-network connectivity for multiple frontoparietal regions: left posterior parietal cortex, right posterior parietal cortex, and right dorsolateral prefrontal cortex in those with FH of AUDs. Many of these functional differences may be more thoroughly explained by investigation into structural, white matter differences between the FHP and FHN groups. Indeed, one study in particular has compared the white matter microstructure of FHP and FHN youth using Diffusion Tensor Imaging (DTI) (Herting et al., 2010). Herting and colleagues specifically found that there was significant disruption in white matter tracts within prefrontal, corticostriatal, and subcortical circuitry of FHP youth. Moreover, these researchers found that reaction times during a delay discounting task negatively correlated with fractional anisotropy (FA; a measure of white matter integrity), leading them to reason that this inherited risk factor relates to inefficient cortical processing. These findings, taken together with the results of current intrinsic connectivity analysis, may imply that decreased functional connectivity of the CEN and SN relates to diminished information processing efficiency, especially during cognitively demanding tasks.

A complementary goal of the current analysis was to further distinguish neural connectivity patterns of inheritance risk subgroups such as FHP males and females. Because FHP males have been historically thought of as more prone to genetic AUD risk, examining differences in intrinsic neural circuitry is potentially informative. Most
notably, the intrinsic connectivity analyses revealed increased coupling between the rFIC and premotor cortex (PMC)/superior frontal gyrus of FHP males (Figure 8) and increased coupling between the lFIC and dorsal prefrontal cortex (dPFC) of FHP females (Figure 9). Shannon et al., 2011 found that abnormal PMC connectivity predicted impulsive behaviors in juvenile offenders. Thus, this FIC-PMC connectivity could possibly underlie impulsivity and should be investigated further. The FIC-dPFC connectivity difference in FHP females is particularly interesting, because this area of the frontal cortex also distinguished FHN adults from FHP adults (Figure 5). This analysis of gender differences in FHP individuals establishes a starting point for future examination into the effects of AUD family history status on both male and female brain connectivity.

**Limitations**

Similarly to DeVito et al., 2013, the current study may lack some degree of generalizability given that the FHP individuals did not differ significantly on any questionnaire measures regarding impulsivity or alcohol consumption and dependence. Since we selectively recruited for “social” drinkers that had no history of psychiatric disorders, we may have been limited in the range of potentially risk prone FHP individuals. Future studies may benefit from including adults with mild alcohol use disorder to better account for this problem.

The current investigation also suffered from the problem of uneven sample sizes: we collected data for 22 FHP adults and 36 FHN adults. As this an ongoing fMRI study continues to accumulate more participants’ data, it will be important to address this issue. Fortunately, however, both samples were of an adequate size relative to other recently
reported fMRI studies and the samples did not differ in terms of questionnaire and demographic measures. Both were evenly weighted in terms of gender, for example.

Also, recognizing that the VTA/midbrain ROI was not constrained to a very small area, future analyses should use a smaller ROI, specifically targeting the VTA. Using ROIs based on subject specific anatomy may also be useful.

Other limitations relate to the operationalization of family history status. Many studies have differed on how they define AUD FHP individuals. While some researchers indicate that FHP adults are only those with first-degree relatives with potential problem drinking, others require the participant to report multiple problematic drinking family members. Additionally, for this set of analyses, the effects of family history of AUD on brain connectivity were measured by using family history status as a categorical variable. Future analyses should explore additional correlational analyses using the number of problematic drinking relatives for each participant as a continuous variable.
Conclusion and Future Directions

In summary, these results suggest dysfunctional interconnectivity between SN, CEN, and DMN is associated with familial risk for AUDs. Specifically, decreased resting state rFIC-CEN connectivity may serve as a neurocognitive biomarker for AUD risk and an intermediate phenotype for AUDs. VTA-mOFC ROI analyses and VTA whole-brain functional connectivity maps indicate the possibility of heightened connectivity between SN and DMN in FHP individuals, which could theoretically underlie difficulties in cognitive control and behavioral inhibition among FHP individuals. Moreover, these data implicate the dysfunctional interactions of “task-positive networks” as a potential risk factor for AUD development. Future research should use effective connectivity methods to assess differences in the casual relations between these intrinsic networks in the FHP and FHN groups.
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