Does substance use during youth cause lasting changes in resting-state neurophysiology and brain functional connectivity? A co-twin control investigation

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Dedication

To mom and dad.

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Overview

This dissertation comprises two studies aimed at disentangling potential causal effects of recreational substance use (alcohol, cannabis, tobacco) on resting-state electroencephalogram (EEG) and functional magnetic resonance imaging (fMRI) brain outcomes in a community sample of young adults. As noted by the introductory text for each study, there is a dearth of causally-informative research designs in published literature regarding whether drug and alcohol use has lasting effects on human EEG and fMRI. These two studies intend to bridge this gap by utilizing a causally-informative co-twin control (CTC) research design which utilizes the fact that twins reared in the same home are matched on many factors (e.g., genes, parental substance use, SES) that contribute to confounding in the hypothetical causal link between substance use and brain outcomes in extant cross-sectional research. As such, within twin-pair differences in use can be exploited to study within twin-pair differences in brain outcome (e.g., EEG, fMRI) to understand possible causal effects.

Although the two studies presented here overlap with regards to aims and methodologies, there are a couple of noticeable discrepancies among them that should be explained. For instance, although both studies focus on the fourth assessment of the Enrichment Sample of the Minnesota Center for Twin and Family Research, the sample size for Study 1 (N = 481) is different from that in Study 2 (N = 304), and neither study matches the sample size of the original 998 subjects (Keyes et al., 2009). The first reason for these sample size discrepancies is that data were drawn from an ongoing assessment that is not yet complete. Study 1 included all subjects with usable resting-state EEG data

as of June 29, 2017 and Study 2 included all subjects with usable resting-state fMRI data as of January 15, 2017. An earlier cutoff date was chosen for Study 2 than that chosen for Study 1 because an MRI scanner upgrade occurred on January 16, 2017, which would have complicated statistical analyses if fMRIs were included that were collected after this date. Additionally, stricter exclusion criteria (e.g., history of metal-working, metal implants, piercings, etc.) were implemented in Study 2 for reasons specific to extra precautions taken in the MRI environment.

While Study 1 focused only on alcohol use with relation to spectral power of the resting-state EEG, Study 2 focused on three substances (alcohol, cannabis, and tobacco use) with relation to ventral striatal functional connectivity as measured with fMRI. The decision to focus only on alcohol use in Study 1 was motivated by an extensive background of animal and human EEG research that suggests alcohol use-related hyperarousal of the central nervous system (CNS), which may be reflected in increased spectral power in beta frequencies (13 to 30 Hz) (Kamarajan & Porjesz, 2015; Rangaswamy & Porjesz, 2014). While I do not presume there to be no impact of cannabis and tobacco use on EEG power, the relation of these substances to resting-state EEG is currently less well-characterized (Herning, Better, & Cadet, 2008; Herning, Better, Tate, & Cadet, 2003; Struve, Straumanis, & Patrick, 1994; Struve et al., 1999; Struve, Straumanis, Patrick, & Price, 1989), and thus forming hypotheses at this stage would be difficult. By contrast, alcohol, cannabis, and tobacco use were investigated in Study 2 because these three substances are thought to similarly affect brain circuitry involving the

ventral striatum (Di Chiara & Imperato, 1988; Sulzer, 2011). Future research should aim to better characterize spectral EEG power in regular cannabis and tobacco users.

Additionally, the personality measures investigated in each study were chosen based on leading hypotheses regarding their involvement in substance use pathology. Specifically, we focused on personality indices reflecting negative affect (e.g., stress reactivity, anhedonia) and inhibitory control (e.g., impulsivity, risk taking) in Study 1 because these two personality domains encompass competing theories on what psychological function is reflected by increased beta power in alcohol users. By contrast, in Study 2 we investigated two measures of inhibitory control thought to tap into different aspects of diminished "top-down" control of addictive behaviors, including impulsivity and compulsive substance use.

Further descriptions for each study are as follows:

1. Alcohol use during sensitive developmental periods such as youth (ages 11 to 25) may have deleterious effects on developing neurophysiology, yet most studies examining drinking-related effects on electroencephalogram (EEG) in humans have been cross-sectional, making claims about causal inference impossible. Here, we examined resting-state EEGs of 481 young adults (mean age = 24.5) and employed a co-twin control (CTC) design, which enables parsing causal effects of alcohol use from confounding effects such as shared vulnerability factors (e.g., genes, rearing environment). We found drinking was associated with elevated absolute beta power (13 to 30 Hz) at frontal and central scalp regions. CTC analyses revealed that these effects were

attributable to within twin-pair differences in drinking, such that the twin who had drank more exhibited greater beta power than his or her co-twin who approximates what the greater-drinking twin might have looked like had he/she not drank as much. Further, we found increased beta power to be partially responsible for the association between drinking and heightened negative affect (stress reactivity, anhedonia) but not the association between drinking and diminished inhibitory control (impulsivity, risk taking). Collectively, these findings are consistent with the notion that drinking may cause brain hyperarousal reflected by increased beta power, and that such beta power may reflect negative affective traits in alcohol users.

2. Use of addictive substances during youth may have lasting effects on brain reward circuitry based in ventral striatum (VST), but due to limitations inherent to cross-sectional research commonly applied in human studies, understanding whether substance use has lasting causal effects on such circuits remains difficult. Here, we examined resting-state functional connectivity of VST in a sample of 304 young adults (mean age = 24.5), and employed a co-twin control (CTC) design, which enables parsing causal effects of substance use from confounding effects such as shared vulnerability factors (e.g., genes, rearing environment). Seed-based connectivity analyses revealed that alcohol, cannabis, and tobacco use were associated with decreased functional connectivity of VST with right superior frontal gyrus (rSFG) and several regions previously implicated in the brain's default mode

network (DMN), and that reduced connectivity in rSFG and DMN regions was associated with scales reflecting trait impulsivity and compulsive substance use (respectively). CTC analyses suggested reduced connectivity with DMN regions may be in part attributed to alcohol, cannabis, and tobacco use, such that the twin who has used more exhibited lesser functional connectivity than his or her co-twin who approximates what the greater-using twin may have looked like had he/she not used as much. By contrast, reduced VST functional connectivity with rSFG appeared to reflect familial vulnerability for substance use. Collectively, results suggest that substance use may cause experience-dependent neuroadaptations in VST-based functional circuits, which may explain behaviors relevant to the onset and maintenance of substance use.

Study 1. Does Youth Alcohol Use Cause Changes in Resting-State Neurophysiology? A Co-Twin Control Study

Alcohol is the most commonly used recreational substance by teenagers in the United States (Johnston, O'Malley, Miech, Bachman, & Schulenberg, 2017); a recent report estimates that among 12th graders, more than one-third have used alcohol in the past month and one-fifth have "binge drank" (consumed more than 4 or 5 drinks on one occasion, for females and males, respectively) in the past two weeks (Patrick & Schulenberg, 2014). Crucially, the potential harms of drinking during youth (ages 11 to 25) on neurophysiology may be especially problematic due to alcohol's ability to directly interact with excitatory and inhibitory neurochemicals essential for shaping still-maturing brain systems (for reviews, see Hermens et al., 2013; Jacobus & Tapert, 2013). Yet, because prior human research examining drinking-related neurophysiology have largely relied on cross-sectional study designs, it is impossible to infer whether drinking causes lasting alterations in neurophysiology or whether drinking-related neurophysiology is caused by preexisting genetic and environmental vulnerabilities. The present study investigated drinking-related associations with resting-state electroencephalogram (EEG) using co-twin control analyses to elucidate possible causal effects of drinking on neurophysiology, and related EEG measures to personality constructs associated with drinking behaviors to clarify functional implications of observed possible drinking-related effects.

EEG spectral power and AUDs

Relative to controls, people with alcohol use disorders (AUDs) exhibit several differences in EEG spectral power (summed area under the curve in the oscillatory signal at different frequencies) observed during idle wakefulness (for reviews, see Kamarajan & Porjesz, 2015; Rangaswamy & Porjesz, 2014), which may reflect differences in brain mechanisms responsible for synchronization among cortical neurons (Lopes da Silva, 2005). Of these differences, the most intensely studied characteristic is an excess of beta frequency (13 to 30 Hz) power in AUD subjects (Bauer, 2001; Costa & Bauer, 1997; Ehlers, Phillips, Gizer, Gilder, & Wilhelmsen, 2010; Fein & Allen, 2005; Herrera-Diaz et al., 2016; Kaplan, Glueck, Hesselbrock, & Reed, 1985; Propping, Kruger, & Mark, 1981; Rangaswamy et al., 2002; Saletu-Zyhlarz et al., 2004), perhaps reflecting a state of central nervous system (CNS) hyperarousal (Begleiter & Porjesz, 1999; Perlis, Merica, Smith, & Giles, 2001; Seevers & Deneau, 1963). Moreover, increased beta power has been associated with alcohol craving (De Ridder, Vanneste, Kovacs, Sunaert, & Dom, 2011) and relapse (Bauer, 2001; Saletu-Zyhlarz et al., 2004) in AUD patients, suggesting that beta indexes processes that contribute to AUD severity.

Generally, two theories have emerged regarding the psychological and behavioral processes reflected by increased beta power in AUDs. The first explanation is reminiscent of negative reinforcement theories on addiction (Koob, 2004, 2013; Kushner, Abrams, & Borchardt, 2000; Markou, Kosten, & Koob, 1998; Seevers & Deneau, 1963; Stasiewicz & Maisto, 1993), suggesting that increased beta power might reflect an aberrant and potentially aversive psychophysiological state that is acutely normalized by the CNS-inhibiting effects of alcohol (Bauer & Hesselbrock, 1993; Cohen, Porjesz, &

Begleiter, 1993; Ehlers, Wall, & Schuckit, 1989; Pian, Criado, Walker, & Ehlers, 2008), but exacerbated (i.e. increased beta power) during withdrawal and abstinence following heavy drinking. Consistent with this notion, beta power in healthy subjects has correlated with aversive cognitive and affective processes theorized to contribute to relapse in AUD patients (Heilig, Egli, Crabbe, & Becker, 2010; Sinha & Li, 2007) such as emotional rumination and anxiety (Andersen, Moore, Venables, & Corr, 2009; Cole & Ray, 1985; Pavlenko, Chernyi, & Goubkina, 2009; Stenberg, 1992).

Alternatively, some researchers (Bauer, 2001; Begleiter & Porjesz, 1999; Nunez-Jaramillo et al., 2015) have proposed that increased beta reflects deficits in inhibitory control and increased liability for "externalizing" psychopathology (Kendler, Prescott, Myers, & Neale, 2003; Krueger, 1999; Krueger et al., 2002; Young et al., 2009). While associations among trait impulsivity (e.g., tendency to act without foresight) and risk-taking (e.g., tendency to participate in dangerous activities) and AUDs are strong (Dick et al., 2010; MacPherson, Magidson, Reynolds, Kahler, & Lejuez, 2010; Verdejo-Garcia, Lawrence, & Clark, 2008; Wills, Vaccaro, & McNamara, 1994), associations between these trait measures and beta power have been elusive (e.g., Lansbergen, Schutter, & Kenemans, 2007; Pollock, Earleywine, & Gabrielli, 1995). Thus, the psychological role of beta power in AUDs must be better delineated.

Etiological factors in the association among spectral power, AUDs, and drinking

Genetic vulnerability factors in part underlie the association among beta power and AUDs. Specifically, genes are strongly influential in the development of beta power (Enoch et al., 2008; Malone, Burwell, et al., 2014; McGuire, Katsanis, Iacono, & McGue,

1998; Smit, Posthuma, Boomsma, & Geus, 2005; Tang et al., 2007; van Beijsterveldt, Molenaar, de Geus, & Boomsma, 1996) and AUDs (Carmelli, Heath, & Robinette, 1993; Heath et al., 1997; Kendler, Heath, Neale, Kessler, & Eaves, 1992; Prescott & Kendler, 1999; True et al., 1999), and some studies suggest that the same genes influence both phenotypes (Edenberg et al., 2004; Enoch et al., 2008). Moreover, compared to individuals having no personal or familial history of AUDs, subjects having relatives with AUDs but no AUD themselves have exhibited increased beta power (Bauer & Hesselbrock, 1993; Ehlers & Schuckit, 1990; Finn & Justus, 1999; Gabrielli et al., 1982; Pollock et al., 1995; Rangaswamy et al., 2004), suggesting that elevated beta power may reflect genetic risk for AUDs regardless of whether the individual has ever developed an AUD. Collectively, these observations have motivated the hypothesis that increased beta power is inherited and predates the onset of AUDs in high risk subjects (Begleiter & Porjesz, 1999), although whether beta power prospectively predicts AUDs remains to be demonstrated (see the following for discussions on "endophenotypes": Almasy & Blangero, 2001; Gottesman & Gould, 2003; Iacono, 1998; Iacono, Malone, & Vrieze, 2017). Importantly, whether increased beta in AUDs is inherited does not preclude the possibility that drinking alters spectral power, but rather demonstrates that familial factors (e.g., genes) confound possible causal effects of drinking on spectral power.

One strategy aimed at studying effects of drinking on spectral power while lessening genetic confounding has been to construct relatively “normal” samples consisting entirely of subjects possessing no AUD and no familial history of AUDs and compare groups based on high- and low-drinking behaviors. Generally, these studies

have found that greater drinkers exhibit higher power in beta frequencies than lesser drinkers (Courtney & Polich, 2010; Ehlers & Schuckit, 1990; Ehlers et al., 1989; Nunez-Jaramillo et al., 2015), revealing that drinking behavior itself correlates positively with beta power similar to EEG characteristics of AUD patients and their families. Yet, while studies of this nature demonstrate a correlation between drinking (rather than AUDs) and EEG rhythms, due to having cross-sectional designs it remains impossible to dissociate possible causal effects of drinking from predisposing factors. In other words, while it is possible that drinking causes neuroadaptive and neurodegenerative effects reflected in altered EEG rhythms (F. T. Crews & Nixon, 2009; Guerri & Pascual, 2010; Jacobus & Tapert, 2013; Spear, 2014), it is equally possible that observed associations between drinking and EEG rhythms result from preexisting environmental and genetic factors which may lead individuals to drink in the first place (e.g., Begleiter & Porjesz, 1999; Iacono, Malone, & McGue, 2008), or some combination of both.

It is unethical and impractical to conduct randomized experiments that would enable causal inference on the neurophysiological consequences of drinking in humans, especially for vulnerable subjects such as youths. However, a natural experiment using observational twin data coupled with the co-twin control (CTC) design utilizes the genetic and environmental similarities among twins reared in the same home to control for factors that might confound the causal link between drinking and brain outcomes (Begg & Parides, 2003; McGue, Osler, & Christensen, 2010; Rutter, 2007). Twins reared in the same home are perfectly matched with respect to early-life environmental risk factors (e.g., parental substance use, socioeconomic status, cohort effects) that may

contribute to confounding the causal association between drinking and brain outcomes, whether such variables have been measured or not. Further, monozygotic (MZ) twins have a common genotype and dizygotic (DZ) twins have 50% genetic overlap; thus, shared genomic material is also accounted for. The CTC tests for possible causal effects of environmental exposure (e.g., drinking) by exploiting the correlation among within twin-pair differences in exposure to within twin-pair differences in outcome (e.g., spectral power). Within this framework, the brain of lesser-drinking twin becomes the "control" for what the brain of the greater-drinking twin might have looked like had he or she not drank as much; a significant correlation among within twin-pair differences in drinking and within twin-pair differences in spectral power would be consistent with a causal role of drinking on neurophysiology, while a nonsignificant within twin-pair drinking deviation effect despite significant individual level effect would suggest that drinking does not causally affect brain outcome, but rather familial factors (e.g., genes) are responsible.

Present study

The present study aimed to characterize individual differences in spontaneous EEG power related to drinking in a community sample of young adults, a population that has passed through the period of greatest substance use (Johnston et al., 2017) and SUD risk (Kessler et al., 2005), and is nearing the culmination of significant brain maturation (Paus, 2005; Spear, 2000). Crucially, this study is the first to have used CTC analyses to investigate the possibly causal nature of drinking on resting-state EEG (although, for a CTC investigation of the effects of drinking on event-related EEG, see Harper, Malone,

& Iacono, 2016). Based on previous research that has examined AUD- and drinking-related differences in spontaneous EEG power, we predicted that greater drinking would be associated with elevated beta power. We followed up initial significant effects, which were based on a correlational research design, with CTC analyses to elucidate etiological influences. Due to the influential role of genes in the development of EEG rhythms and AUDs, we expected that any observed drinking-related EEG effects would be attributable to the familial propensity to drinking behaviors and not the consequences of drinking. Additionally, we utilized a mediation model to elucidate whether spectral power accounted for drinking-related personality traits, and hypothesized that beta power would account for traits associated with negative affect rather than inhibitory control.

Method

Participants

Four-hundred and eighty-one subjects (199 male; 208 complete twin pairs in total) were recruited as part of the age 24 assessment of the Enrichment Sample of the Minnesota Twin and Family Study (for overview of study design and sample characteristics, see Keyes et al., 2009). Subjects first visited when they were about 11 years of age, and followed-up at approximate ages 14, 17, and the current assessment (age *M* [*SD*] = 24.5 [.7]).

Quantitative alcohol use

A quantitative measure of drinking was constructed from two inventories, the Computerized Substance Use Assessment (CSA; Han, McGue, & Iacono, 1999) and expanded version of the Substance Abuse Module (SAM; Robins, Babor, & Cottler,

1987). The CSA and SAM assess multiple aspects of alcohol use. The CSA was administered privately in a sound-attenuated room via computer at ages 11 and 14; SAM interviews were conducted for each subject individually at ages 17 and 24 by trained staff having at least a bachelor's degree in psychology or related field.

The drinking composite used in the present study was constructed from questions assessing several quantitative aspects of use (quantity, frequency, density, and misuse; see **Table 1** for item content) thought to potentially cause brain insult, and was computed in the same manner as in previous studies (Harper et al., 2016; Malone, Luciana, et al., 2014; McGue, Malone, Keyes, & Iacono, 2014; Wilson, Malone, Thomas, & Iacono, 2015). Specifically, responses for each item were transformed into zero (no use) through five or six (greatest use) ordinal values, and then at each of the 11-, 14-, 17- and 24-year-old assessments items were summed to yield four age-specific drinking estimates. Finally, these age-specific alcohol use composites were summed resulting in a single cumulative drinking measure.

Table 1

Alcohol exposure construct inventories and items

Inventory Item	Content
Computerize Substance Use Assessment (CSA; ages 11 and 14)	
1. *How many times in the past 12 months have you been drunk?*	Misuse
2. *During the past 12 months, about how many times did you drink alcohol?*	Frequency
3. *In the past 12 months, when you drank alcohol, how many drinks did you usually have? (a drink is a glass of wine, a bottle or can of beer, a shot glass of liquor, or a mixed drink)*	Quantity
4. *In the past 12 months, what is the largest number of drinks you had at one time? (a drink is a glass of wine, a bottle or can of beer, a shot glass of liquor, or a mixed drink)*	Density
Expanded Substance Use Module (SAM; ages 17 and 24)	
1. *About how many times have you been intoxicated or drunk?*	Misuse
2. *In the past 12 months, how often on average have you drunk any alcohol (had any alcohol to drink)?*	Frequency
3. *How much did you have on average each time you drank during the past 12 months?*	Quantity
4. *What is the largest amount of alcohol you ever consumed in a 24-hour period?*	Density

Note. Alcohol exposure was constructed based on items from two inventories, the CSA (assessed at ages 11 and 14) and SAM (assessed at ages 17, 20, and 24). Response data were transformed into zero (no use) through five or six (greatest use) ordinal values, and then at each of the 11-, 14-, 17- and 24-year-old assessments, responses were summed to yield four age-specific drinking estimates. Finally, these age-specific alcohol use composites were summed resulting in a single cumulative alcohol use measure.

Personality dimensions of negative affectivity and disinhibitory traits

To understand the degree to which spectral power accounted for the relationship between drinking and either negative affective and/or disinhibitory personality traits, we explored effects related to constructs from the Multidimensional Personality Questionnaire (MPQ; Tellegen & Waller, 2008). Subjects completed the Personality Booklet – Youth, Abbreviated (developed specifically for use at the Minnesota Twin and Family Study, for more information see Matteson, McGue, & Iacono, 2013), which contains a subsample of scales from the MPQ. Guided by the hypotheses that beta power in AUDs reflects trait negative affect and/or deficits in inhibitory control, four MPQ scales were examined: Stress Reactivity (a person with a high score is highly anxious and irritable); Anhedonia (the Well-being scale reverse-coded; high score is not naturally cheerful, does not experience fun and excitement); Impulsivity (the Control scale reverse-coded; high score is impulsive and spontaneous, does not plan); and Risk Taking (the Harm Avoidance scale reverse-coded; high score enjoys dangerous activities and experiences). Whereas the former two scales aspects of negative affectivity, the latter two reflect aspects of decreased inhibitory control.

EEG recording and processing

Six minutes of resting-state EEG from each participant was acquired during wakefulness with pre-recorded instructions played over headphones at one-minute intervals instructing the subject to close or open his or her eyes, beginning the recording

session with eyes open. EEGs were recorded continuously with a BioSemi ActiveTwo system (BioSemi, Amsterdam, Netherlands; 61 scalp electrodes; 10/10 placement cap; 1024 sample rate; pass-band, DC to 205 Hz) and down-sampled to 256 Hz. Eye movements were measured with a pair of electrodes placed above and below the right eye and another pair of electrodes placed on left and right temples. Custom MATLAB (The MathWorks Inc., Natick, MA) scripts using EEGLAB (Delorme & Makeig, 2004) functions were used for removing artifacts (e.g., salt-bridging, electrical interference, subject movement, muscle bursts, and ocular activity) in a method previously described elsewhere (Burwell, Malone, Bernat, & Iacono, 2014; Burwell, Malone, & Iacono, 2016). At each time point, the recorded voltage at each electrode was re-referenced to the average potential of two earlobe-situated electrodes. Because artifact deletion may not remove all effects due to muscle (e.g., tonic tension of frontalis and/or temporalis), an additional procedure using blind source separation via canonical correlation analysis (De Clercq, Vergult, Vanrumste, Van Paesschen, & Van Huffel, 2006) was used to remove "source" dimensions of the data reflecting white noise to be expected with muscular activity but not neural oscillations.

Eight subjects (2 females) were excluded from analyses based on having fallen asleep during EEG recording. An additional nine subjects (5 females) were excluded for having self-reported recent use of recreational substances that may acutely affect spontaneous EEG either due to intoxication or withdrawal states; eight of these subjects reported using cannabis within 6 hours of the assessment (Crean, Crane, & Mason, 2011; Sneider et al., 2006); one subject reported use of heroin the evening before the

assessment and was excluded to avoid possible withdrawal effects; one subject reported drinking alcohol the day of the assessment and was excluded. Recent use of caffeine or nicotine were not exclusions, although subjects were asked to consume on the day of the assessment no more or no less than they would consume on an ordinary day.

Spectral power quantification

Continuous EEG time-series for each electrode were segmented into two-second epochs, mean-subtracted within epoch, convolved with a Tukey window, and then converted to spectral power using Fast Fourier Transform. Guided by previous studies examining resting-state EEG associations with AUDs during the eyes-closed task (Ehlers et al., 2010; Fein & Allen, 2005; Kaplan et al., 1985; Propping et al., 1981; Rangaswamy et al., 2003; Rangaswamy et al., 2002; Saletu-Zyhlarz et al., 2004), epochs from the eyes-closed portion of the task were selected and absolute power was averaged across epochs at each electrode for each frequency (DC to 128 Hz, in .5 Hz increments). Power spectra were log-transformed using $\log10(x)$ (Pivik et al., 1993), and mean power within delta (1 to 3 Hz), theta (3 to 8 Hz), alpha (8 to 13 Hz), beta (13 to 30 Hz), low gamma (30 to 58 Hz), mid-gamma (62 to 98), and high gamma (102 to 127.5 Hz) frequency bands were derived for each electrode and subject.

Statistical analyses

Individual level associations among drinking and spectral power. To examine potential associations between drinking and spectral power, we calculated linear mixed models (LMMs) (Bates, Machler, Bolker, & Walker, 2015) which enabled the inclusion of a random intercept for each twin pair, accounting for the family structure of the data.

In the model $POWER_{ij} = B_0 + B_1 DRINK_{ij} + \alpha_i + \varepsilon_{ij}$, $POWER_{ij}$ and $DRINK_{ij}$ reflect spectral power for a given frequency band and drink (respectively) for person j of twin-pair i; B_1 reflects the individual level association between drinking and spectral power which ignores within twin-pair differences in drinking, thus akin to existing correlational research using non-twin designs. The terms B_0 and α_i reflect fixed and twin-pair specific random model intercepts, respectively; ε_{ij} is random noise. Degrees of freedom and *p*-values for LMMs were estimated by Kenwood-Roger approximation (Kuznetsova, Brockhoff, & Bojesen Christensen, 2016). Although not depicted above, all LMMs included main effects of gender and gender × drinking interaction effects in individual level LMMs; interaction terms were subsequently dropped if they were shown to be associated with a *p*-value greater than .05.

Frequency bands and scalp regions of interest for which drinking was significantly related to power were algorithmically determined. Specifically, individual level LMMs were conducted at each electrode site, and scalp regions of interest were defined if the effect of drinking on power was associated with p-values less than .01 at no fewer than 5 contiguous sites. For frequency bands that met these criteria, mean power within the frequency range across all electrodes within the region was derived as a scalar value for each subject to be explored in subsequent analyses.

Co-twin control (CTC) analysis to examine possible causal effects of drinking. Statistically significant ($p < .05$) individual level associations were followed up with co-twin control (CTC) analyses (Begg & Parides, 2003; McGue et al., 2010; Rutter, 2007) which examined the possible causal effect of drinking on spectral power. To

perform CTC analyses, individual drinking scores were re-expressed as within twin-pair difference in drinking, such that in the model $POWER_{ij} = B_0 + B_W(DRINK_{ij} - \overline{DRINK}_{i\cdot}) + \alpha_i + \varepsilon_{ij}$ where $\overline{DRINK}_{i\cdot}$ reflects average drinking within a given twin-pair, the coefficient B_W reflects the degree to which twins from the same family differ in spectral power (for a given frequency band) because of within twin-pair differences in drinking ($DRINK_{ij} - \overline{DRINK}_{i\cdot}$). Unlike the individual level coefficient B_I that enables only correlational inference, observation of a significant B_W coefficient is consistent with the notion that drinking has caused within twin-pair differences in spectral power. For the sake of interpretability, we presented T_I (B_I divided by standard error) and T_W (B_W divided by standard error) test statistics in tables. Because individual level analyses set precedent regarding the direction of anticipated effects and to increase statistical power to detect possible causal effects, one-tailed tests were employed for CTC analyses.

We also tested for zygosity effects in CTC analysis to determine whether it was appropriate to examine effects separately within subsamples of DZ and MZ twins. To test whether zygosity-related effects were of statistical import in the DZ/MZ combined sample, a likelihood ratio test was utilized whereby measures of statistical misfit (-2 log likelihood, or -2LL) were compared for two models: (1) including zygosity (dizygotic vs. monozygotic) and zygosity × drinking covariates, and (2) without these zygosity covariates. The value obtained by subtracting the -2LL of the latter from the former was subsequently referred to a chi-square distribution with 2 degrees of freedom. An observed

change in fit ($\Delta\chi^2$) that is significant ($p < .05$) would suggest differing effects by zygosity, and would deem further inspection separately within DZ and MZ subsamples.

Does spectral power explain the association between drinking and personality?

Generally, there have been two theories regarding what is reflected by spectral power differences in the association between drinking and personality, these include: 1) beta/CNS hyperarousal reflects negative affective traits (e.g., stress and anxiety, anhedonia) (Heilig et al., 2010; Koob, 2004; Seevers & Deneau, 1963), and 2) beta/CNS hyperarousal reflects deficits in inhibitory control (e.g., impulsivity, risk-taking) (Bauer, 2001; Begleiter & Porjesz, 1999; Nunez-Jaramillo et al., 2015). In a mediation framework, the association between drinking and personality can be thought of as the "total effect" of drinking on personality (Impulsivity, Risk Taking, Stress Reactivity, Anhedonia), corresponding to path *c* in **Figure 1A**. However, if the association between alcohol use and a given personality dimension is achieved by way of common neurocircuitry reflected in spectral power, then adjusting the total effect for the association between spectral power and that personality dimension should result in a significant reduction of *c*. We tested the mediation model proposed in **Figure 1B** where the total effect has been partitioned into "direct" (path *c'*) and "indirect" (paths *a* and *b*) effects. Path *c'* is computed as the difference between total effect of drinking on personality and its indirect path via spectral power (or $c - c'$), which is equivalent to the product of *a* and *b*. Spectral power is considered to have partially mediated the

relationship between drinking and personality if the reduction in *c* to *c'* is significant ($p <$ .05); or equivalently, if the indirect effect *ab* is significantly different than zero.

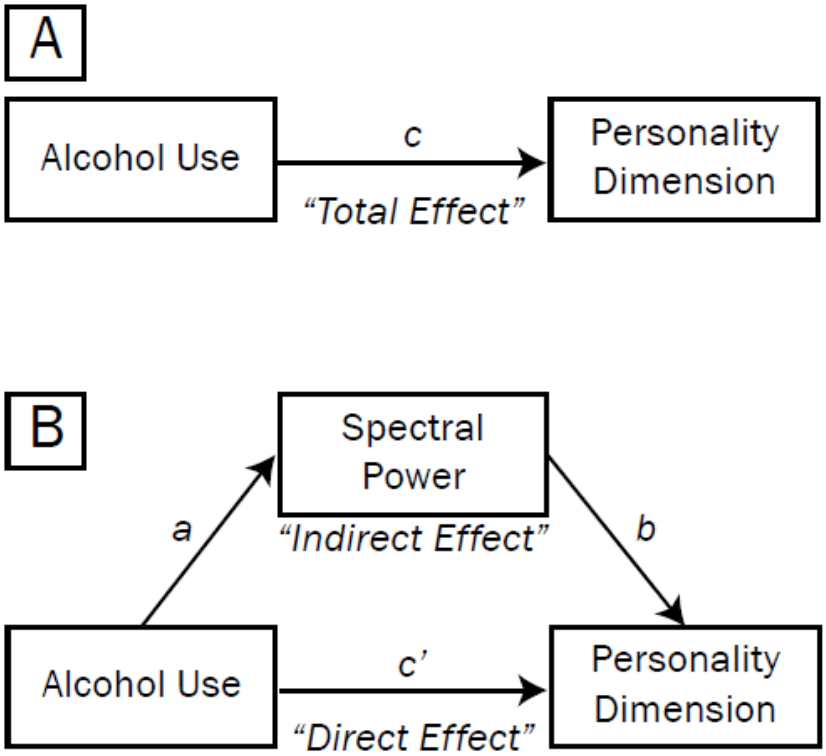

Figure 1. Mediation of the effect of drinking on personality via spectral power. A) The association between drinking and personality can be thought of as the "total effect" of drinking on personality (Impulsivity, Risk Taking, Stress Reactivity, Anhedonia), corresponding to path *c*. However, if the association between alcohol use and a given personality dimension is achieved by way of common neurocircuitry reflected in spectral power, then adjusting the total effect for the association between spectral power and that personality dimension should result in a significant reduction of *c*. B) We tested the mediation model where the total effect has been partitioned into "direct" (path *c'*) and "indirect" (paths *a* and *b*) effects. Path *c'* is computed as the difference between total effect of drinking on personality (path *c*) and its indirect path via spectral power *c'*, which is equivalent to the product of *a* and *b*. Spectral power is considered to have partially mediated the relationship between drinking and personality if the reduction in *c* to *c'* is significant ($p < .05$); or equivalently, if the indirect effect *ab* is significantly different than zero.

Spectral power was considered to have partially mediated the relationship between drinking and personality if significant shrinkage in the value of *c* to *c'* occurred. To estimate whether this reduction was significant, we used a bootstrapping approach

utilized by Burwell et al. (2014) whereby 1,000 indirect effects (*ab*, equivalent to the change in *c* to *c'*) were simulated from the observed data with replacement (Shrout & Bolger, 2002), keeping the proportion of matched- to unmatched-twin-pairs constant. Ninety-five and 99% confidence intervals were estimated from this distribution of simulated *ab* effects to evaluate statistical significance at the .05 and .01 levels; significant mediation occurred when the confidence interval did not include 0.

Results

Participant characteristics

Sample demographic and clinical descriptive statistics with regards to the drinking measure used in the current study are presented in **Table 2** and showed that drinking scores did not significantly correlate with gender or age at the current assessment (*p*s > .430), but did correlate with clinical characteristics relevant to problem drinking. Specifically, one standard deviation increase on the drinking measure were predictive of younger age of first drink, as well as increased odds for binge drinking (consumed ≥5 drinks on one occasion), having been intoxicated ≥100 times, and meeting criteria for an AUD diagnosis (*p*s < .001).

Table 2

Demographic, clinical, and personality descriptive statistics and associations with drinking measure

Demographic, clinical, and personality dependent variables (self-reported at current assessment)	Overall *Mean* or *%*	LMM Association *B* (*SE*) or *OR*	*p*
Gender [a]	59.2% female	.69	.437
Age at current assessment	24.5 years	.0 (.0)	.464
Age at first drink ever	16.7 years	-1.8 (.1)	**<.001**
Ever binge drank (≥5 drinks/occasion) [a]	87.7% yes	55.90	**<.001**
Ever intoxicated ≥100 times [a]	27.0% yes	6.54	**<.001**
Meets criteria for Alcohol Use Disorder [a]	26.8% yes	3.08	**<.001**

Note. Overall means and frequencies, and linear mixed model-estimated (LMM) associations with drinking for demographic and clinical variables. Coefficients (*B*s) reflect the degree to which the listed dependent variable changes with respect to one standard deviation increase on the drinking measure. Odds ratios (*OR*s) are flagged with superscript "a"; positive values reflect increased odds of a "yes" response given one standard deviation increase in drinking. LMMs were adjusted for a main effect of gender. Significant effects ($p < .05$) are highlighted in bold text. Other abbreviations: SE = standard error.

Individual level associations among drinking and spectral power

Scalp topographical associations among drinking and beta power are presented in the top of **Figure 2** and show that drinking was associated with increased beta power spanning frontal and central scalp locations. To further illustrate effects from these scalp regions, LMMs were conducted on power values derived at each frequency, and the results plotted in the bottom of **Figure 2**. Drinking was associated with increased power most noticeably in the beta range (highlighted in grey), as evidenced by greater positivity

for alcohol (red trace, ± standard error) versus intercept (black trace) estimates. Mean beta power across these sites was extracted for everyone and individual level t-statistics (T_Is) are presented left of the vertical in **Table 3**. The individual level association between drinking and beta power was positive and highly significant ($p < .001$). There was no significant gender × drinking interaction so it was dropped from LMMs. We did not observe drinking-related associations of sufficient size ($p < .01$) and spatial extent (more than 5 contiguous electrode sites) in any other frequency band to deem further investigation (see Method for description of inclusion criteria).

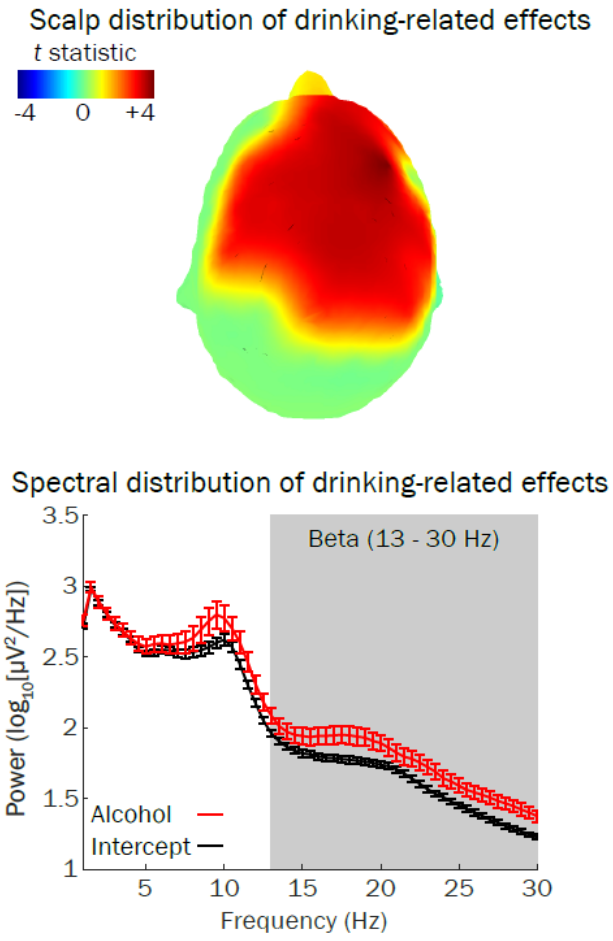

Figure 2. Beta (13 to 30 Hz) power scalp topography (top) and spectral distribution (bottom) associations with youth drinking. In the top panel, results from linear mixed models (LMMs) were plotted depicting the

association between drinking and beta frequency power. Positive (red) hues over frontal and central scalp regions indicate that drinking was associated with increased beta power in these regions where the t-statistic was associated with p-values less than .01. In the bottom panel, estimates and standard error bars from LMMs depicting the association between drinking and spectral power at each frequency were plotted (red trace), and can be compared to the LMM model intercept (black trace).

Table 3

Spectral power associations with alcohol use

ROI	Individual		Co-twin control (CTC)					
Substance	Cumulative Use		Cumulative Use		Adolescent Use		Past 7 Years Use	
Gender	T_I (*df*)	*p*	T_W (*df*)	*p*	T_W (*df*)	*p*	T_W (*df*)	*p*
Beta power	4.3 (457)	**<.001**	3.2 (225)	**<.001**	2.8 (224)	**.003**	1.7 (206)	**.046**

Note. Test statistics (*T*), degrees of freedom (*df*), and significance values (*p*) from linear mixed models depicting main effects of alcohol use on resting-state beta EEG power (13 to 30 Hz) within the scalp region of interest. *T*-values left of the vertical (T_Is) depict the individual-level association, or correlation between drinking and spectral power. Statistically significant ($p < .05$, denoted by boldface) individual level results were followed up using the co-twin control (CTC, right of vertical) design; here, CTC coefficients (T_Ws) depict the main effect of within twin-pair differences in drinking on spectral power, or the possible "causal effect" of alcohol use. Kenwood-Roger approximation determined degrees of freedom and *p*-values. Because individual level analyses set precedent regarding the direction of anticipated CTC effects and to increase statistical power to detect possible causal effects, one-tailed tests were utilized for CTC analyses. All analyses were adjusted for a main effect of gender. We explored gender × drinking interaction effects, but none were significant so they were dropped.

Supplemental statistics.

Co-twin control (CTC) analysis to examine possible causal effects of drinking

Significant individual level associations were followed up with CTC analyses to elucidate possible causal effects of drinking on beta power. As presented right of the vertical in **Table 3**, within twin-pair increases in drinking were significantly associated with within twin-pair increases in beta power ($p < .001$). This T_W effect is depicted in **Figure 3** as a positively-sloped regression line, and shows that after accounting for

vulnerability factors shared by twins (e.g., genes, rearing environment), within twin-pair increased drinking predicts within twin-pair increased beta power, consistent with a possible causal role of drinking on beta power. For example, members from the twin-pair differing greatest with respect to drinking are highlighted by arrows in **Figure 3**; the upper arrow indicates higher beta power for a twin who drinks nearly 15 scale units more alcohol than her co-twin (depicted by lower arrow). Adding zygosity and zygosity × drinking terms did not significantly improve model fit ($\Delta\chi^2(2) = 2.2$, $p = .338$), thus effects between DZ and MZ were deemed statistically equivalent.

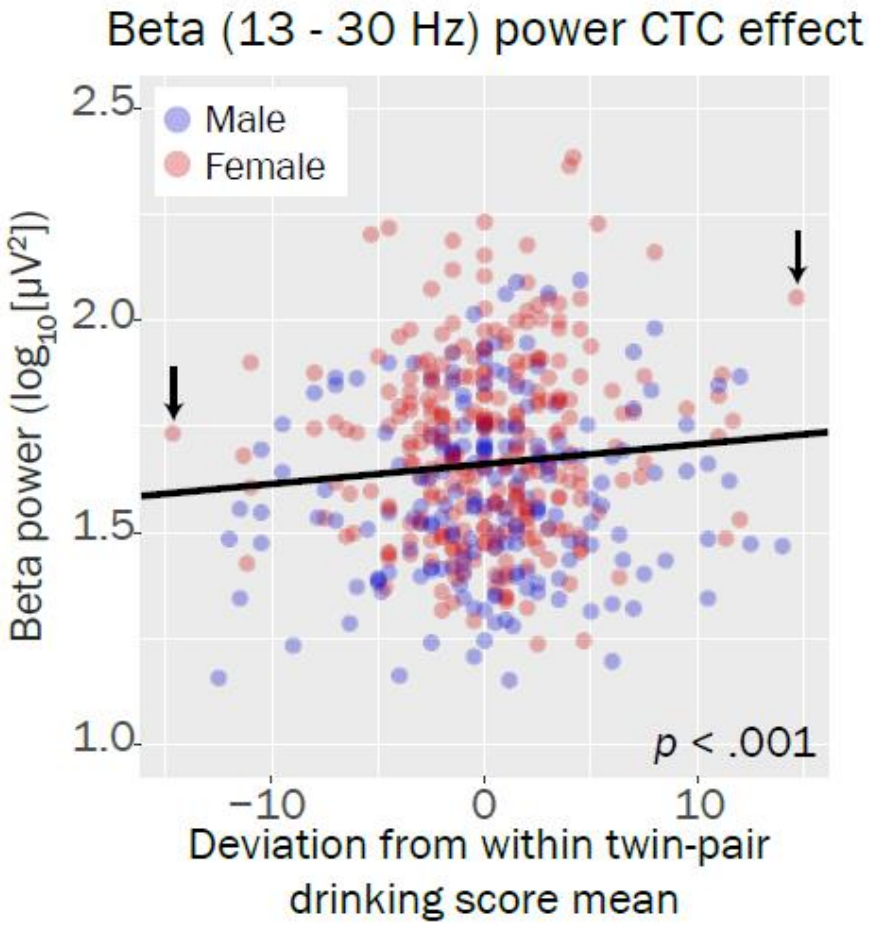

Figure 3. Beta (13 to 30 Hz) power plotted as a function of within twin-pair differences in drinking. Results from co-twin control (CTC) analyses depicting the effect of within twin-pair differences in drinking

on beta power are displayed, and the positively-sloped regression fit line indicates drinking associated with increased beta power, after accounting for vulnerability factors shared among twins (e.g., genes). For example, the twin-pair differing greatest in drinking are highlighted by arrows; the upper arrow indicates higher beta power for a twin who drinks nearly 15 scale unit more alcohol than her co-twin (depicted by lower arrow).

Does beta power explain the association between drinking and personality traits?

To understand whether increased beta power may explain associations between drinking and personality dimensions, we estimated "total effects" of drinking on negative affective (Stress Reactivity and Anhedonia) and disinhibitory (Impulsivity and Risk Taking) personality dimensions (see **Figure 1A**), and followed up these analyses using the mediation model in **Figure 1B** to estimate "direct" and "indirect" effects. As presented in **Table 4** in the column labeled *c*, positive and significant associations were observed between drinking and all personality dimensions examined: Stress Reactivity ($p = .004$), Anhedonia ($p = .022$), Impulsivity ($p < .001$), and Risk-taking ($p = .033$). Indirect (columns labeled *a* and *b*) and direct (column labeled *c'*) effects of drinking on personality dimensions derived from mediation models in **Figure 1B** are also presented in **Table 4**, and in the rightmost column indicate whether beta power significantly mediated the association between drinking and personality, or equivalently whether the reduction in *c* to *c'* was significantly different from zero. Significant partial mediation was observed for Stress Reactivity ($c – c' = .465$, $p < .05$) and Anhedonia ($c – c' = .643$, $p < .01$) but not for Impulsivity ($c – c' = .377$, $p > .05$) or Risk Taking ($c – c' = -.169$, $p > .05$), suggesting that increased beta power is partly responsible for the link between drinking and increased negative affect, but not the link between drinking and reduced inhibitory control.

Table 4

Beta power as a potential mediator between drinking and personality measures

Personality Dimension	*Does drinking predict personality?*	*Does beta power mediate drinking prediction of personality?*			
	Total Effect	Indirect Effect		Direct Effect	Mediation
	c	*a*	*b*	*c'*	$\|c - c'\|$ significant?
<u>Negative affectivity</u>					
Stress Reactivity	3.0**	4.3**	2.0*	2.5*	*
Anhedonia	2.3*	4.3**	3.2**	1.7	**
<u>Reduced inhibitory control</u>					
Impulsivity	7.5**	4.3**	1.3	7.1**	n.s.
Risk-taking	2.2*	4.3**	-1.1	2.3*	n.s.

Note. Results depicting the "total effect" of drinking on personality traits (left), alongside "indirect" and "direct" effects obtained from mediation models (right). Paths a, b, c, and c' correspond to those described in **Figure 1**. The total effect reflects the influence of drinking on personality measures (listed in the leftmost column); within the mediation model, the total effect is partitioned into "indirect" and "direct" effects. Paths *a* and *b* comprise the indirect effect of drinking on personality via EEG beta power, and path *c'* is the effect of drinking on personality adjusted for the indirect effect. The reduction of c to c' is equivalent to the product of a and b, and the rightmost column indicates whether the magnitude of this reduction is significant at $p < .05$ ("*") and $p < .01$ ("**") levels.

Discussion

We characterized drinking-related associations with spectral power of resting-state EEGs in a community sample of young adults and tested whether drinking-related differences in spectral power might be attributed to causal effects of drinking by utilizing CTC analyses. In support of our first hypothesis, drinking was associated with increased beta power, and these effects were observed at frontal and central scalp locations. However, our second hypothesis that any potential drinking-related associations would be attributable to familial factors (e.g., genes) and not causal effects of drinking, was not supported. Within twin-pair excess drinking was significantly associated with within twin-pair increased beta power, consistent with the notion that alcohol use may cause greater beta in resting-state EEG, perhaps indicative of experience-dependent plasticity. Subsequently, in support of our third hypothesis, we found that beta partially accounted for the association between drinking and negative affective personality measures but not the association between drinking and personality measures related to decreased inhibitory control, consistent with the notion that beta power reflects negative affect in drinkers.

Conducting CTC analyses for the first time on resting-state EEG and prospective alcohol use data collected from a large sample of young adult twins, we found evidence in support of the notion that drinking causes lasting increases in spontaneous beta power. Specifically, within a given twin-pair, because the EEG of the twin with lower alcohol use provides an approximation of what the EEG of the twin with higher alcohol use might have looked like had he or she not drank as heavily, the relative increase in beta observed for the greater drinking twin can be ascribed to alcohol exposure effects,

because effects due to genetic and environmental vulnerability confounds were accounted for. Experimental research conducted on rats has found similar results, whereby rats randomly assigned to ethanol exposure during adolescence exhibited greater beta EEG activity as adults compared to rats randomly assigned to placebo (Slawecki, Betancourt, Cole, & Ehlers, 2001). Elevated beta power possibly caused by alcohol exposure may reflect upregulation of cortical excitability (e.g., Whittington, Traub, Kopell, Ermentrout, & Buhl, 2000), perhaps involving alcohol-induced plasticity of glutamatergic and GABAergic systems (Davies, 2003; Dodd, Beckmann, Davidson, & Wilce, 2000; Ron & Wang, 2009) similar to neuroadaptations observed during withdrawal states (Heilig et al., 2010; Hendricson et al., 2007).

We also found increased beta power to mediate the strength of the relationship between drinking and trait negative affect, such that effects of drinking on stress reactivity and anhedonia were reduced by way of the indirect effect of drinking conveyed through increased beta power. These results are consistent with prior studies that have found increased beta power to correlate with cognitive and affective processes theorized to contribute to relapse in AUD patients (Bauer, 2001; Brower, Aldrich, Robinson, Zucker, & Greden, 2001; Heilig et al., 2010; Saletu-Zyhlarz et al., 2004; Sinha & Li, 2007) such as alcohol craving (De Ridder et al., 2011), emotional rumination and anxiety (Andersen et al., 2009; Cole & Ray, 1985; Pavlenko et al., 2009; Stenberg, 1992) and insomnia (Perlis et al., 2001; Riemann et al., 2010). Researchers have hypothesized that beta oscillations signify maintenance of internal cognitive sets that persist despite external stimuli (Engel & Fries, 2010), a theory that is complemented by the fact that beta

has been shown to tap into default mode network activation (Hlinka, Alexakis, Diukova, Liddle, & Auer, 2010; Knyazev, Savostyanov, Volf, Liou, & Bocharov, 2012; Laufs et al., 2003; Mantini, Perrucci, Del Gratta, Romani, & Corbetta, 2007; Neuner et al., 2014), which in the case of overactivation, might result in chronic negative affect (Price & Drevets, 2012).

While results of the present study support the role of increased beta power as a "biomarker" (a biological features that is associated with psychopathology; Iacono, 1985; Iacono et al., 2017) for alcohol use, the finding that drinking may cause increased beta power imposes limitations on the utility of increased beta power in drinking populations as an "endophenotype" (a biomarker that is associated with genetic risk for psychopathology; Almasy & Blangero, 2001; Gottesman & Gould, 2003; Iacono, 1998) for alcohol use (Begleiter & Porjesz, 1999; Kamarajan & Porjesz, 2015; Porjesz & Rangaswamy, 2007; Rangaswamy & Porjesz, 2008, 2014). Specifically, increased beta power in relation to alcohol use and AUDs fulfills several necessary endophenotype criteria (for a discussion of endophenotype best practices, see Iacono et al., 2017): it has a well-established association with drinking and AUDs (Bauer, 2001; Costa & Bauer, 1997; Courtney & Polich, 2010; Ehlers et al., 2010; Ehlers & Schuckit, 1990; Ehlers et al., 1989; Fein & Allen, 2005; Herrera-Diaz et al., 2016; Kaplan et al., 1985; Nunez-Jaramillo et al., 2015; Propping et al., 1981; Rangaswamy et al., 2002; Saletu-Zyhlarz et al., 2004), it is heritable (Enoch et al., 2008; Malone, Burwell, et al., 2014; McGuire et al., 1998; Smit et al., 2005; Tang et al., 2007; van Beijsterveldt et al., 1996) and present in unaffected relatives of subjects with AUDs (Bauer & Hesselbrock, 1993; Ehlers &

Schuckit, 1990; Finn & Justus, 1999; Gabrielli et al., 1982; Pollock et al., 1995; Rangaswamy et al., 2004), and has been linked to common molecular genetic substrates thought to underlie AUDs (Edenberg et al., 2004; Enoch et al., 2008). Yet, to the degree that increased beta power is environmentally-induced by drinking, the association between biomarker and genetic risk for alcohol use may be confounded. In other words, in drinking populations it is not known whether genes caused increased beta or whether drinking caused increased beta. Future developmental research should investigate the degree to which increased beta is rank-stable across brain maturation (for discussion on developmental stability, see Burwell et al., 2016; Iacono & Malone, 2011) and reflects genetic risk prior to the onset of alcohol use.

Limitations

Although the CTC design employed here is a powerful method to elucidate cause and effect in observational data, it does not account for possible confounding due to substance use-related factors that are unshared within twin-pairs, such as within twin-pair differences in environment or genes (for DZ twins only) related to drinking. Similarities among twins with regards to alcohol use were moderate (intra-class correlation [ICC], ICC_{DZ} = .38) to strong (ICC_{MZ} = .75) for DZ and MZ pairs (respectively, $p < .001$), and this pattern of similarities suggested that only a minor portion of the variance in alcohol use was attributable to environment differences (E = $1 - ICC_{MZ}$ = .25) whereas the majority of variance (i.e. about 3 times that of E) was due to additive genetics (A = $2[ICC_{MZ} - ICC_{DZ}]$ = .74) (Falconer & Mackay, 1996). Moreover, unshared additive genetics within DZ pairs appeared to have no significant effect on the possible causal

association between drinking and beta power, as we observed no significant zygosity effects (i.e. DZ vs. MZ pairs). Possible issues with statistical power cannot be ruled out, although, the sample size for the current study was large (N = 481). Collectively, alcohol use was well-matched within twin-pairs and largely attributable to genetic factors accounted for by CTC analyses; and, CTC results were similar across DZ and MZ pairs, suggesting possible causal effects of drinking on beta power regardless of genotype.

Critically, results in the present study are limited to spectral power of spontaneous EEG collapsed over several minutes of recording and do not rule out the possibility that other neurophysiological (e.g., event-related EEG; Harper et al., 2016) or and/or neurobiological features (e.g., brain tissue morphometry; Wilson et al., 2015) reflect possible causal effects of drinking or familial propensity. However, our focus on spontaneous EEG rhythms was motivated by extensive research suggesting that CNS hyperarousal (defined by increased high frequency [e.g., >13 Hz] EEG power during baseline/resting-state recording) is a potential biomarker for alcohol use and AUDs (for reviews, see Begleiter & Porjesz, 1999; Kamarajan & Porjesz, 2015; Porjesz & Rangaswamy, 2007; Rangaswamy & Porjesz, 2014). The results of the present study were consistent with previous research and further inform etiological hypotheses regarding a potential neurophysiological mechanism underlying drinking behaviors and possibly AUDs, and link such neurophysiological processes to clinically-relevant phenotypes involving trait negative affect (Heilig et al., 2010). Based on the limited spatiotemporal information provided by spectral EEG estimates, future research should focus on understanding which brain structures might be involved in drinking-related beta

power differences; although, see (De Ridder et al., 2011) for a case study linking drinking- and alcohol craving-related beta power to default mode network brain regions (e.g., medial prefrontal/anterior cingulate cortex).

Conclusion

The present study investigated EEG of young adults with respect to alcohol use prospectively reported several times since age 11, and is the first study to have used a CTC design to elucidate whether drinking-related spectral power differences may be attributable to possible causal effects of alcohol use. Despite previous research suggesting that drinking/AUDs are linked with increased beta power as the result of common familial vulnerability factors (e.g., genes), we found that within twin-pair differences in alcohol use were related to within twin-pair differences in beta power, such that the greater drinking twin possessed greater beta power than his or her lesser drinking co-twin. These results are consistent with the notion that drinking may cause lasting CNS hyperarousal reflected in elevated resting-state beta power, regardless of genetic and environmental vulnerability factors shared within twin-pairs. Additionally, we demonstrated that increased beta power mediated the positive correlation between drinking and negative affective personality dimensions (stress reactivity and anhedonia) but not the correlation between drinking and diminished inhibitory control (impulsivity and risk-taking), suggesting that elevated beta power may index negative affective but not behavioral disinhibitory processes in alcohol users. Because beta power may confer risk for drinking/AUDs as well as reflect a neurophysiological consequence of alcohol use, future research using developmental samples should delineate the association

between alcohol use, beta power, and such personality characteristics at different ages to better understand in what circumstances beta power reflects risk and/or consequence of alcohol use. Nonetheless, results of the present study suggest that alcohol use may cause lasting CNS hyperarousal reflected in increased beta power, which may be linked to deleterious affective processes in alcohol users.

Study 2. Does Alcohol, Cannabis, and Tobacco Use Alter Ventral Striatal Functional Connectivity in Youths? A Co-Twin Control Study

Recreational use of addictive substances among youths aged 11 to 25 is a weighty public health concern. Among 12th graders in the United States, 33% have drank alcohol in the past month, 22% have used cannabis, and 10% have smoked cigarettes (Johnston et al., 2017). Crucially, early-life substance use increases risk for later-life substance use disorders (SUDs) (e.g., Irons, Iacono, & McGue, 2015; Kendler, Myers, Damaj, & Chen, 2013; Lynskey et al., 2003) and addiction, the most severe form of SUD characterized by impaired self-control over the motivation to use drugs and alcohol (Volkow, Koob, & McLellan, 2016). Yet, putative brain mechanisms that mediate the transition from normative use (e.g., social drinking) to problematic use (e.g., alcoholism) remain difficult to study in humans because it is unethical and impractical to conduct a randomized experiment of possible substance use-related brain insult. Thus, whether substance use itself causes lasting neuroadaptations, or whether predisposing factors (e.g., genes) are responsible remains in question. Nonetheless, experiments on animal subjects suggest that ventral striatum (VST) and its connectivity are altered by substance use, and that the youth brain is hypersensitive to such experience-dependent neuroplasticity (Bossong & Niesink, 2010; F. Crews, He, & Hodge, 2007; Dwyer, McQuown, & Leslie, 2009; Lubman, Cheetham, & Yucel, 2015; Monti et al., 2005; O'Dell, 2009; Witt, 2010). Here, we examined connectivity of VST using functional magnetic resonance imaging (fMRI) with regards to youth alcohol, cannabis, and tobacco use, employing a co-twin control design to shed light on possible causal effects of substance use, and related functional

connectivity to measures of trait impulsivity and compulsive substance use to inform pathological processes.

VST functional connectivity, prefrontal cortex, and inhibitory control

Reward signals in the brain elicited by natural (e.g., palatable food) and drug (e.g., alcohol, nicotine) reinforcers are thought to be encoded by dopamine circuits in VST and frontal cortex (Di Chiara & Imperato, 1988; Ikemoto, 2010; Nestler, 2005; Sulzer, 2011), and fMRI research conducted on SUD and SUD prone populations suggest that VST reward circuitry plays multiple roles in the manifestation of SUDs. For instance, prior to the development of SUD, increased activation of VST during potentially rewarding situations predicts high levels of approach behavior (Beaver et al., 2006), sensation seeking (Abler, Walter, Erk, Kammerer, & Spitzer, 2006; Bjork, Knutson, & Hommer, 2008), temporal delay discounting of rewards (Hoogman et al., 2011), and impulsivity (Forbes et al., 2009), which may reflect genetic susceptibility for SUDs (Dalley, Everitt, & Robbins, 2011; de Wit, 2009; Iacono et al., 2008; Verdejo-Garcia et al., 2008). Such activation of VST is thought to be central to the initial pleasurable subjective experience of substance use, but also thought to be responsible for drug and alcohol wanting (e.g., craving) after repeated uses (Robinson & Berridge, 1993) when the actual pleasure received from using the substance may be diminished or absent (Keiflin & Janak, 2015; Redish, 2004). Consistent with this theory, individuals with SUDs exhibit relatively increased activation for drug- than non-drug-related cues (Filbey et al., 2016; Li et al., 2012), which may signify incentive salience for drug-related cues and actions.

Top-down control of motivational drives by frontal cortex is thought to be critical for inhibition of impulsive and/or inappropriate behaviors, and this circuitry may be impaired in addiction (Dalley et al., 2011; Feil et al., 2010; Jahanshahi, Obeso, Rothwell, & Obeso, 2015; Tomasi & Volkow, 2013){Everitt, 2008 #48;Robbins, 2012 #4}{Graybiel, 2008 #7}. Studies employing resting-state fMRI functional connectivity (interregional correlated blood flow) support this hypothesis. For example, relative to control subjects, frontostriatal connectivity is reduced in SUDs including tobacco (Hong et al., 2009; Hong et al., 2010; Yuan et al., 2016), opioids (Upadhyay et al., 2010; Wang et al., 2013), cocaine (Hu, Salmeron, Gu, Stein, & Yang, 2015), and polysubstance use (Motzkin, Baskin-Sommers, Newman, Kiehl, & Koenigs, 2014), although some contradictions exist (Camchong, Stenger, & Fein, 2013; Gu et al., 2010; Ma et al., 2011). Additionally, other disorders characterized by impaired inhibitory control have been associated with reduced frontostriatal functional connectivity such as obesity (e.g., Tomasi & Volkow, 2013), internet gaming disorder (e.g., Yuan et al., 2017), attention-deficit hyperactivity disorder (ADHD; e.g., Cao et al., 2009), obsessive compulsive disorder (e.g., Vaghi et al., 2017), and comorbid Parkinson's Disease with impulse control disorder (e.g., Rao et al., 2010) and pathological gambling (e.g., Cilia et al., 2011). Thus, reduced frontostriatal functional connectivity may not be specific to SUDs, but instead reflect cognitive and behavioral disinhibition across several diagnoses.

Importantly, specific brain regions for which striatal functional connectivity is reduced in SUDs may provide insight into pathophysiological processes. For example, aberrant connectivity with dorsal frontal cortex might reflect impaired attention and

planning that contribute to impulsive substance use (e.g., using without foresight into possible consequences) while aberrant connectivity with more anterior and ventral regions of frontal cortex might reflect impaired motivational and affective control that contribute to compulsive use (e.g., using despite foresight of negative consequences) (cf. Dalley et al., 2011; Everitt et al., 2008; Goldstein & Volkow, 2011; Robbins, Gillan, Smith, de Wit, & Ersche, 2012). In support of this idea, reduced functional connectivity between VST and dorsal frontal cortex has predicted response-inhibition errors during selective attention and correlates with personality dimension scores reflecting trait impulsivity (Davis et al., 2013; Motzkin et al., 2014). By comparison, diminished functional interactions between VST and anteroventral prefrontal cortex during goal-driven decision-making has been associated with choosing immediate rewards instead of long-term goals (e.g., as in choosing acute use [relapse] over long-term abstinence) (Diekhof & Gruber, 2010). Thus, further investigation of possibly dissociable roles of frontostriatal functional connectivity in SUDs may be valuable for understanding the development and maintenance of SUD and addiction.

Etiological factors in the association among VST functional connectivity and substance use

Due to cross-sectional research designs employed in existing literature, previous studies have been limited to establishing only correlational evidence of the association between substance use and frontostriatal functional connectivity, and have not been able to dissociate causal effects from predisposing factors. For instance, while it is possible that substance use causes neuroplastic and neurodegenerative effects reflected in altered

connectivity (e.g., Everitt et al., 2008; Koob & Volkow, 2010; Volkow, Wang, Tomasi, & Baler, 2013), it is also possible that observed associations between substance use and frontostriatal functional connectivity are caused by preexisting environmental and genetic factors which may lead individuals to use substances in the first place (e.g., via trait impulsivity), or some combination of both (e.g., Iacono et al., 2008).

Familial factors are certainly thought to partially explain the link between SUDs and functional connectivity; youth with family history of SUDs but no SUD themselves have exhibited functional connectivity anomalies (Cservenka, Casimo, Fair, & Nagel, 2014; Herting, Fair, & Nagel, 2011; Qiao et al., 2015; Weiland et al., 2013; Wetherill et al., 2012). To date, however, too few studies have been conducted to establish a cohesive narrative of what familial risk might be reflected in frontostriatal connectivity. Notably, Ersche et al. (2012) examined fractional anisotropy (FA; a measure of white matter connectivity) in stimulant-dependent subjects, their non-substance-dependent biological siblings, and age-/intelligence-matched controls; they found that stimulant-dependent subjects and their siblings had highly similar FA values, and that frontostriatal FA in these groups was significantly decreased relative to controls, suggesting that diminished frontostriatal FA reflects a predisposing familial risk for stimulant use rather than causal effects from taking the drug (Ersche et al., 2012). Yet, it is not known whether such findings regarding brain structure carry over to brain function, albeit FA and functional connectivity have correlated positively in the past (Damoiseaux & Greicius, 2009).

To distinguish causal effects of substance use on human brain outcomes from familial confounds, a randomized experiment would be desirable; but, such an approach

is not ethical nor practical, especially when studying vulnerable populations such as youths. However, a *natural experiment* using observational twin data via the co-twin control (CTC) design utilizes the genetic and environmental similarities among twins reared in the same home to control for factors that might confound the causal link between substance use and brain outcomes (Begg & Parides, 2003; McGue et al., 2010; Rutter, 2007). Twins reared in the same home are perfectly matched with respect to early-life environmental risk factors (e.g., parental substance use, socioeconomic status, cohort effects) that may contribute to confounding the causal association between substance use and brain outcomes, whether such variables have been measured or not. Further, monozygotic (MZ) twins have a common genotype and dizygotic (DZ) twins have 50% genetic overlap; thus, shared genomic material is also accounted for. The CTC tests for possible causal effects of environmental exposure (e.g., substance use) by exploiting the correlation between within twin-pair differences in exposure to within twin-pair differences in brain outcome (e.g., functional connectivity). Within this framework, the brain of twin with lesser substance use becomes the "control" for what the brain of the twin with greater substance use might have looked like had he or she not used as much; a significant correlation between within twin-pair differences in substance use and within twin-pair differences in brain outcome is consistent with a causal role of substance use on brain outcome, while a nonsignificant correlation suggest that substance use does not affect brain outcome, but rather familial factors (e.g., genes) are responsible for observed use-related effects.

Present study

The present study aimed to characterize VST functional connectivity associated with quantitative measures of alcohol, cannabis, and tobacco use in a community sample of young adults, a population that has passed through the period of greatest substance use (Johnston et al., 2017) and SUD risk (Kessler et al., 2005), and nearing the culmination of significant brain maturation (Paus, 2005; Spear, 2000). Crucially, this study is the first to have used CTC analyses to investigate the potential causal nature of substance use on resting-state connectivity. Based on previous studies that have characterized SUD-related striatal functional connectivity, we predicted that higher amounts of substance use would be associated with reduced functional connectivity between VST and regions of frontal cortex, and that due to these three substances theorized common interactions with striatal dopamine (Di Chiara & Imperato, 1988; Ikemoto, 2010; Nestler, 2005; Sulzer, 2011), potential effects would be ascribed to similar regions of the brain across substances. We followed up such effects, which were discovered based on correlational analyses, with CTC analyses to elucidate etiological influences. Due to the heritable nature of SUDs (Goldman, Oroszi, & Ducci, 2005) and brain connectivity (Bohlken et al., 2014; Glahn et al., 2010), we predicted that any use-related effects would be attributable to the familial propensity to use rather than a consequence of use. Finally, we explored the degree to which VST functional connectivity correlated with measures of trait impulsivity and compulsive substance use, and predicted that both scales would be inversely related to frontostriatal connectivity, but that impulsivity would be stronger related to reduced VST functional connectivity with dorsal frontal cortex while compulsive use would be stronger associated with reduced connectivity in regions of ventral prefrontal cortex.

Method

Participants

Three-hundred and four subjects (111 male; 136 twin pairs in total) were recruited as part of the age 24 assessment of the Enrichment Sample of the Minnesota Twin and Family Study (for overview of study design and sample characteristics, see Keyes et al., 2009). Subjects first visited when they were about 11 years of age, and followed-up at approximate ages 14, 17, and the current assessment (age *M* [*SD*] = 24.5 [.7]).

Quantitative alcohol, cannabis, and tobacco use measures

Quantitative substance use measures were constructed from two inventories, the Computerized Substance Use Assessment (CSA; Han et al., 1999) and expanded version of the Substance Abuse Module (SAM; Robins et al., 1987) which assess multiple aspects of alcohol, cannabis, and tobacco use. The CSA was administered privately in a sound-attenuated room via computer at ages 11 and 14; SAM interviews were conducted for each subject individually at ages 17 and 24 by trained staff having at least a bachelor's degree in psychology or related field.

Alcohol, cannabis, and tobacco use measures were generated from questions assessing several quantitative aspects of use (see **Table 5** for item content). The alcohol use measure combines information about quantity, frequency, density of use, and abuse of alcohol (see **Table 5** for item content), and has been validated previously in studies of similar focus (Harper et al., 2016; Malone, Luciana, et al., 2014; McGue et al., 2014; Wilson et al., 2015). Specifically, responses for each item were transformed into zero (no use) through five or six (greatest use) ordinal values, and then at each of the 11-, 14-, 17-

and 24-year-old assessments, responses were summed to yield four age-specific drinking estimates. Finally, these age-specific alcohol use composites were summed resulting in a single cumulative alcohol use measure. Composite measures for cannabis use and tobacco use were constructed in a similar manner, such that ordinal-transformed responses were summed within age, and then across ages to yield cumulative cannabis use and tobacco use (respectively).

Two subjects were excluded for having self-reported use of recreational substances that may have acute effects on fMRI; one subject reported using cannabis within 6 hours of the scan (Crean et al., 2011; Sneider et al., 2006) and another reported using heroin the evening before the assessment and was excluded to avoid possible withdrawal effects. Recent use of caffeine or nicotine were not exclusions, although subjects were asked to consume on the day of the assessment no more or no less than they would consume on an ordinary day.

Table 5

Substance use construct inventories and items

Inventory Item	Content
Computerize Substance Use Assessment (CSA; ages 11 and 14)	
Alcohol	
1. *How many times in the past 12 months have you been drunk?*	Misuse
2. *During the past 12 months, about how many times did you drink alcohol?*	Frequency
3. *In the past 12 months, when you drank alcohol, how many drinks did you usually have? (a drink is a glass of wine, a bottle or can of beer, a shot glass of liquor, or a mixed drink)*	Quantity
4. *In the past 12 months, what is the largest number of drinks you had at one time? (a drink is a glass of wine, a bottle or can of beer, a shot glass of liquor, or a mixed drink)*	Density
Cannabis	
1. *During the past 12 months, about how many times did you use marijuana or hashish?*	Frequency
Tobacco	
1. *During the past 12 months, how many days did you smoke cigarettes during a typical month?*	Frequency
2. *During the past 12 months, how much have you smoked (cigarettes) on a typical day that you smoked?*	Quantity
Expanded Substance Use Module (SAM; ages 17 and 24)	
Alcohol	
1. *About how many times have you been intoxicated or drunk?*	Misuse
2. *In the past 12 months, how often on average have you drunk any alcohol (had any alcohol to drink)?*	Frequency
3. *How much did you have on average each time you drank during the past 12 months?*	Quantity
4. *What is the largest amount of alcohol you ever consumed in a 24-hour period?*	Density
Cannabis	
1. *In your lifetime, how many times have you used marijuana?*	Quantity
2. *During your period of heaviest use how often did you use marijuana?*	Density
3. *In the past 12 months, how often have you used marijuana?*	Frequency
Tobacco	
1. *In past 12 months, how many days did you smoke (use tobacco) during a typical month?*	Frequency
2. *In past 12 months, how much have you smoked (used tobacco)*	Quantity

on a typical day that you smoked?	

Note. Quantitative measures of alcohol, cannabis and tobacco use were constructed based on items from two inventories, the CSA (assessed at ages 11 and 14) and SAM (assessed at ages 17 and 24). Responses for each item were transformed into zero (no use) through six (greatest use) ordinal values, and then at each of the 11-, 14-, 17- and 24-year-old assessments, responses were summed to yield four age-specific estimates. Finally, these age-specific substance use composites were summed resulting in a single cumulative use measure for each substance.

Trait impulsivity and compulsive substance use

To understand the degree to which VST functional connectivity was associated with individual differences in *trait impulsivity* we examined the Control scale from the Multidimensional Personality Questionnaire (MPQ; Tellegen & Waller, 2008) which subjects completed as part of the Personality Booklet – Youth, Abbreviated (developed specifically for use at the Minnesota Twin and Family Study, for more information see Matteson et al., 2013). High scores on MPQ Control reflects tendencies for cautious planning and sensibility in decision-making whereby low scores reflect tendencies for impulsivity, spontaneity, and recklessness; thus, impulsivity was defined by reverse-coding MPQ Control.

We also investigated the degree to which VST functional connectivity was associated with individual differences in *compulsive substance use*, which may be defined as continued substance use despite having experienced negative consequences or the desire to use less or quit. Similar to a previous report (Hu et al., 2015), we defined compulsive substance use for each individual as the number of symptoms met for alcohol, cannabis, or nicotine use disorder in the *Diagnostic and Statistical Manual of Mental Disorders* (5^{th} ed.; *DSM-5*; American Psychiatric Association, 2013), excluding withdrawal and tolerance criteria.

Neuroimaging assessment

MRI assessments took place at the University of Minnesota's Center for Magnetic Resonance Research. Due to facility equipment upgrades that occurred at the time of the study, fifty twin pairs were scanned on a 3T Siemens Trio and the remaining subjects

were scanned on a 3T Prisma scanner. For each subject, anatomic T1-weighted images were acquired with magnetization-prepared rapid gradient-echo sequence: MPRAGE Trio, TE = 3.65 milliseconds, TR = 2530 milliseconds, flip-angle = 7°, FOV = 256 mm, matrix = 256 x 256, slice-thickness = 1 mm, 240 sagittal slices. Functional T2*-weighted images were acquired during eyes-open resting wakefulness (Trio = 9:56 minutes; Prisma = 10:10 minutes) with the following echo-planar (EPI) sequence [Trio, Prisma]: TE = [25.4, 30.0] milliseconds, TR = [1395, 1500] milliseconds, flip-angle = [90°, 70°], FOV = 212 millimeters, matrix = 106 x 106, slice-thickness = 2 millimeters with no gap, in-plane resolution = 2 x 2 millimeters, 72 axial slices with interleaved multiband (factor = 4) slice acquisition.

In the interest of comfort and to minimize subject movement, vacuum cushions were situated in the receiver coil on the back, right, and left of the head for each subject. After the resting-state scan, participants were asked to rate their subjective level of fatigue and the response to this question was used as a covariate in subsequent analyses.

FMRI preprocessing and functional connectivity computation

Spatial preprocessing of functional data was conducted using open-source software (see fsl.fmrib.ox.ac.uk/ and fil.ion.ucl.ac.uk/spm/software/spm12/); these steps included realignment of functional data to the first volume, slice-time correction, warping to Montreal Neurological Institute (MNI) standard space, re-slicing to 2 millimeter isotropic voxels, and spatial filtering with a 6-millimeter full-width half-maximum Gaussian kernel. The CONN toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012) was used for temporal filtering .009 to .08 hertz, and to diminish effects due to nuisance

variables thought to generate spurious functional connectivity among voxels. The following nuisance variables were constructed for each fMRI session: six head motion parameters (3 translation, 3 rotation) and their first-order temporal derivatives, and signals from eroded white matter and cerebrospinal fluid masks (Behzadi, Restom, Liau, & Liu, 2007; Chai, Castanon, Ongur, & Whitfield-Gabrieli, 2012). Additionally, the censoring procedure described by Power, Barnes, Snyder, Schlaggar, and Petersen (2012) was used to remove the influence of instantaneous excessive motion; these instances were identified when the *frame-wise displacement* (overall subject movement) exceeded .5 mm, or the *DVAR* (global fluctuations in blood-oxygen-level dependent time-course) was statistically deviant (here, exceeding 4 normalized median absolute deviations).

With the above nuisance variables as covariates, functional connectivity (Fischer's z-transformed bivariate correlations) was computed for each subject within the VST seed region (depicted in the left panel of **Figure 4**) and the rest of the brain. The VST seed, which encompasses the bilateral nucleus accumbens and ventral pre-commissural caudate and putamen regions, was previously delineated on the basis of having white matter connectivity with medial orbitofrontal and anterior cingulate cortices and shown to have high dopamine D2/D3-like receptor availability (Tziortzi et al., 2014); such receptors have been posited as a mechanism of disinhibitory traits in addiction (Volkow et al., 2013). Like previous studies where VST functional connectivity is examined (Choi, Yeo, & Buckner, 2012; Di Martino et al., 2008; Jaspers, Balsters, Kassraian Fard, Mantini, & Wenderoth, 2017), we found substantial positive connectivity (illustrated in **Figure 4**, right) in bilateral striatum, cingulate cortex, paracingulate cortex,

insular cortex, orbitofrontal cortex, as well as other ventral and dorsal medial frontal regions (e.g., superior frontal gyrus) and subcortical structures (e.g., thalamus, cerebellum).

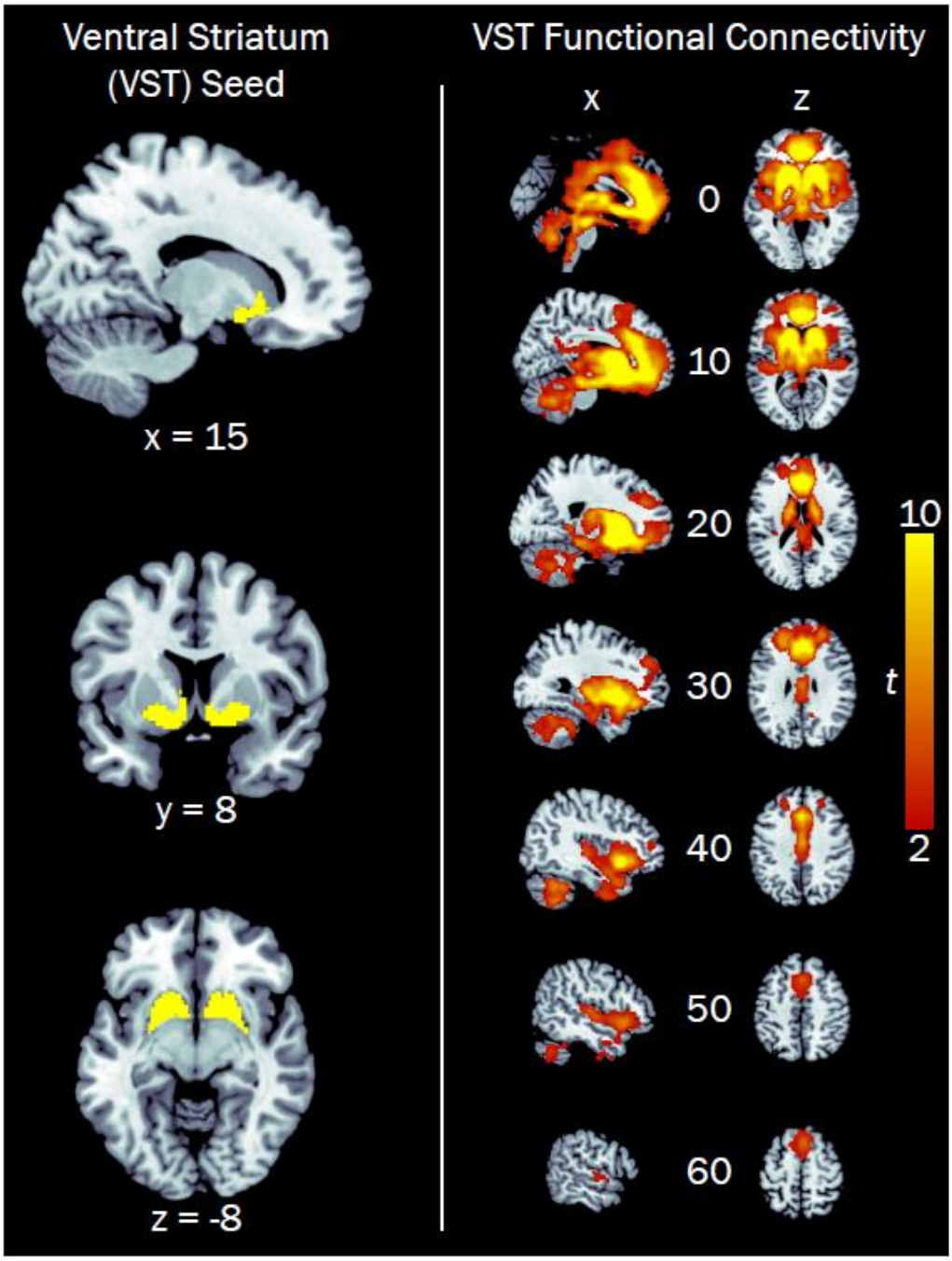

Figure 4. Ventral striatum (VST) seed region and whole-brain resting-state functional connectivity. The left panel shows the VST seed (yellow) that was previously defined by Tziortzi et al. (2014). Positive VST functional connectivity values ($t > 2$) is shown in the right panel, and indicates strong functional connectivity in medial frontal, orbitofrontal, insula, and cingulate structures. X, Y, and Z values correspond to Montreal Neurological Institute coordinates.

Statistical analyses

To examine the effects of alcohol, cannabis, and tobacco use on VST functional connectivity, we calculated linear mixed models (LMMs) implemented in the AFNI program *3dLME* (for whole-brain analyses; Chen, Saad, Britton, Pine, & Cox, 2013) and R program *lmer* (for "region of interest" analyses; Bates et al., 2015) which enabled the inclusion of a random intercept for each twin pair, accounting for the family structure of the data. LMMs tested for main effects of substance use (alcohol, cannabis, or tobacco, separately), main effects of gender (male vs. female), and interaction effects (substance use × gender). Although we only focused on effects pertaining to substance use (main effect, use × gender), the average whole-brain functional connectivity value (cf. "global correlation"; Saad et al., 2013), average number of censored time-points (*M* = 7.5%, *SD* = 5.8%), fatigue ratings (*M* = 5.9, *SD* = 2.3; 1 = least, 10 = most tired), and acquisition scanner (Prisma or Trio) for each subject were included as nuisance covariates. Although not depicted in the below equations, all LMMs included main effects of gender and gender × use interaction effects in individual level LMMs; interaction terms were subsequently dropped if they were shown to be associated with a *p*-value greater than .05.

Individual level associations among substance use and functional connectivity. To determine which brain region(s) in VST functional connectivity maps were significantly associated with substance use, we first regressed whole-brain VST functional connectivity onto individual level measures of alcohol, cannabis, and tobacco use (each separately) using the model, $FC_{ij} = B_0 + B_1 USE_{ij} + \alpha_i + \varepsilon_{ij}$ (other covariates not shown for simplicity). Here, FC_{ij} and USE_{ij} reflect functional connectivity and

(alcohol, cannabis, or tobacco) use for person j of twin-pair i; B_1 reflects the individual level association between substance use and functional connectivity which ignores within twin-pair differences in substance use, thus akin to existing correlational research using non-twin designs. The terms B_0 and α_i reflect fixed and twin-pair-specific random model intercepts, respectively; ε_{ij} is random noise. We plotted masks depicting associations for alcohol, cannabis, and tobacco use with VST functional connectivity in **Figure 5** (voxel-wise $p < .005$, minimum cluster size ≥ 200 voxels) to highlight regions of overlap for substance use association functional connectivity maps. Only reduced VST functional connectivity is shown as these thresholds did not reveal any region to be associated with substance-use related increases in functional connectivity. Blue voxels correspond to regions where use-related reductions in functional connectivity were specific to alcohol (green = cannabis, red = tobacco), cyan voxels correspond to regions where masks for alcohol and cannabis masks intersected (yellow = intersection of cannabis and tobacco, magenta = intersection of tobacco and alcohol), and white voxels correspond to regions where masks for all three substances intersect.

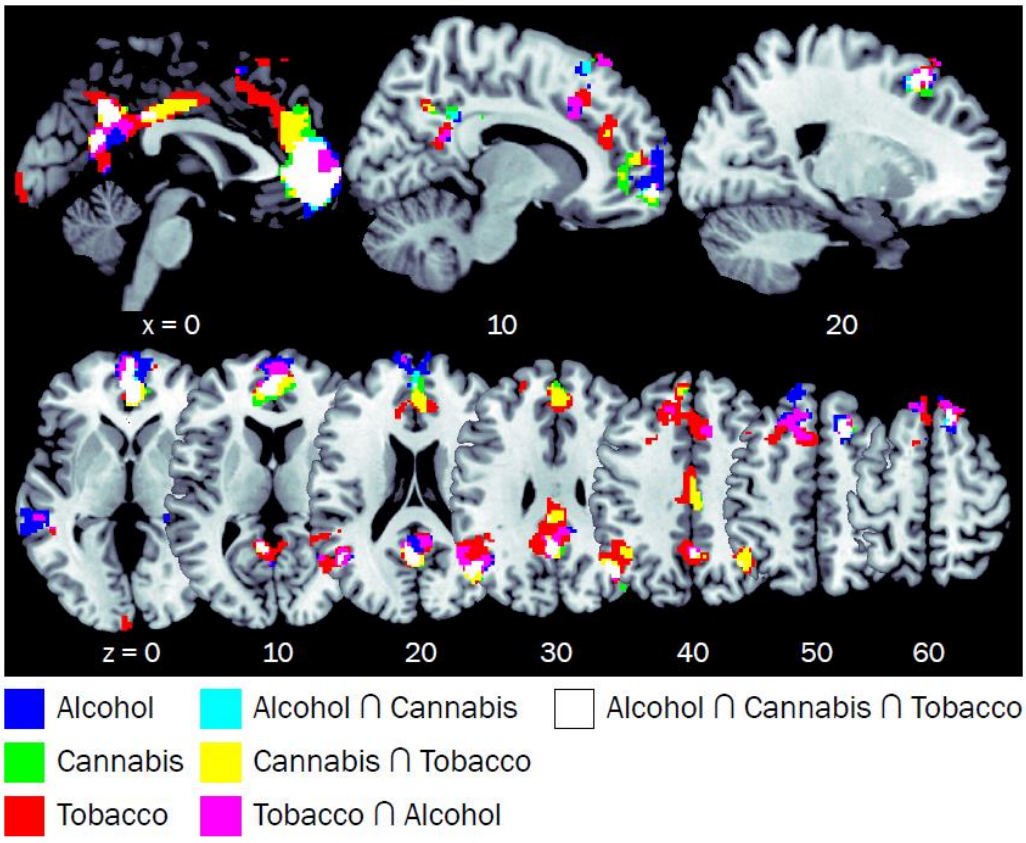

Figure 5. Ventral striatal (VST) functional connectivity maps associations with alcohol, cannabis, and tobacco use. Each mask depicts the individual level VST functional connectivity association for alcohol, cannabis, and tobacco use (voxel-wise $p < .005$, minimum cluster size ≥ 200 voxels). Only reduced VST functional connectivity is shown as these thresholds did not reveal any region to be associated with substance-use related increases in functional connectivity. Blue voxels correspond to regions where use-related reductions in functional connectivity were specific to alcohol (green = cannabis, red = tobacco), cyan voxels correspond to regions where masks for alcohol and cannabis masks intersected (yellow = intersection of cannabis and tobacco, magenta = intersection of tobacco and alcohol), and white voxels correspond to regions where masks for all three substances intersect.

In the interest of extracting functional connectivity scores for each person within regions of interest (ROIs) for further exploration, we conducted ROI identification as described below. Due to high correlations among substance use measures (*r*s ranged .63 to .67) and to focus on regions in VST functional connectivity maps showing high convergence across substances, we averaged the three substance use composites into a single variable and regressed whole-brain VST functional connectivity onto this variable.

ROIs were determined by the program *3dClustSim* in AFNI (version 16.3.08; Cox, 1996), which uses Monte Carlo simulation (10,000 iterations) to estimate the probability of false positive clusters at different voxel-wise and cluster-size cutoffs based on smoothness properties (i.e., the "-acf" option; Cox, Chen, Glen, Reynolds, & Taylor, 2017) computed from temporally-concatenated resting-state fMRI time-series by the program *3dFWHMx*. A priori voxel-wise and cluster size thresholds were chosen to be .001 and .05, respectively (e.g., Addicott, Sweitzer, Froeliger, Rose, & McClernon, 2015; Camchong et al., 2013). Finally, for each subject the mean functional connectivity within ROIs was calculated, and these values were used for statistical exploration. Degrees of freedom and *p*-values for LMMs were estimated by Kenwood-Roger approximation (Kuznetsova et al., 2016).

Co-twin control (CTC) analysis to examine possible causal effects of substance use. Statistically significant ($p < .05$) individual level associations between substance use and ROI-derived connectivity scores were followed up with co-twin control (CTC) analyses (Begg & Parides, 2003; McGue et al., 2010; Rutter, 2007) which enabled testing for possible causal effect(s) of substance use on functional connectivity. To perform CTC analyses, for each alcohol, cannabis, and tobacco use measure, scores were re-expressed as within twin-pair difference in use, such that in the model $FC_{ij} = B_0 + B_W(USE_{ij} - \overline{USE}_{i\cdot}) + \alpha_i + \varepsilon_{ij}$ where $\overline{USE}_{i\cdot}$ reflects average alcohol, cannabis, or tobacco use within a given twin-pair, the coefficient B_W reflects the causal effect on functional connectivity as a result of within twin-pair differences in use. Unlike the individual level coefficient B_I that enables only correlational inference, observation of

a significant B_W coefficient is consistent with the notion that substance use has caused within twin-pair differences in functional connectivity. For the sake of interpretability across substance use measures that have different scales, we presented T_I (B_I divided by standard error) and T_W (B_W divided by standard error) statistics in tables. Because individual level analyses set precedent regarding the direction of anticipated effects and to increase statistical power to detect possible causal effects, one-tailed tests were employed for CTC analyses.

We also tested for zygosity effects in CTC analysis to determine whether it was appropriate to examine effects separately within subsamples of DZ and MZ twins. To test whether zygosity-related effects were of statistical import in the MZ/DZ combined sample, a likelihood ratio test was utilized whereby measures of statistical misfit (-2 log likelihood, or -2LL) were compared for two models: (1) including zygosity (DZ vs. MZ), zygosity × use, and zygosity × gender × use interaction covariates, and (2) without these zygosity covariates. The absolute difference value obtained by subtracting one model from the other was subsequently referred to a chi-square distribution. A significant change in fit ($\Delta\chi^2$) was consistent with differential effects by zygosity, and deemed further inspection separately within DZ and MZ subsamples.

Results

Participant characteristics

Sample demographic, clinical, and personality descriptive statistics with regards to each substance use measure are presented in **Table 6** and show that alcohol, cannabis, and tobacco use measures did not significantly correlate with gender or age at the current

assessment (*p*s > .05), but they did significantly correlate with clinical and personality characteristics relevant to SUDs and addiction. Specifically, all substance use measures were associated with younger age of first (alcohol, cannabis, or tobacco) use (*p*s <.001), approximately two- to six-fold increased odds of alcohol, cannabis, or tobacco use disorders (*p*s < .05), as well as high trait impulsivity and compulsive substance use (*p*s < .001).

Table 6

Demographic, clinical, and personality descriptive statistics and associations with alcohol, cannabis, and tobacco use

Demographic, clinical, and personality variables	Overall	LMM Association with Substance Use					
		Alcohol		Cannabis		Tobacco	
	Mean or *%*	B_I (*SE*) or *OR*	*p*	B_I (*SE*) or *OR*	*p*	B_I (*SE*) or *OR*	*p*
Gender [a]	63.2% female	.60	.418	.58	.370	.66	.491
Age at current assessment	24.4 years	.0 (.0)	.112	.0 (.0)	.920	.0 (.0)	>.05
Age first use (alc., can., or tob.)	16.1 years	-1.8 (.2)	**<.001**	-1.6 (.2)	**<.001**	-1.5 (.2)	**<.001**
Meets criteria for AUD [a]	24.5% yes	2.76	**<.001**	2.33	**<.001**	2.31	**<.001**
Meets criteria for CUD [a]	25.8% yes	1.77	**.013**	6.04	**<.001**	1.94	**<.001**
Meets criteria for NUD [a]	19.9% yes	3.33	**<.001**	5.12	**<.001**	4.62	**<.001**
Trait impulsivity	35.9	2.3 (.5)	**<.001**	2.1 (.5)	**<.001**	1.9 (.5)	**<.001**
Compulsive substance use	2.1	1.4 (.2)	**<.001**	1.9 (.1)	**<.001**	1.6 (.1)	**<.001**

Note. Overall means and frequencies, and linear mixed model-estimated (LMM) associations with substance use measures for demographic, clinical, and personality variables. Out of the entire sample, 100% subjects have reported having used alcohol, 69% cannabis, and 58% tobacco. Coefficients (*B*s) reflect the degree to which the listed dependent variable changes with respect to one standard deviation increase on the use measures. Variables for which odds ratios (*OR*s) were presented instead of coefficients are flagged with superscript "a"; positive values reflect increased odds of a "yes" response given one standard deviation increase in substance use measure. LMMs were adjusted for a main effect of gender. Significant effects ($p < .05$) are highlighted in bold text. Other abbreviations: SE = standard error; AUD = alcohol use disorder; CUD = cannabis use disorder; NUD = nicotine use disorder.

Individual level effects of substance use on VST functional connectivity

As depicted in **Figure 6**, five ROIs were identified where the association among substance use (alcohol, cannabis, and tobacco use scales *z*-scored and averaged) and VST functional connectivity was strong ($p < .001$) and spanned at least 167 contiguous voxels, preserving the family-wise error rate of $p < .05$. Labels for each ROI, hemispheric locations, center-of-mass MNI coordinates, and anatomical references are presented in **Table 7**. Generally, these ROIs correspond spatially to regions where alcohol, cannabis, and tobacco use-related maps intersected in **Figure 5** (depicted by white voxels).

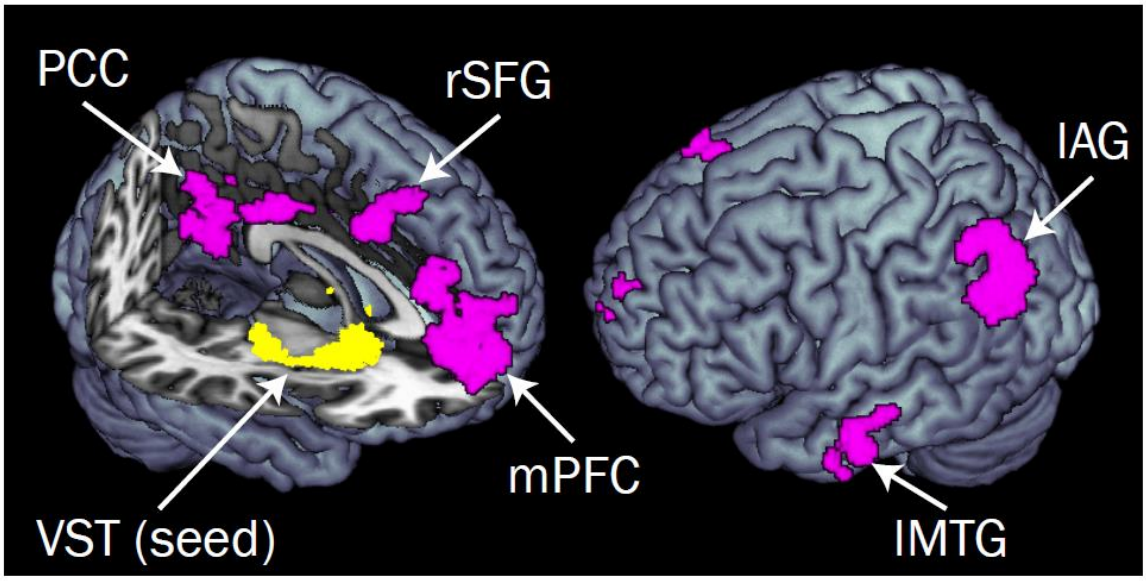

Figure 6. Ventral striatal seed (VST, yellow) and regions of interest (ROIs, magenta) determined by whole-brain cluster analysis. Cluster size was corrected for family-wise error at $p < .05$, using a voxel-wise threshold of $p < .001$ and spatial autocorrelation smoothness parameters estimated from the data (see text for more details).

Table 7

Target regions of interest showing substance use-related reductions in functional connectivity with ventral striatum

Label	Hemisphere	MNI position			Anatomical reference (percentage overlap)
		x	*y*	*z*	
mPFC	Right, Left	2	46	0	Para-cingulate gyrus (36%), frontal pole (29%), anterior cingulate cortex (11%), medial frontal cortex (7%)
PCC	Right, Left	0	-32	32	Posterior cingulate cortex (58%), precuneus (32%), anterior cingulate cortex (4%)
lAG	Left	-50	-66	40	Superior lateral occipital cortex (56%), angular gyrus (35%)
rSFG	Right	18	22	48	Superior frontal gyrus (60%)
lMTG	Left	-64	-10	-24	Posterior (52%) and anterior (44%) medial temporal gyrus

Note. Whole-brain ventral striatal (VST) resting-state functional connectivity (rsFC) was regressed onto the substance use (alcohol, cannabis, and tobacco measures, averaged) to examine the regional use-related associations. Target regions of interest (ROIs, bold text) were delineated by a thresholding process described in the text that protects against family-wise error (voxel-wise $p < .001$, cluster size $p < .05$) and accounts for the non-uniform smoothness of functional magnetic resonance imaging data. All ROIs were associated with significant decreased VST functional connectivity as a function of substance use; no use-related increases in functional connectivity were observed. ROIs are listed in order of their size, and presented alongside Montreal Neurological Institute (MNI) coordinates of the ROIs' center of mass as well as description of anatomical characteristics as referenced to a standard atlas. Percent overlap was calculated by the number of voxels overlapping with the anatomical reference divided by the total number of voxels in the ROI. Mean rsFC was derived within each ROI mask and used for subsequent statistics reported in tables. Other abbreviations: mPFC = medial prefrontal cortex; PCC = posterior cingulate cortex; lAG = left angular gyrus; rSFG = right superior frontal gyrus; lMTG = left temporal gyrus.

As depicted by negatively-signed T_1 statistics left of the vertical in **Table 8**, alcohol, cannabis, and tobacco use were all associated with reduced VST functional connectivity for all five ROIs. Also, these associations were significant for all three substances at all ROIs: medial prefrontal cortex (mPFC, *p*s <.001), posterior cingulate cortex (PCC, *p*s < .002), left angular gyrus (lAG, *p*s <.002), right superior frontal gyrus (rSFG, *p*s < .02), and left middle temporal gyrus (lMTG, *p*s <.007). Significant gender × use interactions were observed in four instances (denoted by superscript "a" in the table); in these instances, effects for males were more negative than that for females (*p*s < .05) but main effects were negatively-signed for both genders, so we presented the main effects for alcohol, cannabis, or tobacco use adjusted for gender interactions.

Table 8

Ventral striatal functional connectivity associations with alcohol, cannabis, and tobacco use

ROI	Individual		Co-twin control (CTC)					
Substance	Cumulative Use		Cumulative Use		Adolescent Use		Past 7 Years Use	
Gender	T_I (*df*)	*p*	T_W (*df*)	*p*	T_W (*df*)	*p*	T_W (*df*)	*p*
mPFC								
Alcohol	-3.9 (239)	**<.001**	-2.6 (138)	**.005**	-2.1 (134)	**.018**	-2.5 (124)	**.006**
Cannabis	-3.5 (231)	**<.001**	-1.9 (133)	**.031**	-2.0 (134)	**.025**	-1.3 (119)	.101
Tobacco	-3.6 (225)	**<.001**	-1.7 (139)	**.047**	-.6 (138)	.262	-1.9 (133)	**.031**
PCC								
Alcohol	-4.2 (239)	**<.001**	-2.1 (138)	**.019**	-1.9 (133)	**.031**	-2.4 (123)	**.009**
Cannabis	-3.4 (231)	**.001**	-.8 (132)	.202	-1.1 (134)	.137	-.2 (189)	.404
Tobacco	-4.1 (223)	**<.001**	-.1 (139)	.447	.5 (137)	.310	-1.0 (132)	.170
lAG								
Alcohol	-3.8 (243)	**<.001**	-2.0 (137)	**.025**	-1.3 (133)	.103	-1.8 (123)	**.034**
Cannabis	-3.3 (237)	**.001**	-1.3 (132)	.091	-1.2 (133)	.126	-.6 (119)	.269
Tobacco[a]	-3.5 (218)	**<.001**	-.9 (127)	.179	-.4 (127)	.343	-1.0 (121)	.151
rSFG								
Alcohol[a]	-3.3 (235)	**.001**	-1.2 (133)	.125	-.4 (133)	.359	-2.2 (124)	**.016**
Cannabis	-3.0 (232)	**.003**	-1.6 (133)	.060	-1.4 (134)	.083	-.9 (120)	.172
Tobacco[a]	-2.7 (218)	**.009**	.4 (127)	.361	-.0 (128)	.481	.2 (122)	.416
lMTG								
Alcohol	-3.9 (230)	**<.001**	-2.4 (140)	**.008**	-1.8 (135)	**.035**	-2.0 (125)	**.023**
Cannabis	-3.2 (224)	**.002**	-1.9 (134)	**.028**	-1.4 (135)	.086	-1.7 (120)	**.045**
Tobacco[a]	-3.0 (208)	**.004**	-.3 (128)	.366	.0 (128)	.486	-.5 (123)	.309

Note. Test statistics (*T*), degrees of freedom (*df*), and significance values (*p*) from linear mixed models depicting main effects of alcohol, cannabis, and tobacco use on ventral striatal (VST) functional connectivity within regions of interest (ROIs, underlined). *T*-values left of the vertical (T_Is) depict the individual-level association, or correlation between substance use and functional connectivity. Statistically significant ($p < .05$, denoted by boldface) individual level results were followed up using the co-twin control (CTC, right of vertical) design; here, CTC coefficients (T_Ws) depict the main effect of within twin-pair differences in substance use on functional connectivity, or the possible "causal effect" of substance use. Kenwood-Roger approximation determined degrees of freedom and *p*-values. Because individual level analyses set precedent regarding the direction of anticipated CTC effects and to increase statistical power to detect possible causal effects, one-tailed tests were utilized for CTC analyses. All analyses were adjusted for a main effect of gender. We explored gender × use interaction effects and flagged rows with superscript "a" where the interaction was significant ($p < .05$); gender × use interaction effects were dropped in rows without superscript "a". Main effects of alcohol, cannabis, and tobacco use were negatively-signed for both males and females, so we did not present results separately by gender.

Supplemental statistics.

Co-twin control (CTC) analysis to examine causality of individual level effects

To understand whether reduced functional connectivity may be attributed to possible causal effects of alcohol, cannabis, or tobacco use, we followed up with CTC analyses that were conducted on a total of 136 complete pairs (DZ = 51, MZ = 85) presented right of the vertical in **Table 8**. In line with individual level effects, nearly all CTC effects (T_Ws) were negatively-signed, supporting the possibility that within twin-pair differences in substance use may be responsible for reduced functional connectivity. Moreover, 7 of the 15 CTC effects were significant; these were for mPFC (all three substances, *p*s < .048), PCC (alcohol, $p = .019$), lAG (alcohol, $p = .025$), and lMTG (alcohol and cannabis, *ps* < .029), suggesting a causal role of substance use in reduced functional connectivity at these ROIs. Notably, CTC effects were nominally strongest for mPFC (mean $T_W = -2.1$, all *p*s < .05) and weakest for rSFG (mean $T_W = -.8$, all *p*s > .05), and the range of CTC effects at either ROI did not overlap, indicating that substance use may cause reduced VST functional connectivity in a regionally-selective manner, rather than uniformly across all regions of the brain. Additionally, CTC effects were nominally strongest for alcohol (mean $T_W = -2.1$), followed by cannabis (mean $T_W = -1.5$), and then tobacco (mean $T_W = -.5$), indicating that some substances (e.g., alcohol) may have greater influences on brain connectivity relative to others (e.g., tobacco). CTC effects did not differ among DZ and MZ twins; adding zygosity and zygosity × twin-pair use effect terms to the models did not improve model fit at any ROI (all *p*s > .173).

Functional connectivity associations with trait impulsivity and compulsive substance use

Finally, we sought to understand whether reduced VST functional connectivity was associated with measures of trait impulsivity and compulsive substance use. Turning to **Figure 7A**, *t*-statistics are plotted reflecting the degree to which impulsivity and compulsive substance use were associated with reduced VST functional connectivity at each of the five ROIs. Trait impulsivity was associated with reduced VST functional connectivity with rSFG only ($p = .008$, other ps $> .486$), while compulsive substance use was associated with reduced functional connectivity at all ROIs (ps $< .05$). However, given that trait impulsivity and compulsive substance use variables correlated with one another ($r = .35$, $p < .001$) and to account for the possibility that observed associations with functional connectivity for either scale may be due to variance common among the two scales, we adjusted *t*-statistics in **Figure 7B**, such that both variables were included as co-predictors in the same model. After adjusting for compulsive substance use, the unique association between impulsivity and reduced VST functional connectivity with rSFG persisted ($p = .005$); and, after adjusting for impulsivity, unique associations for mPFC, PCC, lAG, and lMTG persisted (ps $< .05$). Collectively, reduced connectivity within the ROI that exhibited least evidence of substance use causal effects above (i.e., rSFG) was strongest associated with trait impulsivity, while reduced connectivity in other ROIs that exhibited significant CTC effects (i.e., mPFC, PCC, lAG, and lMTG) were not associated with trait impulsivity but rather compulsive substance use.

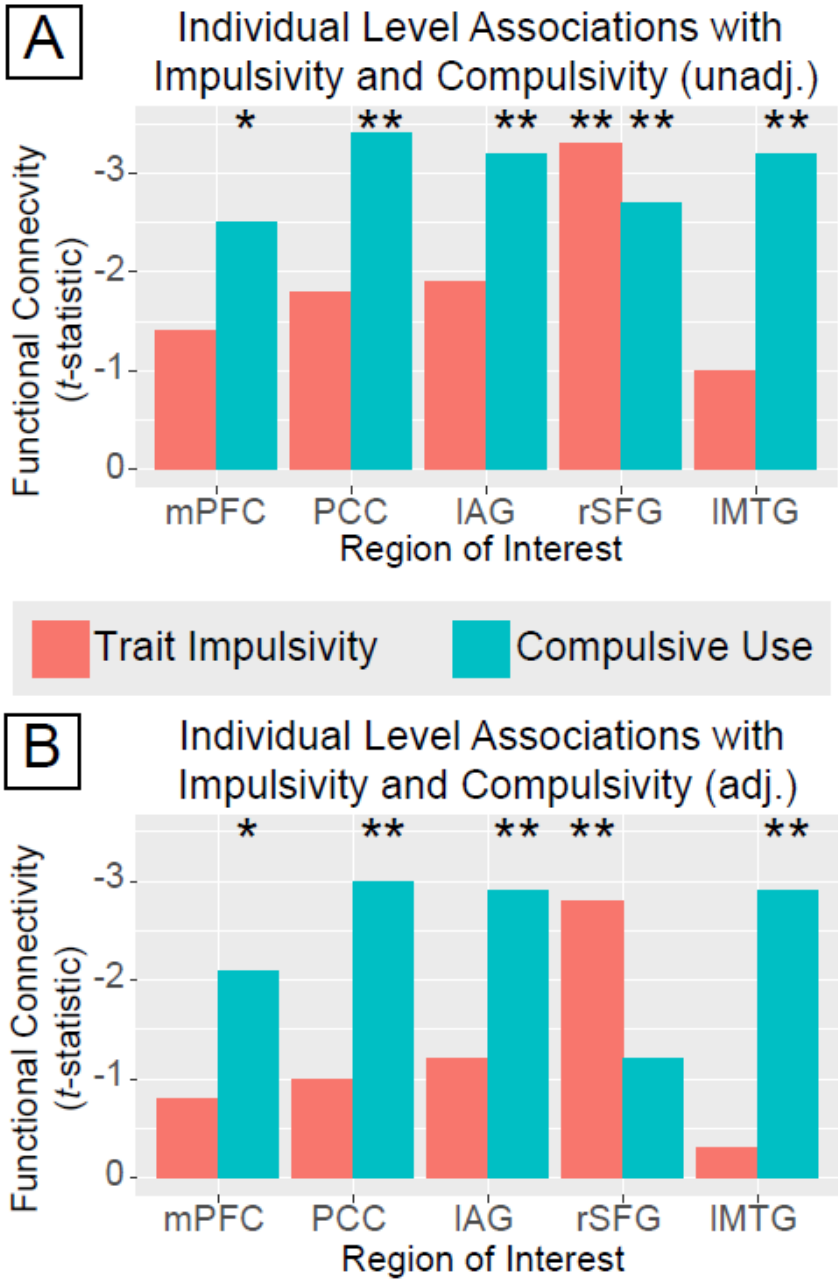

Figure 7. Bars depicting t-statistics reflecting associations of reduced ventral striatum (VST) functional connectivity with trait impulsivity (red) and compulsive substance use (blue). A) associations for trait impulsivity and compulsive substance use, within separate regression models. B) trait impulsivity and compulsive substance use (r = .35, p < .001) were entered into the same regression to clarify their unique contributions to VST functional connectivity. * p < .05, ** p < .01.

Discussion

We examined individual differences in VST functional connectivity related to quantitative measures of alcohol, cannabis, and tobacco use in a community sample of young adults, and tested whether connectivity differences may be attributable to causal exposure effects of substance use by employing CTC analyses. Our hypothesis that functional connectivity would be inversely related to substance use was supported, and is consistent with prior research comparing groups of severely affected individuals exhibiting lower connectivity for SUDs relative to control subjects. Upon investigating the etiology of such effects using CTC analyses, we found reduced VST functional connectivity in some brain regions (e.g., rSFG) to be attributable to familial confounding factors, whereas in other brain regions (e.g., mPFC) substance use appeared possibly to have caused reductions in VST functional connectivity. Thus, our second hypothesis was partly supported and revealed substantial confounding by familial factors in the association between substance use and functional connectivity, but also revealed that substance use (especially alcohol use) may cause regionally-specific and lasting alterations in resting-state VST functional connectivity, perhaps via experience-dependent plasticity (cf. Graybiel, 2008). In support of our third hypothesis, reduced VST functional connectivity was linked to high trait impulsivity and compulsive substance use in dorsal and ventral frontal cortex (respectively), which is discussed below with regards to implications for SUD-related pathophysiology.

We observed several significant CTC results, suggesting that substance use may have caused reduced VST functional connectivity with mPFC, PCC, lAG, and lMTG. These

regions have been interrelated as part of the brain's default mode network (DMN) (Davey et al., 2016; Fox et al., 2005; Raichle et al., 2001), which is thought to mediate self-referential cognition regarding future and past situations (Amodio & Frith, 2006; Buckner & Carroll, 2007; Northoff & Bermpohl, 2004). As such, functional connectivity between VST and DMN (for other examples, see Choi et al., 2012; Di Martino et al., 2008) might indicate incorporation of motivational drives in DMN processes, perhaps encoding subjective representations of potential rewards (Kable & Glimcher, 2007; Kolling, Behrens, Mars, & Rushworth, 2012; McNamee, Rangel, & O'Doherty, 2013). Moreover, strength of interaction between VST and DMN activities has predicted individual differences in delayed gratification (Diekhof & Gruber, 2010; McClure, Laibson, Loewenstein, & Cohen, 2004), or for example, selecting to attain a large reward later in lieu of a small reward now. Deficits in functional connectivity between VST and DMN regions associated with compulsive substance use behaviors in our study might tap into the hypothesized shortsightedness characteristic of some addicted individuals (Bechara, Dolan, & Hindes, 2002), or tendency to choose immediate rewards (e.g., substance use) instead of long-term goals (e.g., abstinence). Crucially, our results suggest that such reductions in functional connectivity may be caused by substance use, and perhaps lend insight into how frontostriatal functional connectivity may change with repeated substance use to facilitate compulsive substance use behaviors.

Unlike the above VST connectivity with DMN regions, reduced VST functional connectivity with rSFG was not significantly related to within twin-pair differences in substance use, suggesting that VST functional connectivity with rSFG is less susceptible

to causal effects from use and that such reductions likely instead reflect familial propensity to use substances. Given that rSFG is important for response-inhibition (Floden & Stuss, 2006), especially via pre-supplementary motor area's (contained within SFG; Nachev, Kennard, & Husain, 2008) involvement in a right-lateralized fronto-basal-ganglia "stopping" network (Aron et al., 2007; Aron, Robbins, & Poldrack, 2014), it is not surprising that we found reduced frontostriatal functional connectivity with this region to be indicative of individual differences in impulsivity. Trait impulsivity is thought to precede substance use (e.g., in childhood and adolescence) and confer risk for SUDs (de Wit, 2009; Tarter et al., 2003; Verdejo-Garcia et al., 2008), perhaps reflecting familial vulnerability (Iacono et al., 2008). Future research should delineate the extent to which reduced dorsal frontostriatal functional connectivity prospectively predicts substance use and SUDs, and further characterize the degree to which such connectivity reflects genetic liability for disinhibitory psychopathology.

Different CTC results among DMN regions (mPFC, PCC, lAG, lMTG) versus dorsal frontal cortex (rSFG) may highlight a distinction in the specific ways VST functional connectivity (and corresponding brain function) may be causally affected by substance use, versus ways VST functional connectivity may be less susceptible to changes but nonetheless reflect a familial predilection. Dysfunction of dorsal versus ventral frontal cortex has been posited to underlie deficits in "cool" (cognition independent of one's emotional/ motivational state) versus "hot" (cognition influenced by one's emotional/ motivational state) executive functions in addiction, respectively (Goldstein & Volkow, 2011). Consistent with this theory, we found that reduced VST

functional connectivity with dorsal frontal cortex corresponded to greater trait impulsivity (e.g., acting without foresight) whereas connectivity with ventromedial prefrontal cortex (among other DMN regions) corresponded to greater compulsive use (e.g., using despite negative consequences). Thus, cool executive dysfunction such as impulsivity might be less affected by substance use, whereas hot executive dysfunction such as compulsivity might be greater affected. Future research should aim to disentangle the overlapping but dissociable roles cool/hot executive functions and impulsivity/compulsivity might play in the familial predilection for substance use, as well as the role of substance use in altering such functions (for reviews, see Dalley et al., 2011; Everitt et al., 2008; Robbins et al., 2012).

Limitations

Although the CTC design employed here is a powerful method to elucidate cause and effect in observational data, it does not account for possible confounding due to substance use-related factors that are unshared within twin-pairs, such as within twin-pair differences in environment or genes (for DZ twins only) related to substance use. Similarities among twins with regards to alcohol, cannabis, and tobacco use were moderate (intra-class correlation [ICC], ICC_{DZ} = .39, .38, .50, respectively) to strong (ICC_{MZ} = .75, .80, .76) for DZ and MZ pairs (respectively, all *p*s < .01), and this pattern of similarities suggested that only a minor portion of the variance in substance use was attributable to environment differences (E = $1 - ICC_{MZ}$ = .25, .20, .24) whereas the majority of variance (i.e. 2 to 4 times that of E) was due to additive genetics (A = $2[ICC_{MZ} - ICC_{DZ}]$ = .72, .84, .52) (Falconer & Mackay, 1996). Moreover, unshared

additive genetics within DZ pairs appeared to have no significant effect on the possible causal association between use and VST functional connectivity, as we observed no significant zygosity effects (i.e. DZ vs. MZ pairs). Possible issues with statistical power cannot be ruled out, although, the sample size for the current study (N = 304) is many times larger than what is typical in fMRI literature (e.g., Ns ~ 15; Carp, 2012). Collectively, substance use was well-matched within twin-pairs and largely attributable to genetic factors accounted for by CTC analyses; and, CTC results were similar across DZ and MZ pairs, suggesting possible causal effects of substance use on VST functional connectivity regardless of genotype.

Critically, results in the present study are limited to VST functional connectivity and do not rule out the possibility that other functional circuits (e.g., functional connectivity seeded in different brain regions) and/or neurobiological features (e.g., tissue morphology) reflect possible causal effects of substance use or familial propensity. Moreover, the process used for deriving ROIs in the present study focused on brain regions where VST functional connectivity was reduced for all three substances, rather than regions where VST functional connectivity was affected by only one substance. However, our rationale for using VST as seed region was motivated by the fact that VST is central to many addiction theories (Koob & Volkow, 2010, 2016; Volkow et al., 2016; Volkow et al., 2013; Wise & Koob, 2014) and theories on cognitive and behavioral control (Aron et al., 2014; Feil et al., 2010; Graybiel, 2008; Jahanshahi et al., 2015). And, VST circuitry is thought to be similarly affected by drugs and alcohol via common interactions with dopamine (Di Chiara & Imperato, 1988; Ikemoto, 2010; Nestler, 2005;

Sulzer, 2011). Thus, this study advances knowledge on a possible common brain mechanism of alcohol, cannabis, and tobacco SUDs, and suggests that despite the role of familial factors in shaping VST functional connectivity, substance use may cause lasting alterations in connectivity that could contribute to the development of future problems such as SUDs. Future studies should focus on other functional networks (e.g., using other seed regions or independent components analysis) and examine possible effects that are specific to individual substances, rather than common effects across substances.

Conclusion

Results from the present study complement a growing body of literature that suggests a role for frontostriatal circuits in substance use and behavioral disinhibition (Feil et al., 2010; Graybiel, 2008; Jahanshahi et al., 2015; Sutherland, McHugh, Pariyadath, & Stein, 2012; Tomasi & Volkow, 2013; Volkow et al., 2013), and this study is the first to use a CTC design to inform etiological hypotheses regarding potential consequences of substance use on resting-state brain connectivity. As revealed by CTC analyses, within twin-pair differences in alcohol, cannabis, and tobacco use were associated with within twin-pair differences in VST functional connectivity, such that the twin that used more possessed lower functional connectivity than his or her co-twin, accounting for shared familial factors (e.g., genes) that have confounded causal inference in previous cross-sectional research. Moreover, potential causal effects of substance use on reduced VST functional connectivity appeared to be region-specific; such that CTC effects were stronger in some regions (e.g., DMN regions such as mPFC) relative to others (e.g., rSFG). Interestingly, trait impulsivity (e.g., acting without foresight) and

compulsive substance use (e.g., using despite negative consequences) self-report measures were differentially associated with reductions in VST functional connectivity, such that reduced connectivity with rSFG was associated with trait impulsivity and reduced connectivity with DMN regions was associated with compulsive substance use. Nonetheless, results suggest potential neuroadaptations in VST functional connectivity insult caused by substance use, which relates to deficits in inhibitory control.

Final remarks

Use of recreational substances during youth is common and considered a risk factor for deleterious outcomes, such as substance use disorders (SUDs) and addiction. However, whether substance use itself causes alterations in brain functions (measured with EEG and fMRI) has been difficult to determine based on extant studies, which are mostly based on cross-sectional data and lack causally-informative research designs. We studied two resting-state brain function phenotypes frequently associated with SUDs and thought to be related to SUD pathophysiology, beta EEG power and reduced frontostriatal fMRI connectivity, and hypothesized that differences related to substance use would be attributable to familial vulnerability factors (e.g., genes) rather than substance use exposure effects.

In both Study 1 and Study 2, we found evidence to suggest that resting-state brain arousal (EEG beta power) and connectivity (frontostriatal fMRI correlations) are causally influenced by substance use (particularly alcohol) during young adulthood. Moreover, we found these brain measures to be related to personality and clinical characteristics that may contribute to further substance use and perhaps SUDs, such as negative affectivity

and impaired inhibitory control. Future research should examine the degree to which these brain characteristics predict future use, especially in vulnerable populations such as youths and treatment-seeking individuals.

I believe that resting-state EEG and fMRI hold promising roles in the future of psychiatric research and practice due to the ability of these instruments to characterize *in vivo* the intrinsic functional organization of brain networks in their modus operandi. Specifically, by employing these technologies to tap into pathological brain processes that putatively underlie clinical conditions such as SUDs, it is my hope that researchers and clinicians will be better equipped to design and assign interventions, and improve clinical outcomes and public health.

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