Prediction of adolescent substance use by individual differences in GABA and glutamate levels in the lateral prefrontal cortex: A preliminary exploration

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Abstract

Adolescence is a period of time associated with lower levels of impulse control and is often the time during which youth begin to engage in substance use. Prior research has found that higher levels of impulsivity are associated with increased adolescent substance use. The brain structure responsible for impulse control, the prefrontal cortex (PFC), is underdeveloped in adolescents, which may help to explain increased levels of impulsivity observed during adolescence. As such, research has been conducted on the neural correlates of impulsivity in the PFC, including magnetic resonance spectroscopy (MRS) studies that measure levels of relevant neurotransmitters in relation to levels of impulsivity. However, the majority of this research has been focused on neurotransmitter levels in the medial PFC in adult populations – no previous studies have assessed the relationship between *lateral* PFC neurotransmitter levels and substance use in adolescents. This exploratory study examines whether individual differences in lateral PFC neurotransmitters γ-aminobutyric acid (GABA) and glutamate can significantly predict the age of onset and frequency of alcohol use in a nonclinical adolescent population. MRS was used to measure proxy values for GABA (GABA+) and glutamate (Glx; glutamate + glutamine) levels in the lateral PFC, and data on disinhibition and substance use were collected via self-report questionnaires. Results showed that higher levels of impulsivity were associated with earlier and more frequent substance use, as predicted per extensive prior literature. While the hypothesized relationship with GABA+ was not observed, higher Glx levels were associated with earlier onset of substance use while accounting for the joint relationships between both Glx and substance use with impulsivity. As a result, we concluded that the relationship between excitatory-inhibitory signaling in the lPFC and impulsivity levels is likely different in the developing adolescent brain than in adults, and future research in this area will help to expand upon the findings of this preliminary study.

1. Introduction

The initiation and increased frequency of substance use during adolescence has been largely attributed to increased levels of impulsivity during adolescent development (Blakemore & Robbins, 2012). The region of the brain that is responsible for decision-making and impulse control, the prefrontal cortex (PFC), is also one of the last cortical brain structures to fully mature, and is therefore underdeveloped in youth (Siddiqui et al., 2008). Thus, some prior research studies have examined the neural correlates of impulsivity by using magnetic resonance spectroscopy (MRS) to measure the levels of neurotransmitters in the medial PFC (Silveri et al., 2013; Wang et al., 2017). However, very few of these studies have been focused on the effect of neurotransmitter levels on the initiation and frequency of substance use, and no research has been done on neurotransmitter levels in the *lateral* PFC during adolescence. The aim of this exploratory study is to examine the predictive relationship between substance use in a nonclinical adolescent population and individual differences in the levels of neurotransmitters γ-aminobutyric acid (GABA) and glutamate (Glx) in the lateral PFC.

1.1. Impulsivity and Substance Use

Impulsivity is defined as the tendency to act on urges without regard as to the potential consequences (Shin et al., 2013). Whiteside & Lynam developed the UPPS scale as a measure of the four facets of impulsivity: Urgency (acting rashly to regulate negative emotions), lack of Premeditation (preference for immediate reward), lack of Perseverance (poor concentration on tasks), and Sensation-seeking (preference for novel situations) (Whiteside & Lynam, 2001). Along with the UPPS scale, other established self-report questionnaires are often used to measure these impulsive traits with a Likert-type agreement response scale; examples include the Behavioral Inhibition/Activation Scale (BIS/BAS; Carver & White, 1994), the Sensitivity to Punishment and

Sensitivity to Reward Questionnaire (SPSRQ; Torrubia et al., 2001), the Sensation Seeking Scale (SSS; Zuckerman et al., 1978), and the Barratt Impulsiveness Scale (BIS-11; Patton et al., 1995).

Numerous studies have elucidated a relationship between impulsivity during adolescent development and the initiation and frequency of substance use. Greater levels of impulsivity, as measured by the BIS-11 and SSS, have been established as a significant predictor of later alcohol, tobacco, and cannabis use in young adolescents (Gullo & Dawe, 2008; Brook et al., 1999; Lynskey et al., 1998). Note that substance use can be defined as either any consumption of alcohol or drugs, without clinical diagnosis, *or* as a Substance Use Disorder (SUD), in which the recurrent use of substances causes "clinically significant impairment" (SAMHSA, n.d.). Prior studies have provided evidence for the correlation between higher impulsivity levels and adolescent substance use according to *both* of these definitions. A significant correlation was shown between impulsivity and age of onset of alcohol use in adolescent DSM-IV-classified alcohol abusers (von Diemen et al., 2008), and Shin et al.'s study found impulsivity to be associated with frequency of alcohol use, as measured by the WHO's Alcohol Use Disorders Identification Test (AUDIT: Saunders et al., 1993), in healthy 18-25 year-olds (Shin et al., 2013). The majority of the research on the relationship between impulsivity and substance use has been focused on the adolescent and young adult populations, but a limited number of preliminary studies have also revealed a similar relationship in middle-aged adults (Liu et al., 2020; Bertin et al., 2021).

1.2. The Role of the PFC in Impulse Control

Cognitive control is the ability to voluntarily and adaptively organize behavior in a goal-oriented manner (Luna et al., 2015). In adults, higher-order cognition allows for successful decision-making, governed by cognitive processes including, but not limited to, working memory, selective attention, response inhibition, and performance monitoring (Luna et al., 2015; Larsen &

Luna, 2018). In addition to general decision-making, impulse control is also an ability mediated by higher-order cognition, as the development of cognitive control processes is central to the ability to inhibit impulsive urges. These processes have been found to be primarily controlled by the prefrontal cortex (PFC).

The PFC is a neocortical region in the anterior frontal lobe that contains several overlapping patterns of connection with the motor, sensory, and visuospatial cortical areas, as well as subcortical structures involved in emotion, memory, and homeostasis (Miller, 2000). This wide-spread interconnectedness allows the PFC to synthesize a range of disparate information, making it an optimal structure for the control of complex cognitive processes, including impulse control. Using functional magnetic resonance imaging (fMRI), several studies have established a connection between impulse control and PFC activation in healthy adults. For example, Rubia et al. linked successful completion of cognitive control-focused tasks like 'go/no-go' and 'stop tasks' to activation of both the lateral PFC (lPFC) and the anterior cingulate cortex (ACC), a medial PFC (mPFC) region, in healthy adult males (Rubia et al., 2001). This connection has been further solidified by evidence from studies in clinical populations: positron emission tomography (PET) studies found decreased mPFC activity in adults with disorders that feature lack of impulse control as a prominent symptom, including Borderline Personality Disorder (BPD; Soloff et al., 2000) and Traumatic Brain Injury (TBI; Varney et al., 2001), when compared to healthy controls.

The PFC undergoes significant development during adolescence and is one of the last cortical structures to fully mature (Siddiqui et al., 2008; Silveri et al., 2013). Therefore, cognitive control is not yet fully developed in the adolescent population. The underdevelopment of the PFC is thought to contribute to the increased impulsivity observed in adolescents (Blakemore &

Robbins, 2012). It follows that PFC function may be related to the onset and frequency of risk-taking behaviors in adolescence, such as substance use.

1.3. Impulsivity and its Neural Correlates

Neural correlates of impulsivity have been studied using magnetic resonance spectroscopy (MRS) to measure the levels of relevant neurotransmitters (NTs) in the PFC. The primary inhibitory and excitatory NTs in the brain are γ-aminobutyric acid (GABA) and glutamate (Glx), respectively. MRS is a relatively cutting-edge technique: one that measures neurochemical markers of chemical concentration in the brain rather than its structure, function, or connectivity. It's also important to note that MRS is an inferential measure of NT concentration in neuron tissue, rather than a directly quantifiable measure of NT activity. As such, the existing literature on the relationship between impulsivity and GABA/Glx levels is limited.

However, a few prior studies using MRS have consistently found that higher levels of impulsivity are negatively correlated with GABA levels in medial regions of the PFC, in both non-clinical adolescents (Silveri et al., 2013) and adult women with BPD (Wang et al., 2017). These findings suggest that lower levels of GABA in the PFC may predict greater disinhibition. Notably, these studies have focused almost exclusively on structures in the mPFC, such as the ACC, which play an integral role in cognitive control and risk-related decision-making (Euston et al., 2010). Though the mPFC is a valuable target of research on disinhibition, the lPFC also plays a significant role in impulse control and thus needs to be examined in this context as well.

1.4. Aims of the Current Study

While the two relationships described above – impulsivity to substance use and impulsivity to GABA/Glx levels – have been established, comparatively little research has been done on the transitive relationship of GABA/Glx levels to substance use.

Some preliminary studies looking at populations with SUDs have identified an effect of sustained substance use on levels of these NTs. Epperson et al. found that cortical GABA levels decreased in adult female smokers after a period of long-term abstinence from nicotine (Epperson et al., 2005). Similarly, in alcohol-dependent adult males, decreased ACC GABA and Glx levels were observed after 2 weeks of sobriety (Chen et al., 2020; Hermann et al., 2011), and Abé et al. found that lower dorsolateral PFC (DLPFC) GABA levels were associated with greater cocaine consumption in polysubstance abusers (Abé et al., 2013). However, no research has been done on the effect of differential GABA/Glx levels on the initiation and frequency of substance use, particularly in nonclinical adolescent populations.

Additionally, to our current knowledge, research on this connection in the lPFC – particularly in the ventrolateral PFC (VLPFC) – is as of yet unexplored. The majority of MRS studies measuring PFC NT levels in relation to substance use have been confined to the mPFC. Though both the mPFC and lPFC play a role in higher-order cognition and impulse control, they are distinct in the psychological concepts they are associated with. The mPFC has been found to control the motor, motivation, and social aspects of cognitive control, while the lPFC is involved in the more abstract, large-scale concepts such as semantics and working memory (de la Vega et al., 2016; de la Vega et al., 2017). In the context of adolescent substance use, impulse control involves planning and consideration of long-term consequences in the face of a novel urge. These large-scale aspects of cognitive control will be at least in part associated with activity in the lPFC, so it is important that the lPFC NT levels be studied in relation to substance use, as they have been in the mPFC.

The aim of this study is therefore to examine the predictive relationship between individual differences in GABA and Glx levels in the lPFC and adolescent substance use. Given a relative

dearth of data on marijuana (THC) usage in the target sample, the focus of this exploratory study will be alcohol use as self-reported by adolescents. Behavioral self-report data on disinhibition will also be collected as an additional independent variable, in order to determine whether our sample demonstrates the previously established relationship between impulsivity and substance use.

1.5. Hypotheses

Following the findings of the prior literature cited above, lower levels of inhibitory GABA and higher levels of excitatory Glx are expected to predict greater impulsivity, which may in turn have a positive correlation with rates of alcohol usage, particularly during the developmental age range examined (16-24). Thus, our hypotheses are as follows:

H1. Prediction of a positive relationship between Impulsivity and substance use

These hypotheses will be examined in order to determine whether our sample shows the typical relationship between impulsivity and substance use that has been reported previously.

H1a. Greater levels of impulsivity will be reported in adolescents that drink alcohol compared to adolescents that do not drink alcohol.

H1b. Impulsivity levels will be negatively correlated with Age of Initiation of Alcohol Consumption amongst adolescents that drink alcohol.

H1c. Impulsivity levels will be positively correlated with Frequency of Alcohol Consumption amongst adolescents that drink alcohol.

H2. Prediction of a negative relationship between lPFC GABA levels and substance use

These hypotheses will be examined in order to test the novel aspects of our current investigation with regards to the inhibitory NT, GABA.

H2a. Lower levels of lPFC GABA+ will be found in adolescents that drink alcohol compared to adolescents that do not drink alcohol.

H2b. lPFC GABA+ levels will be positively correlated with Age of Initiation of Alcohol Consumption amongst adolescents that drink alcohol.

H2c. lPFC GABA+ levels will be negatively correlated with Frequency of Alcohol Consumption amongst adolescents that drink alcohol.

H3. Prediction of a positive relationship between lPFC glutamate (Glx) levels and substance use

These hypotheses will be examined in order to test the novel aspects of our current investigation with regards to the excitatory NT, glutamate (Glx).

H3a. Higher levels of lPFC Glx will be found in adolescents that drink alcohol compared to adolescents that do not drink alcohol.

H3b. lPFC Glx levels will be positively correlated with Frequency of Alcohol Consumption amongst adolescents that drink alcohol.

H3c. lPFC Glx levels will be negatively correlated with Age of Initiation of Alcohol Consumption amongst adolescents that drink alcohol.

Figure 1. Research Model

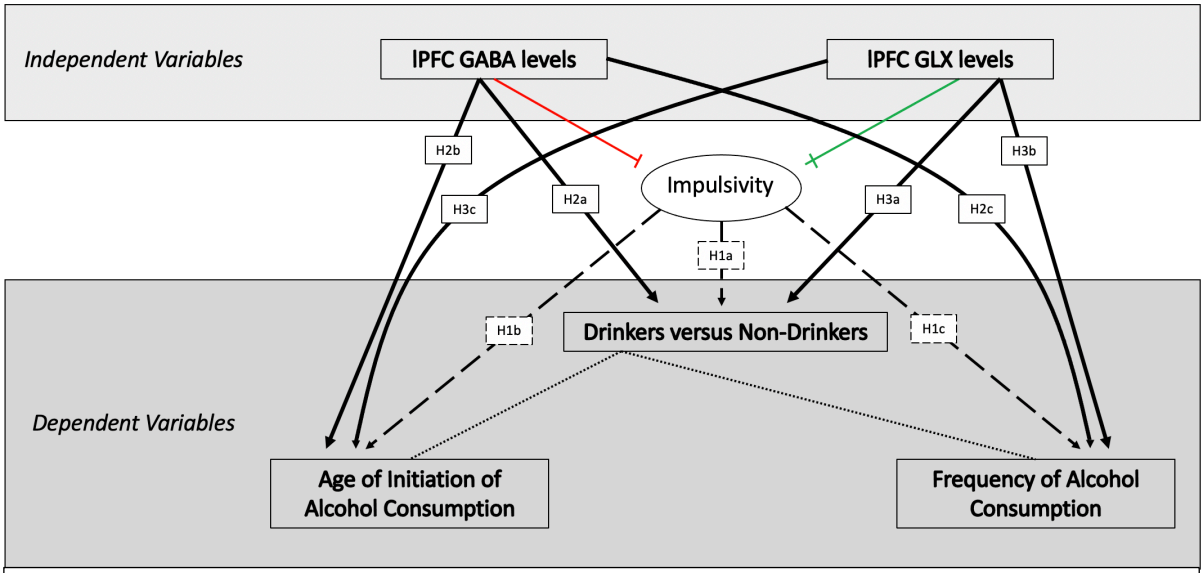

Figure 1. Research model and hypotheses predicting the relationships between lPFC NT levels, impulsivity, and substance use. Dashed lines indicate confirmatory hypotheses (H1) regarding the relationship between impulsivity and substance use that has been established in prior literature. Solid lines indicate novel hypotheses (H2, H3) regarding the relationship between lPFC NT levels and substance use that is the central focus of this study. Dotted lines indicate the division of this study's 2 approaches to the dependent variable of interest: *Group Analyses* and *Individual Analyses* (described in further detail in Methods below). The colored lines indicate the relationship found between PFC NT levels and impulsivity in previous studies (notably in the mPFC only).

See Figure 1 above for a depiction of how each of our hypotheses fits into the overall research model for this study.

2. Methods

2.1. Participants

Participants were 108 adolescents who participated in the Colorado Cognitive Neuroimaging Family Emotion Research (CoNiFER) study conducted through the Banich Laboratory at the University of Colorado Boulder. 26 participants were excluded due to incomplete spectroscopy data; spectroscopy data points were excluded if the spectra (1) did not have discernable peaks coinciding with the NTs of interest, (2) were saturated with a signal indicative of considerable fat within the voxel, or (3) showed artifacts suggestive of motion or other causes. This process resulted in a final sample of 82 participants aged 16-24 (m = 19.3, sd = 1.65). Participants were from families drawn from an unselected community sample that was originally recruited for two different studies in the Genes and Environment Mood (GEM) Lab (Benjamin Hankin, P.I.). These community samples were recruited from the Denver metro area via public schools and using direct mail to target zip codes to maximize demographic and socioeconomic diversity.

All participants were screened to be free of history of neurological insult. Participant demographic information is shown in Table 1. All participants spoke English as their first language and were reasonably able to complete the given written questionnaires and tasks. Informed consent was obtained from all adult participants, and adolescent assent with parent permission were obtained for participants under the age of 18. All procedures were approved by the University of Colorado Institutional Review Board.

Table 1. Participant characteristics

Demographic information for all participants at TP2	
Sample Size	82
Gender (% male)	48.2%
Average Age (yrs)	19.3 ± 1.65 (range 16-24)

2.2. Data Acquisition

The data for this research comes from the CoNiFER study, a large-scale longitudinal study that examines how the brain processes involved in executive function and cognitive control develop across adolescence. The data reported in this paper are thus a subset of a larger set of data collected on emotional regulation, prefrontal cortex neurotransmitter (PFC NT) levels, regional brain activation, and behavioral performance.

The data for the CoNiFER study was collected at two separate timepoints, spaced 20-24 months apart: timepoint 1 (TP1) and timepoint 2 (TP2). At each timepoint, participants completed two visits to the University of Colorado Boulder. The first visit lasted approximately 3.5-4 hours, and it included computerized behavioral tasks, paper questionnaires on self-report measures, and a clinical interview. The second visit consisted of MRI scanning for fMRI and MRS data that lasted approximately 2 hours and 15 minutes.

As described in the Results below, initial data analyses revealed cause for concern with regard to the validity of the TP1 self-report behavioral data. Due to these concerns, the research conducted in this study was limited to the data collected at TP2. See Results below for further details.

2.2.1. Magnetic Resonance Spectroscopy (MRS)

MRS was used to obtain an inferential measure of NT concentrations in the neuron tissue of the PFC to serve as this study's primary independent variable of interest. MRS data were acquired on a SIEMENS MAGNETOM PRISMA (3-Tesla) MRI system with a 32-channel head

coil at the Intermountain Neuroimaging Consortium (INC) on the University of Colorado Boulder campus for all participants, except for nineteen participants who were scanned on the pre-upgrade version of the same magnet (TIM TRIO). To reduce head motion during MRS data acquisition, foam padding was placed around participants' heads.

Two MRS voxels were placed following visible inspection of each individual subject's T1 structural image. Because this was a study of individual differences in NT levels, the order of acquisition of each of the voxels was consistent across individuals so it would not be a confounding factor: data for the VLPFC voxel was always acquired first, followed by the DLPFC voxel. Voxels were positioned with respect to an individual's unique neuroanatomical characteristics of the PFC, as we were specifically interested in deriving signal from particular neuroanatomical regions. As such, the size of the spectroscopy voxel was not uniform across all participants. As shown in Figure 2, the VLPFC voxel was positioned in the left inferior frontal gyrus (IFG), anterior to the precentral gyrus and posterior to the frontopolar cortex. The DLPFC voxel was positioned in the left middle frontal gyrus (MFG), anterior to the precentral gyrus and posterior to the frontopolar cortex (Fig. 2).

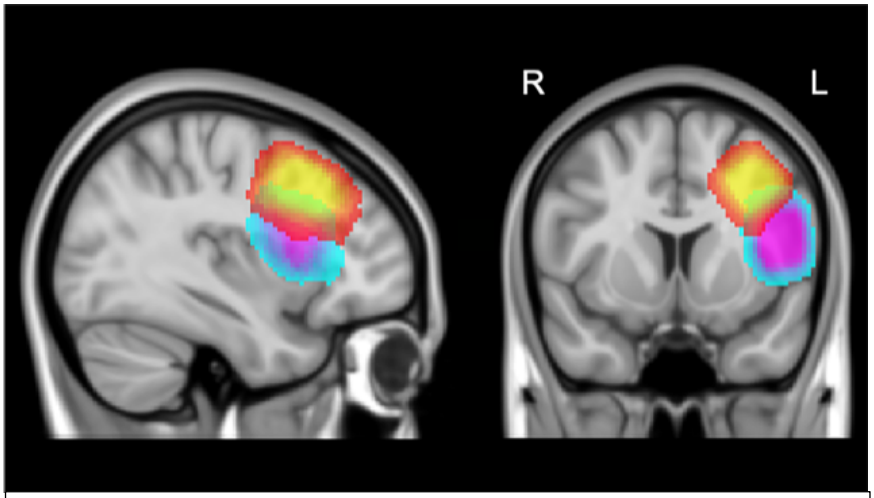

Figure 2. Extent of MRS sample coverage for voxel placement in the DLPFC (red-yellow) and VLPFC (cyan-magenta) for the original TP1 sample. Areas in red and cyan fell within the MRS voxel for 90% of all participants (i.e. the voxel extended past these regions in 10% or less of tested individuals.

Because of the relatively poor ability to separate their distinct peaks in the spectra, MRS provides a measure of the sum of glutamate (Glu) and

glutamine (Gln), a molecule associated and strongly correlated with Glu. The resulting measure is often referred to as Glx (Glu + Gln), or the glutamate complex. Similarly, it is difficult to isolate the peak for GABA specifically, so the measure of GABAergic function derived from MRS is often referred to as GABA+, indicating the presence of the GABA peak as well as related nearby peaks. In the present study, levels of Glx and GABA+ were obtained for each individual as proxies for glutamatergic and GABAergic activity. Therefore, the two voxels were used to determine concentration levels of GABA+ and Glx in the DLPFC and VLPFC, measured against the baseline of water and divided by the volume of each respective voxel to control for grey matter.

Spectroscopy data on the concentration levels of the NTs choline (Cho) and N-acetylaspartate (NAA) were also collected at these voxels as both monoamine and non-monoamine control measures of general neurochemistry. These values were later used to determine the specificity of any significant findings to the common neurochemical pathway for GABA+/Glx.

As this study had no specific hypotheses about the NT concentrations in the DLPFC versus the VLPFC, data from each voxel were averaged to create measures of average GABA+, Glx, Cho, and NAA concentrations in the entire lPFC. However, the voxel-specific data were retained in the event of any significant findings with the average NT variables, so that the effect could be examined and compared in the DLPFC and VLPFC separately. Throughout the rest of the paper, unless otherwise noted, all NT levels discussed are average measures across the dorsal and ventral voxels.

2.2.2. Substance Use Measures

The CoNiFER study included 2 self-report scales to assess participants' use, initiation, and frequency of use of several substances, including alcohol, marijuana, cigarettes, and recreational drugs such as ecstasy, stimulants, hallucinogens, and narcotic painkillers: the Substance Use

Questionnaire (SUQ) and the Alcohol Use Disorders Identification Test (AUDIT). The SUQ was modeled on Molina & Pelham's substance use survey, adapted for the CoNiFER study, and the AUDIT was developed by the WHO as a screening instrument for hazardous alcohol consumption (Molina & Pelham, 2003; Saunders et al., 1993). The scales were presented to participants as paper questionnaires at the first visit of each timepoint.

Three measures of interest were selected from the SUQ/AUDIT in order to focus this study on reported alcohol use: (1) use of alcohol, (2) age of initiation of alcohol use, and (3) frequency of alcohol use. For purposes of this study, "use" of alcohol was defined as having ever had a "drink" of alcohol, in which the participants had their "own glass or bottle" (as opposed to a "sip of alcohol from someone else's glass"). This measure was assessed by item SUQ_3, shown below. If participants endorsed alcohol use in SUQ_3, they were asked to respond to items SUQ_4 and AUDIT_1 to assess age of initiation of alcohol use and frequency of alcohol use, respectively.

Substance Use: Alcohol (SUQ_3)
"Have you *ever* had a drink of beer, wine, wine cooler, or liquor – not just a sip or taste of someone else's drink?"
Response Set:
(0) No – (1) Yes, but only once – (2) A few times – (3) More than a few times

Age of Initiation: Alcohol (SUQ_4)
"How old (in years) were you when you *first* had a drink of beer, wine, wine cooler, or liquor – not just a sip or taste of someone else's drink?"

Frequency of Use: Alcohol (AUDIT_1)
"How often *on average in the last year* do you have a drink containing alcohol?"
Response Set:
(0) Never – (1) Once a month or less – (2) 2-4 times a month – (3) 2-3 times a week – (4) 4+ times a week

2.2.3. Impulsivity Measures

Behavioral control measures on disinhibition and impulsivity were collected via self-report questionnaires. Measures of interest included Zuckerman et al.'s Sensation Seeking Scale (SSS),

the Impulse Control subscale of the Weinberger Adjustment Inventory (WAI), and Steinberg & Monahan's Resistance to Peer Influence (RPI) scale (Zuckerman et al., 1978; Weinberger & Schwartz, 1990; Steinberg & Monahan, 2007). See Appendix A for details on items selected from each of these scales. Table 2 shows the correlations between the z-scores of these measures, tested to ensure that they all measured impulsivity as the construct of interest. As shown in Appendix A, *lower* values on each of these scales indicate greater levels of impulsivity. For purposes of clarity in this study, we operationalized impulsivity levels with a score that indicated greater levels of impulsivity with *higher* values. Therefore, the z-scores for these three measures were averaged and reversed-scored (made negative) to create a composite Impulsivity Score (IS) for each participant, where higher scores represented greater levels of impulsivity.

	SSS z-score	*WAI z-score*	*RPI z-score*	*Composite IS*
SSS z-score	1.00			
WAI z-score	**0.54**	1.00		
RPI z-score	0.23	0.28	1.00	
Composite IS	**-0.78**	**-0.81**	**-0.67**	1.00

*SSS = Sensation Seeking Scale, WAI = Weinberger Adjustment Inventory, RPI = Resistance to Peer Influence Scale, IS = Impulsivity Score

Table 2. Correlations between z-scores of selected measures of disinhibition at TP2. The correlations shown confirm the positive correlation between all three individual scales at TP2, indicating that all three scales represent higher levels of impulsivity with *lower* scores (see Appendix A). The negative correlations between all three individual scales and the final composite IS confirms successful reverse-scoring to ensure that the composite IS represents higher levels of impulsivity with *higher* scores. All correlations shown have a p-value < 0.05; bolded values represent correlations with a p-value < 0.001.

2.3. Statistical Analyses

All statistical analyses were conducted using RStudio software version 1.2.5033. As described below, the data were analyzed using 2 main statistical approaches to examine the effects of both impulsivity (*H1*) and lPFC NT levels (*H2, H3*) on alcohol use:

2.3.1.1. Group Analyses

Based on their responses to item SUQ_3, participants were divided into 2 groups: Drinkers and Non-Drinkers. Drinkers were defined as participants that responded 1, 2, or 3 to SUQ_3 – i.e. participants that had *ever* had a drink of alcohol in their lives. Non-Drinkers were defined as participants that responded 0 to SUQ_3 – i.e. participants that had never had a drink of alcohol in their lives. Demographic information on the two groups is shown in Table 3.

Independent 2-group unpaired t-tests were run to examine the differences in impulsivity and NT levels between Drinkers and Non-Drinkers.

Table 3. Drinkers vs Non-Drinkers

	Sample Size	*Average Age (yrs)*	*Gender (% male)*
Drinkers	69	19.4 ± 1.67	43.2%
Non-Drinkers	13	18.6 ± 1.39	46.2%

2.3.1.2. Individual Analyses

Amongst only the 69 participants in the Drinkers group, linear regression models were run to further examine the effects of personality measures and lPFC NT levels on both the *age of onset* and *frequency* of alcohol use amongst alcohol-using adolescents. Though it wasn't involved in the study's *a priori* hypotheses, Gender was included as a covariate in these analyses. Unless otherwise noted, Gender had no significant effect in the results reported below.

2.3.2. Measures of NT Levels

A number of different measures of NT concentration were examined in this study, rather than just the raw average GABA+ and Glx values described above.

The first set of measures focused on the unique contributions of each of the GABA+ and Glx concentrations to brain activation, while taking into account the other NT's contribution and Age. Throughout the paper, these measures are referred to as the GABA+-specific concentration and the Glx-specific concentration. For the *Group Analyses*, these measures were obtained by

calculating the residual value for each NT, while controlling for the other NT and age in months. In the *Individual Analyses*, these measures were examined in the linear regressions by including each NT as a regressor of interest, with Age serving as a nuisance covariate.

The second measure focuses on the combined concentration of neurotransmitter to understand the covariant contributions of both GABA+ and Glx to brain activation. This measure is conceptually justified by the fact that both GABA+ and Glx belong to the GABA shunt, the neurochemical pathway in which glutamine is a precursor for both GABA and glutamate (Reubi et al., 1978; Bak et al., 2006). Throughout the paper, this measure is referred to as the average NT concentration. For the *Group Analyses*, this measure was obtained by averaging the z-scores of each participants' average lPFC GABA+ and lPFC Glx concentrations, and then calculating the residual value of this average while controlling for Age. For the *Individual Analyses*, the average value was included as a regressor of interest, with Age as a nuisance covariate.

The third measure is focused on the contribution of the relative ratio of excitatory to inhibitory NT concentration to patterns of brain activation. Throughout the paper, this measure is referred to as the NT concentration ratio. For the *Group Analyses*, the residual value of the z-scored ratio of lPFC Glx to lPFC GABA+ was calculated, while controlling for Age. In the *Individual Analyses,* the z-scored ratio was included in the linear regressions as the regressor of interest, with Age serving as a nuisance covariate.

The first set of measures represents the lPFC NT levels referenced in this study's *a priori* hypotheses, *H2* and *H3*, which posit specific effects of GABA+ and Glx on adolescent substance. However, the driving research question broached in this study has to do with the broader relationship between lPFC neurochemistry and adolescent substance use. Therefore, while we hypothesized specific effects of GABA+ and Glx on substance use, it may turn out that there *is* a

relationship between lPFC GABA+/Glx levels and substance use but that it is slightly different in nature. The latter two sets of measures were therefore explored to examine whether it is the activity of the GABA shunt in general (including *both* GABA and glutamate) that has a relationship with adolescent substance use.

To control for the effect of general neurochemical activity and determine if any significant findings with the above measures were specific to GABA+ and Glx, the NTs Cho and NAA were included in our analyses, as they belong to different signaling pathways than the GABA shunt. For the *Group Analyses*, the residual values of both Cho and NAA were calculated while controlling for Age. For the *Individual Analyses*, average Cho and average NAA were included as regressors of interest in their respective models, with Age included as a nuisance covariate.

These procedures resulted in four constructs within the overall independent variable "lPFC NT levels," as well as two control measures. These six constructs are shown in Table 4.

Table 4. Measures of lPFC NT Levels

Measure	Definition	Purpose
GABA+-specific concentration	lPFC GABA+ concentration, accounting for lPFC Glx concentration and Age	to represent the unique contribution of GABA+
Glx-specific concentration	lPFC Glx concentration, accounting for lPFC GABA+ concentration and Age	to represent the unique contribution of Glx
Average NT concentration	combined lPFC GABA+ and lPFC Glx concentration, accounting for Age	to represent the covariant contribution of both GABA+ and Glx
NT concentration ratio	Ratio of lPFC Glx:lPFC GABA+, accounting for Age	to represent the contribution of the relative excitatory:inhibitory concentrations
Cho concentration	lPFC Cho, accounting for Age	to control for the general contribution of monoamine NTs
NAA concentration	lPFC NAA, accounting for Age	To control for the general contribution of non-monoamine NTs

3. Results

3.1. Operationalization of Age of Initiation of Alcohol Consumption

Preparatory data cleaning and analyses revealed significant discrepancies between the Age of Initiation of Alcohol Consumption reported at TP1 and TP2. Figure 3 below demonstrates these discrepancies in a comparative violin plot (Fig. 3a) and a connected spaghetti box plot (Fig. 3b). As the survey item SUQ_4 is designed to measure the participants' age when they had their first drink, we would expect consistency in every participant's answer between TP1 and TP2. The only difference between the responses given to SUQ_4 across timepoints should have been a greater number of responses at TP2, assuming more participants endorsed alcohol use when they were older. Thus, the comparative violin plot would ideally show identical plots for TP1 and TP2, other than a larger number of data points at TP2. As seen in Fig. 3a, the plots do not show consistency from TP1 to TP2. In fact, some individuals reported an earlier Age of First Drink at TP2 than they did at TP1, further indicating a lack of consistency. Likewise, if participants gave the same answer

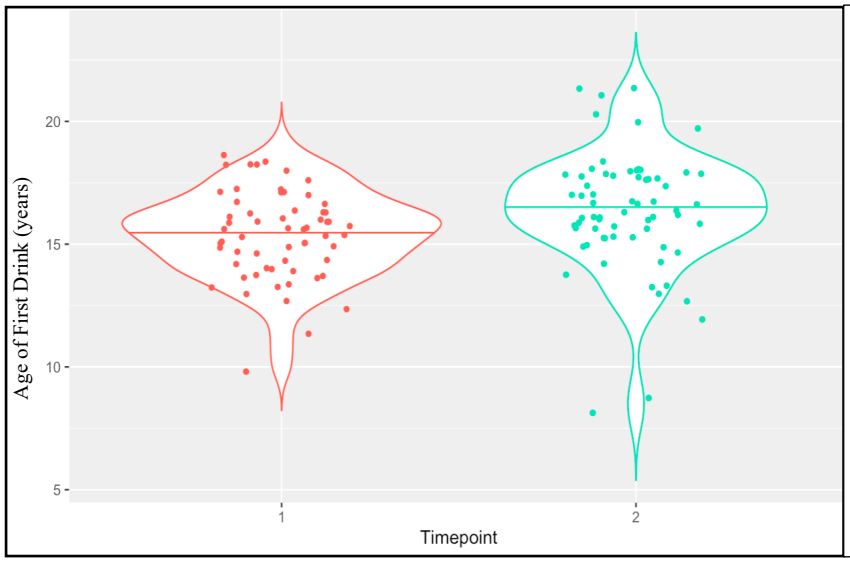

Figure 3a. Comparative violin plot: Age of First Drink at TP1 and TP2. Differences between individual subjects' responses to the same question at different timepoints represent a cause for concern about the validity of the Age of Initiation of Alcohol measure.

to SUQ_4 at TP1 and TP2, the connected spaghetti box plot in Fig. 3b would show straight horizontal lines connecting each individual participant's responses at each timepoint.

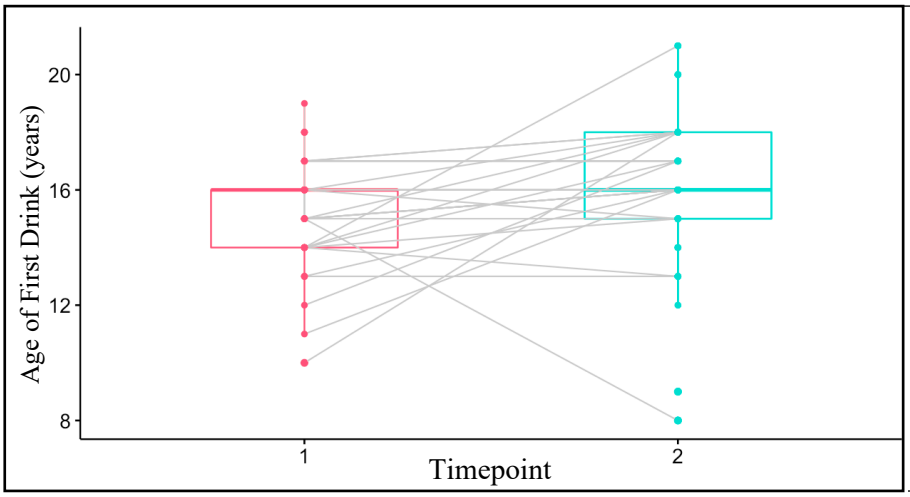

Figure 3b. Connected spaghetti box plot: Age of First Drink at TP1 and TP2. Differences between individual subjects' responses to the same question at different timepoints represent a cause for concern about the validity of the Age of Initiation of Alcohol measure.

The inconsistency across timepoints demonstrated in Figure 3 represented a cause for concern regarding the validity of the TP1 data. This led us to consider the possibility that SUQ_4 was interpreted differently by participants at TP1 compared to TP2. Given the nature of the substance use questions as posed to adolescents under the legal drinking age in the U.S., we considered it likely that younger participants might have conflated their responses to the Age of First Drink and Age of First Sip survey items. Both items are shown below:

Age of First Drink (SUQ_4)

"How old (in years) were you when you *first* had a drink of beer, wine, wine cooler, or liquor – not just a sip or taste of someone else's drink?"

Age of First Sip (SUQ_2)

"How old (in years) were you when you *first* had a sip or taste of alcohol?"

To explore this possibility, the correlation between the responses to these two items were compared at both timepoints. As displayed in Figure 4 below, the correlation between responses to Age of First Drink and Age of First Sip was less robust at TP2 than at TP1, indicating a greater level of distinction between the participants' conceptions of their First Sip versus their First Drink at TP2. For purposes of this study, alcohol use was defined as a "drink" of alcohol, not a "sip." Therefore, responses given to the Age of First Drink item at TP2 were selected as the best estimate for operationalization of the dependent variable: Age of Initiation of Alcohol Consumption. Due to the same concerns surrounding the validity of the TP1 substance use data, all of the analyses conducted in this study were limited to the data collected at TP2 only.

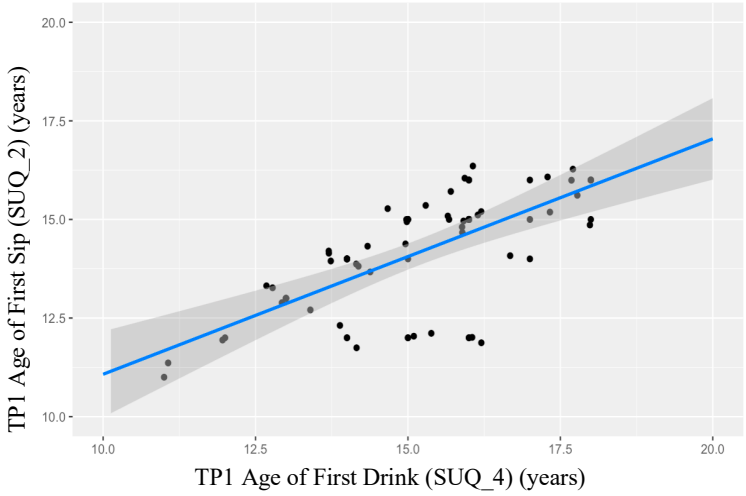

Figure 4a. Correlation between Age of First Drink (years) and Age of First Sip (years) at TP1. At TP1, the correlation between responses to Age of First Drink and Age of First Sip ($r = 0.692$, $t = 5.99$, $df = 39$, $p = 5.37E\text{-}7$) was more robust than that at TP2.

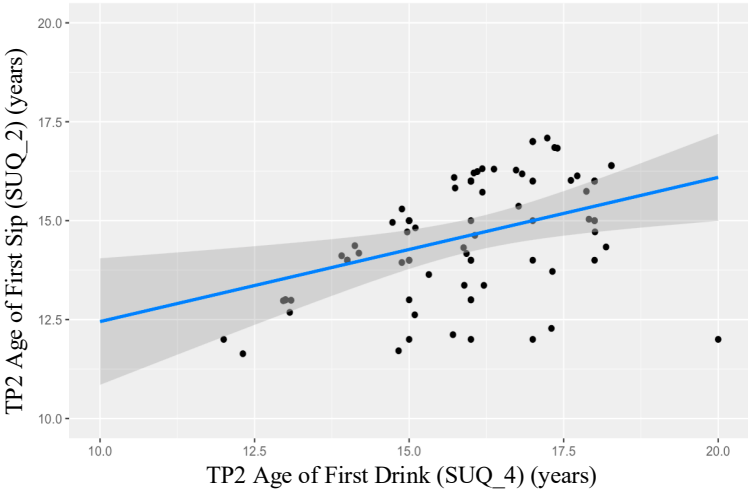

Figure 4b. Correlation between Age of First Drink (years) and Age of First Sip (years) at TP2. At TP2, the correlation between responses to Age of First Drink and Age of First Sip ($r = 0.450$, $t = 3.34$, $df = 44$, $p = 1.71E\text{-}3$) was less robust than that at TP1.

3.2. Group Analyses

In order to test the hypotheses that adolescents who have started drinking alcohol would have higher levels of Impulsivity, lower lPFC GABA+ concentrations, and higher lPFC Glx concentrations than adolescents who have not started drinking, responses to SUQ_3 were used to create two groups of Drinkers and Non-Drinkers at TP2. Independent, unpaired t-tests were run to determine if there were any significant differences in impulsivity or NT levels between the groups. See Table 5 for demographic details on each group. Given the small sample size of Non-Drinkers compared to Drinkers, the analyses based on group comparisons described below should be interpreted with caution.

Table 5. TP2 Group Demographics

	Drinkers	***Non-Drinkers***
Sample Size	69	13
Mean Age (yr)	19.4	18.6
Gender (% male)	43.2%	46.2%
Mean GABA+-specific concentration	2.47	2.59
Mean Glx-specific concentration	21.9	22.7
Mean Average NT concentration	-0.036	0.23
Mean NT concentration ratio	9.394	9.387
Mean Composite Impulsivity Score	0.061	-0.37
Mean Choline	-0.0076	-0.052
Mean NAA	-0.030	0.12

3.2.1. H1a: Are there higher levels of Impulsivity in Drinkers compared to Non-Drinkers?

T-test analyses revealed a significant difference between composite Impulsivity Score (IS) in the Drinkers and Non-Drinkers groups. As shown in Figure 5, Drinkers' IS was significantly higher than Non-Drinkers' IS ($t = 2.27$, $p = 0.035$, $df = 18.8$).

3.2.2. H2a & H3a: Are there lower GABA+-specific concentrations or higher Glx-specific concentrations in Drinkers compared to Non-Drinkers?

In order to test the hypothesis that Drinkers would have lower lPFC GABA+ levels, t-tests were run to examine the difference in NT levels between Drinkers and Non-Drinkers. No significant differences in GABA+-specific concentration, Glx-specific concentration, average NT concentration, or NT concentration ratio between Drinkers and Non-Drinkers were found.

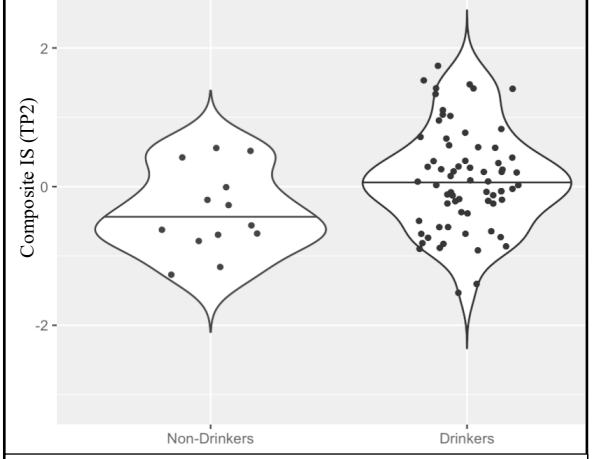

Figure 5. Comparative violin plot of composite IS in Drinkers and Non-Drinkers. The composite Impulsivity Score in the group of Drinkers was significantly higher than that in the Non-Drinkers (t = 2.27, p = 0.035, df = 18.8).

3.2.3. Is there a difference in Cho or NAA concentrations in Drinkers compared to Non-Drinkers?

To examine whether there was a difference in general neurochemistry between Drinkers and Non-Drinkers, t-tests were run to examine the difference in lPFC Cho and NAA levels between groups. No significant difference in Cho concentration or NAA concentration was found between Drinkers and Non-Drinkers.

3.3. Individual Analyses within Drinkers Only

3.3.1. Individual Analyses – Age of Initiation of Alcohol Consumption

To test the hypotheses that, amongst adolescents who drink, higher levels of Impulsivity, lower levels of lPFC GABA+, and higher levels of lPFC Glx would be associated with an earlier Age of Initiation, linear regressions were run predicting Age of Initiation of Alcohol Consumption by personality measures and NT levels.

3.3.1.1. H1b: Amongst Drinkers, are higher levels of Impulsivity associated with an earlier Age of Initiation?

Linear regressions revealed a significant negative relationship between composite IS and Age of Initiation of Alcohol Consumption (t = -2.80, p = 0.0069, df = 61). This indicates that participants with higher levels of impulsivity began drinking at an earlier age (Fig. 6), consistent with prior reports in the literature.

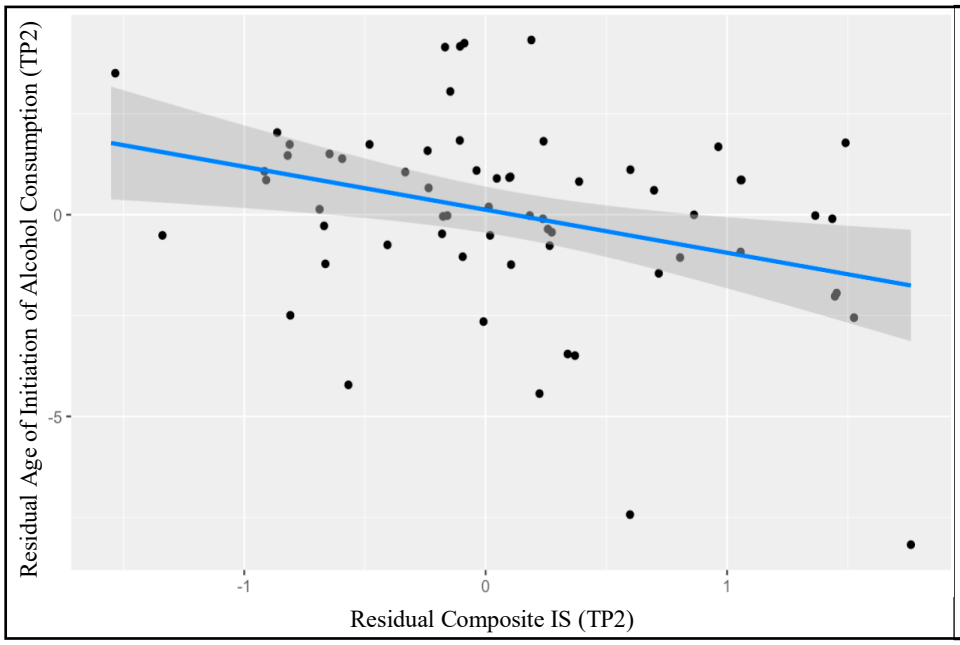

Figure 6. Residuals of composite Impulsivity Score and Age of Initiation of Alcohol, controlling for Age. The negative prediction of Age of Initiation of Alcohol Consumption by composite IS demonstrated through linear regression analyses is maintained when accounting for Age in the residuals of these variables.

Given that impulsivity demonstrated a negative relationship with Age of Initiation of Alcohol Consumption, our exploration of the relationship between lPFC NT levels and Age of Initiation below will include models both without and with composite IS as a covariate. The latter models will test whether neurochemistry provides any predictive power for Age of Initiation of Alcohol Consumption above and beyond levels of impulsivity.

3.3.1.2. H2b & H3c: Amongst Drinkers, are lower GABA+-specific concentrations or higher Glx-specific concentrations associated with an earlier Age of Initiation?

Contrary to our original hypothesis, linear regressions revealed a trend towards lower levels of GABA+-specific concentration being associated with a later Age of Initiation of Alcohol Consumption (t = -1.93, p = 0.063, df = 28). Notably, this relationship was not observed when

controlling for composite IS, suggesting that an individual's levels of GABA+ does not provide any information above and beyond their levels of impulsivity in predicting their age of onset of drinking. However, individuals with higher Glx-specific concentration exhibited a lower Age of Initiation of Alcohol Consumption when controlling for composite IS ($t = -0.445$, $p = 0.040$, $df = 27$), indicating that increased levels of excitatory NT predict a younger age of onset of drinking above and beyond individuals' levels of impulsivity. In addition, increasing levels of average NT concentration were associated with an earlier Age of Initiation ($t = -2.70$, $p = 0.011$, $df = 29$), which remained significant when controlling for composite IS, once again suggesting that average NT concentration had an effect on age of onset of drinking above and beyond impulsivity.

In order to determine whether these significant findings were driven by a particular region of the lPFC, identical linear regressions were run with the spectroscopy data from the separate DLPFC and VLPFC voxels. GABA+-specific concentration in the DLPFC significantly negatively predicted Age of Initiation of Alcohol Consumption ($t = -3.12$, $p = 0.004$, $df = 29$), while there was no significant relationship between these variables in the VLPFC ($t = 0.33$, $p = 0.75$, $df = 28$).

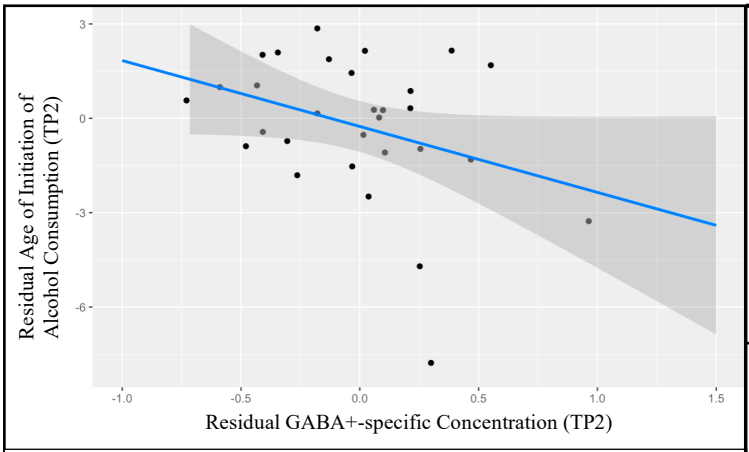

Figure 7a. Residuals of GABA+-specific concentration and Age of Initiation of Alcohol, controlling for Glx-specific concentration and Age.
The negative prediction of Age of Initiation of Alcohol by GABA+-specific concentration demonstrated through linear regression analyses is maintained when accounting for Glx-specific concentration and Age in the residuals of these variables.

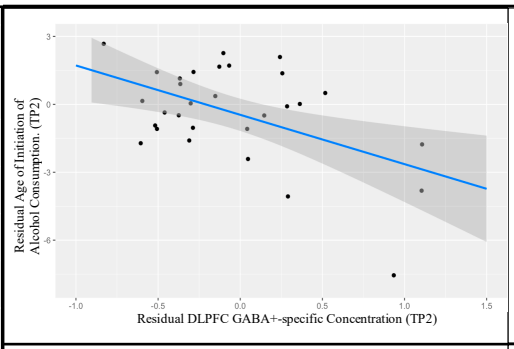

Figure 7b. Residuals of DLPFC GABA+-specific concentration and Age of Initiation of Alcohol, controlling for DLPFC Glx-specific concentration and Age.
The negative prediction of Age of Initiation of Alcohol by GABA+-specific concentration demonstrated in the whole lPFC remains significant in the DLPFC alone.

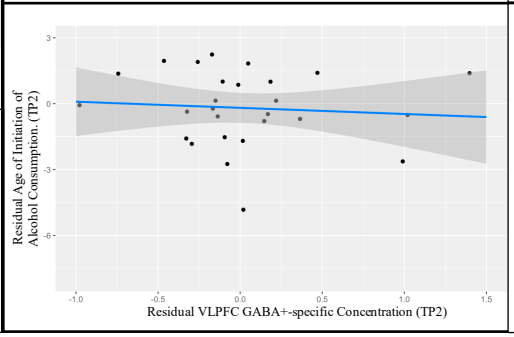

Figure 7c. Residuals of VLPFC GABA+-specific concentration and Age of Initiation of Alcohol, controlling for VLPFC Glx-specific concentration and Age.
The negative prediction of Age of Initiation of Alcohol by GABA+-specific concentration demonstrated in the whole lPFC is not significant in the VLPFC alone.

The significant relationship between DLPFC GABA+-specific concentration and Age of Initiation of Alcohol Consumption was not observed when accounting for composite IS. Glx-specific concentration's negative relationship with Age of Initiation of Alcohol Consumption while controlling for composite IS was trending towards significance in the DLPFC (t = -0.33, p = 0.075, df = 28), with no significant relationship between these variables in the VLPFC (t = -0.186, p = 0.369, df = 27).

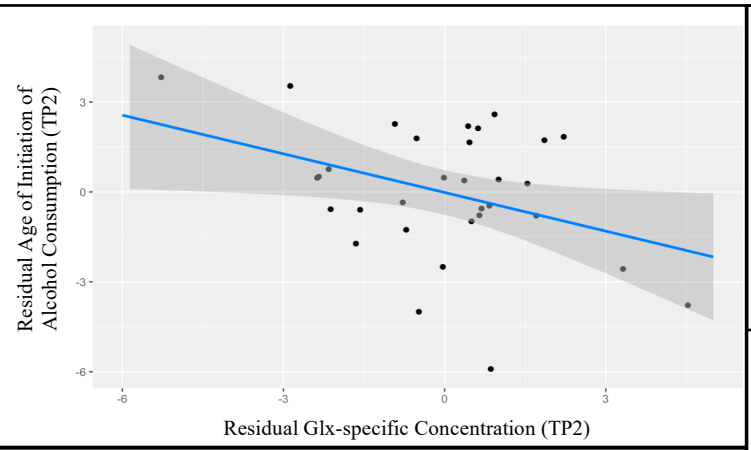

Figure 8a. Residuals of Glx-specific concentration and Age of Initiation of Alcohol, controlling for GABA+-specific concentration, Age, and composite Impulsivity Score.
The negative prediction of Age of Initiation of Alcohol by Glx-specific concentration demonstrated through linear regression analyses is maintained when accounting for GABA+-specific concentration, Age, and composite IS in the residuals of these variables.

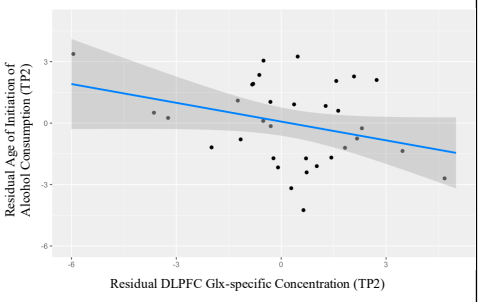

Figure 8b. Residuals of DLPFC Glx-specific concentration and Age of Initiation of Alcohol, controlling for DLPFC GABA+-specific concentration, Age, and composite IS.
The negative prediction of Age of Initiation of Alcohol by Glx-specific concentration when accounting for composite IS that was demonstrated in the whole lPFC is trending towards significance (p = 0.075) in the DLPFC alone.

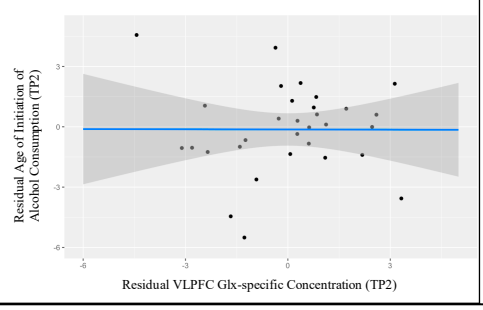

Figure 8c. Residuals of VLPFC Glx-specific concentration and Age of Initiation of Alcohol, controlling for VLPFC GABA+-specific concentration, Age, and composite IS.
The negative prediction of Age of Initiation of Alcohol by Glx-specific concentration when accounting for composite IS that was demonstrated in the whole lPFC is not significant in the VLPFC alone.

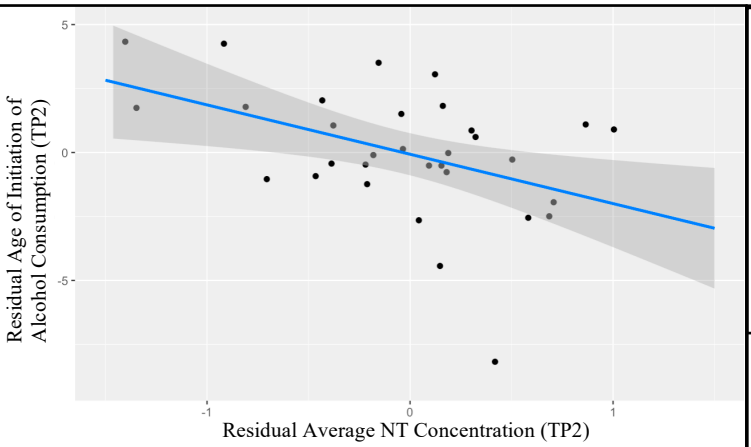

Figure 9a. Residuals of average NT concentration and Age of Initiation of Alcohol, controlling for Age.
The negative prediction of Age of Initiation of Alcohol by average NT concentration demonstrated through linear regression analyses is maintained when accounting for Age in the residuals of these variables.

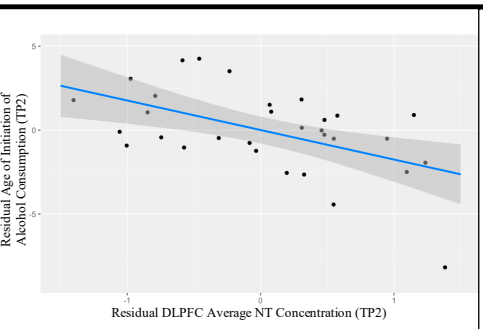

Figure 9b. Residuals of DLPFC average NT concentration and Age of Initiation of Alcohol, controlling for Age.
The negative prediction of Age of Initiation of Alcohol by average NT concentration demonstrated in the whole lPFC remains significant in the DLPFC alone.

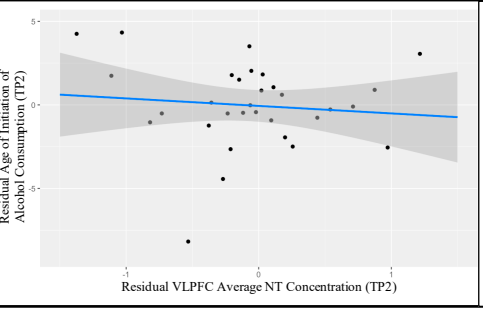

Figure 9c. Residuals of VLPFC average NT concentration and Age of Initiation of Alcohol, controlling for Age.
The negative prediction of Age of Initiation of Alcohol by average NT concentration demonstrated in the whole lPFC is not significant in the VLPFC alone.

Likewise, average NT concentration in the DLPFC demonstrated a significant negative relationship with Age of Initiation ($t = -3.88$, $p = 0.00053$, $df = 30$), but average NT concentration in the VLPFC showed no significant relationship ($t = -0.30$, $p = 0.77$, $df = 29$). In Figures 7, 8, and 9, the negative relationships between Age of Initiation of Alcohol Consumption and GABA+-specific concentration, Glx-specific concentration when controlling for composite IS, and average NT concentration can be seen next to their respective voxel-specific relationships.

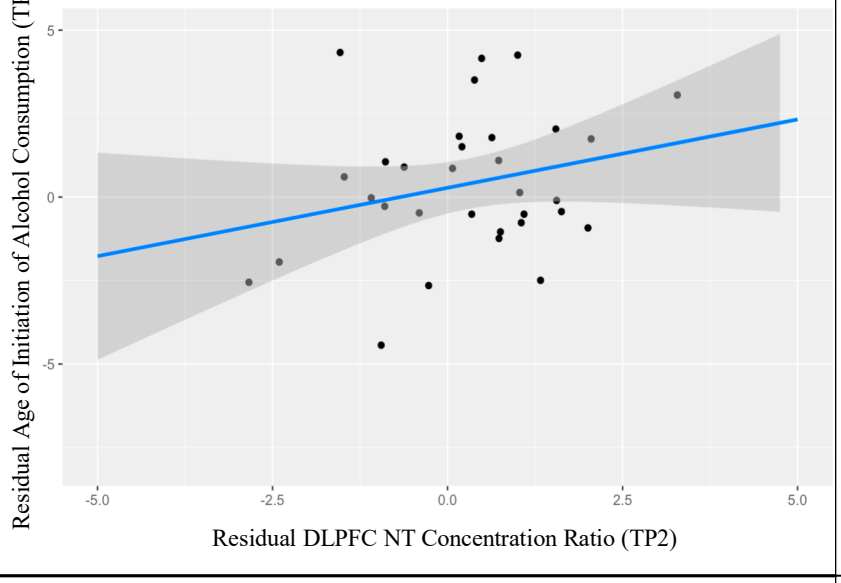

Figure 10a. Residuals of DLPFC NT concentration ratio and Age of Initiation of Alcohol, controlling for Age.
The positive prediction of Age of Initiation of Alcohol Consumption by DLPFC NT concentration ratio demonstrated through linear regression analyses is maintained when accounting for Age in the residuals of these variables.

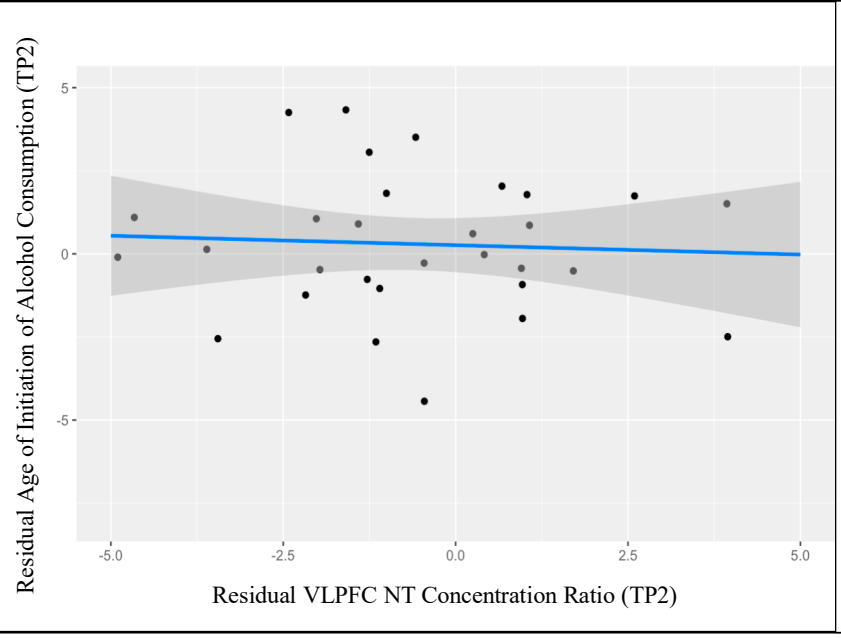

Figure 10b. Residuals of VLPFC NT concentration ratio and Age of Initiation of Alcohol, controlling for Age.
The positive prediction of Age of Initiation of Alcohol Consumption by NT concentration ratio demonstrated in the DLPFC is not significant in the VLPFC.

Furthermore, although there was no significant relationship with the NT concentration ratio averaged across voxels, the DLPFC NT concentration ratio did demonstrate a significant positive relationship with Age of Initiation of Alcohol Consumption ($t = 0.696$, $p = 0.035$, $df = 30$). This relationship in the DLPFC did not remain significant when accounting for composite IS. The VLPFC NT concentration ratio showed no significant relationship with Age of Initiation (Fig. 10).

In general, these voxel-specific analyses are suggestive that neurochemistry in the DLPFC, compared to the VLPFC, may be more associated with Age of Initiation of Alcohol Consumption.

3.3.1.3. Amongst Drinkers, are Cho or NAA concentrations associated with Age of Initiation?

To determine whether the significant effects with Age of Initiation observed for lPFC NT levels associated with the GABA shunt (GABA+-specific concentration, Glx-specific concentration, average NT concentration, and NT concentration ratio) were specific to these NTs, linear regressions were run predicting Age of Initiation by Cho concentration and NAA concentration. No significant relationships were demonstrated between either Cho concentration or NAA concentration and Age of Initiation of Alcohol Consumption, suggesting some specificity to our findings.

3.3.2. Individual Analyses – Frequency of Alcohol Consumption

To test the hypothesis that, amongst adolescents who drink, higher levels of Impulsivity, lower levels of lPFC GABA+, and higher levels of lPFC Glx would be associated with greater frequency of alcohol use, linear regressions were run predicting Frequency of Alcohol Consumption by impulsivity and NT levels.

3.3.2.1. H1c: Amongst Drinkers, are higher levels of Impulsivity associated with a higher Frequency of Alcohol Use?

No significant relationship was shown between composite IS and Frequency of Alcohol Use.

3.3.2.2. H2c & H3b: Amongst Drinkers, are lower GABA+-specific concentrations or higher Glx-specific concentrations associated with a higher Frequency of Alcohol Use?

Linear regressions revealed no significant relationships between Frequency of Alcohol Use and GABA+-specific concentration, Glx-specific concentration, average NT concentration, or NT concentration ratio.

3.3.2.3. Amongst Drinkers, are Cho or NAA concentrations associated with Frequency of Alcohol Use?

Linear regressions revealed no significant relationships between Frequency of Alcohol Use and Cho concentration or NAA concentration.

3.4. Cross-Measure Investigation

	*Age of Initiation**	*GABA+**	*Glx**	*Average NT**	*Ratio**	*Composite IS**
*Age of Initiation**	1.00					
*GABA+**	-0.34	1.00				
*Glx**	-0.31	0.20	1.00			
*Average NT**	**-0.39**	**0.75**	**0.79**	1.00		
*Ratio**	-0.04	**-0.69**	**0.42**	-0.15	1.00	
*Composite IS**	**-0.34**	**0.34**	-0.02	0.21	**-0.29**	1.00

*Age of Initiation = Age of Initiation of Alcohol Consumption, GABA+ = GABA+-specific concentration, Glx = Glx-specific concentration, Average NT = Average NT concentration, Ratio = NT concentration ratio, IS = Impulsivity Score

Table 6. Correlations between Age of Initiation of Alcohol Consumption, lPFC NT levels, and composite Impulsivity Score.
Correlations between the measures tested in the Cross-Measure Investigation are shown above. Bolded values represent correlations with a p-value < 0.05.

Though this study had no *a priori* hypotheses regarding the relationship between impulsivity and lPFC NT levels, we conducted linear regressions to investigate this relationship in light of the significant findings noted above. As composite IS, GABA+-specific concentration,

Glx-specific concentration, and average NT concentration were all found to have significant negative relationships with Age of Initiation of Alcohol Consumption, linear regressions were run to determine whether lPFC NT levels significantly predicted composite IS. Correlations between these measures are shown in Table 6.

Higher GABA+-specific concentration significantly predicted higher composite IS ($t = 0.763$, $p = 0.023$, $df = 42$). This relationship was maintained when looking at GABA+-specific concentration in the DLPFC ($t = 0.508$, $p = 0.028$, $df = 43$) but not in the VLPFC ($t = 0.380$, $p = 0.15$, $df = 42$). See Figure 11 for the relationship between GABA+-specific concentration and composite IS, alongside its voxel-specific relationships. No significant relationship was found between composite IS and Glx-specific concentration, average NT concentration, NT concentration ratio, Cho concentration, or NAA concentration.

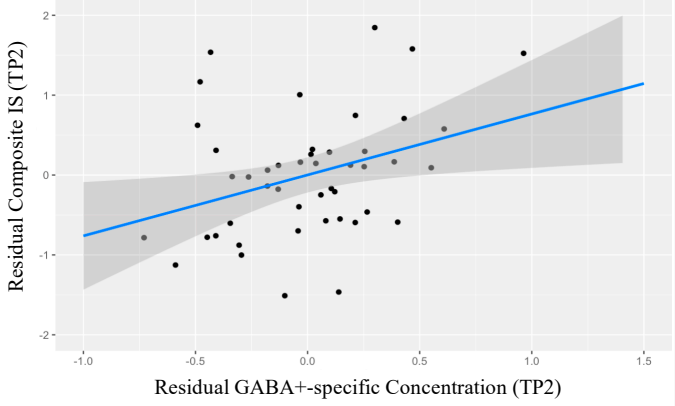

Figure 11a. Residuals of GABA+-specific concentration and Composite Impulsivity Score, controlling for Glx-specific concentration and Age. The positive prediction of composite IS by GABA+-specific concentration demonstrated through linear regression analyses is maintained when accounting for Glx-specific concentration and Age in the residuals of these variables.

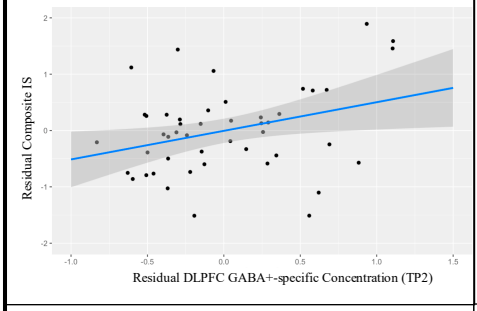

Figure 11b. Residuals of DLPFC GABA+-specific concentration and Composite Impulsivity Score, controlling for DLPFC Glx-specific concentration and Age. The positive prediction of composite IS by GABA+-specific concentration demonstrated in the whole lPFC is significant in the DLPFC alone.

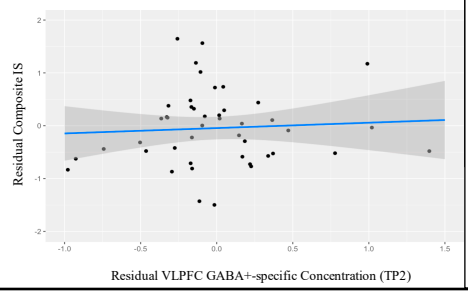

Figure 11c. Residuals of VLPFC GABA+-specific concentration and Composite Impulsivity Score, controlling for VLPFC Glx-specific concentration and Age. The positive prediction of composite IS by GABA+-specific concentration demonstrated in the whole lPFC is not significant in the VLPFC alone.

Table 7. Summary of Significant Results

Hypothesis	Findings	Hypothesis supported?	Figures
H1a	Composite IS was significantly higher in Drinkers compared to Non-Drinkers (t = 2.27, p = 0.035, df = 18.8).	Yes	Fig. 5
H1b	Composite IS significantly negatively predicted Age of Initiation of Alcohol (t = -2.80, p = 0.0069, df = 61).	Yes	Fig. 6
H2b & H3c	GABA+-specific concentration significantly negatively predicted Age of Initiation of Alcohol (t = -1.93, p = 0.063, df = 28). ***** Relationship did not remain significant when accounting for composite IS as a covariate.	No	Fig. 7
	Glx-specific concentration significantly negatively predicted Age of Initiation of Alcohol (t = -0.445, p = 0.04, df = 27) when controlling for composite IS. *****	Yes	Fig. 8
	Average NT concentration significantly negatively predicted Age of Initiation of Alcohol (t = -2.70, p = 0.011, df = 29). *****	N/A †	Fig. 9
	DLPFC NT concentration ratio significantly positively predicted Age of Initiation of Alcohol (t = 0.696, p = 0.035, df = 30). Relationship did not remain significant when accounting for composite IS as a covariate.	N/A †	Fig. 10
N/A †	GABA+-specific concentration significantly positively predicted composite IS (t = 0.763, p = 0.023, df = 42). *****	N/A †	Fig. 11

***** All of these findings were driven by NT levels in the DLPFC.
† N/A indicates that the finding is not related to any of the study's *a priori* hypotheses, as we made no particular predictions about the Average NT concentration or NT concentration ratio's relationship with substance use, nor the relationship between lPFC NT levels and composite IS.

4. Discussion

The results of this preliminary study provide a starting point for future research into the relationship between excitatory and inhibitory NTs in the lPFC and adolescent substance use. Existing literature points to a negative relationship between lPFC GABA+ and substance, as well as a positive relationship between lPFC Glx and substance use, based on the theory that higher

lPFC GABA+ levels inhibit impulsivity while higher lPFC Glx levels promote impulsivity (see Fig. 1). While the results of the present research lend some support to the hypothesis that lPFC Glx has a positive relationship with substance use, they also suggest that both lPFC GABA+ and lPFC Glx have the opposite relationship with impulsivity than was predicted. Furthermore, voxel-specific analyses revealed that NT levels in the DLPFC played a larger contributory role in the significant results than those in the VLPFC, a distinction that should be explored further in future studies.

4.1. Hypothesis Review

Recall the original overarching hypotheses:

H1. Prediction of a positive relationship between Impulsivity and substance use

H2. Prediction of a negative relationship between lPFC GABA+ levels and substance use

H3. Prediction of a positive relationship between lPFC Glx levels and substance use

Hypothesis 1 was largely supported by the findings of this study. Composite IS was significantly higher in adolescents that drink alcohol than those that do not, as predicted by *H1a* (see Fig. 5). However, this finding was the result of *Group Analyses* in which t-tests were used to compare independent groups that were not of comparable sample sizes (Drinkers = 69 participants, Non-Drinkers = 13 participants). Given the small sample size in the Non-Drinkers group, this finding has relatively low statistical power. The lack of support shown for hypotheses *H2a* and *H3a* may also be attributed to these unequal sample sizes. Consistent with *H1b*, composite IS significantly negatively predicted Age of Initiation of Alcohol Consumption (Fig. 6), indicating that greater levels of impulsivity were associated with an earlier onset of alcohol use. Notably, however, there was no significant relationship between impulsivity and Frequency of Alcohol Consumption, as predicted in *H1c*.

Hypothesis 2 was partially refuted by this study's results: lPFC GABA+ levels were not negatively associated with substance use as expected. Rather, GABA+-specific concentration trended towards *negatively* predicting Age of Initiation of Alcohol Consumption, indicating (contrary to *H2b*) that participants with higher levels of lPFC GABA+ began drinking alcohol earlier. However, this finding did not remain trending towards significance when composite IS was included as a covariate. This suggests that GABA+-specific concentration did not have a significant relationship with Age of Initiation of Alcohol above and beyond impulsivity levels. Given that GABA-+specific concentration and composite IS were significantly positively correlated (see Table 6), the finding demonstrated in Figure 7a was likely driven instead by the significant negative relationship between impulsivity and Age of Initiation shown in Figure 6. Once again, there was no significant relationship found between lPFC GABA+ levels and Frequency of Alcohol Consumption, as predicted in *H2c*.

Hypothesis 3 was partially supported by the results of this study. While there was no relationship demonstrated between Glx-specific concentration and Age of Initiation of Alcohol Consumption without controlling for composite IS, a significant negative relationship did emerge when composite IS was included as a covariate. This indicates that composite IS was suppressing the negative relationship between lPFC Glx levels and Age of Initiation, in which higher lPFC Glx levels are associated with earlier drinking. While this finding is ultimately consistent with *H3c*, the suppressor effect demonstrated by composite IS suggests that impulsivity may have a negative relationship with lPFC Glx levels, contrary to the theory that our *a priori* hypotheses were based on. This possibility is supported by the positive correlation between composite IS and GABA+-specific concentration and the negative, although insignificant, relationship between composite IS and Glx-specific concentration (Table 6). It is further supported by the positive prediction of

composite IS by GABA+-specific concentration (Fig. 11). In Figure 1a above, the relationships between impulsivity and lPFC NT levels suggested by this study's findings can be seen in comparison to the relationships theorized in our original research model.

Though they were not in the directions we predicted, the fact that lPFC GABA+ and Glx were still oppositely (albeit not both significantly) correlated with composite IS does indicate that the inhibitory and excitatory signaling systems have opposite relationships with impulsivity levels, lending some internal validity to our findings. One possible explanation for this finding is that the original theories of an inhibitory effect of GABA+ and excitatory effect of Glx on impulsivity were based on MRS research that was confined to the mPFC, rather than the lPFC. However, it is unlikely that this fully explains the observed findings; while the functions of the mPFC and lPFC are distinct in the specific aspects of cognitive control they involve, the regions are jointly responsible for impulse control and are therefore unlikely to have completely opposite mechanisms of neurochemistry (de la Vega et al., 2016; de la Vega et al., 2017).

Another explanation lies in the fact that inhibitory and excitatory NT levels undergo significant changes during adolescent development (Larsen & Luna, 2018). Our *a priori* hypotheses were based in the assumption that different NT levels reflect individual differences between participants that represent differences in "personality." Therefore, higher inhibitory lPFC GABA+ levels were hypothesized to confer a higher degree of impulse control and later/less frequent substance use, and vice versa for excitatory lPFC Glx levels. But given that GABA and Glx levels in the PFC are changing during development and are involved in neuronal pruning and critical period plasticity, it is also possible that the relative levels of these NTs in adolescents do not reflect individual differences in impulse control, but rather the progress of their developmental state (Larsen & Luna, 2018). We included chronological age as a nuisance covariate in all of the

analyses in this study to mitigate the potential confounding effect of NT changes during adolescence as much as possible, but chronological age is known to be an imperfect representation of developmental age during adolescence (Steinberg et al., 2008). Therefore, it is possible that higher lPFC GABA+ levels and lower lPFC Glx levels are indicative of an "older" developmental age (or being further along on the developmental trajectory), during which sensation-seeking, risk-taking, and susceptibility to tempting rewards like alcohol are thought to be at their peak levels (Steinberg et al., 2008; Shulman et al., 2016). This may help to explain the unexpected relationship observed between lPFC NT levels and impulsivity (Fig. 1a).

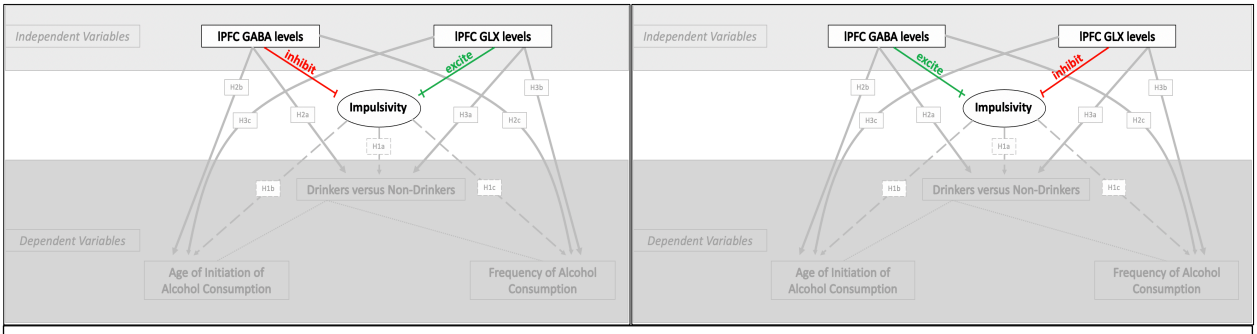

Figure 1a. Relationship between lPFC NT levels and Impulsivity: Predicted versus Observed.
Our original research model (Figure 1, pictured left) predicted a negative relationship between lPFC GABA+ levels and Impulsivity and a positive relationship between lPFC Glx levels and Impulsivity, per prior literature focused on mPFC NT levels in both adolescents and adults. Our results instead suggest an opposite relationship (pictured right), in which lPFC GABA+ levels are positively correlated with Impulsivity and lPFC Glx levels are negatively correlated with Impulsivity.

Though they weren't included in the study's *a priori* hypotheses, the average NT concentration and NT concentration ratio were also included in our analyses to examine whether the GABA shunt or relative ratio of excitatory:inhibitory NT had a relationship with adolescent substance use. As shown in Figure 9a, average NT concentration demonstrated a significant negative relationship with Age of Initiation of Alcohol Consumption that remained significant beyond impulsivity levels when composite IS was included as a covariate. This finding suggests that there might not be a significant effect of GABA+ or Glx alone, but rather that higher levels of

any NTs belonging to the GABA shunt predict an earlier age of onset of drinking. This possibility is further supported by the fact that neither Cho concentration nor NAA concentration had any significant effect on substance use in our analyses, suggesting that the significant results of this study are specific to inhibitory and excitatory NTs.

4.1.1. Voxel-Specific Findings

The significant negative effects of GABA+-specific concentration (without controlling for composite IS only), Glx-specific concentration (while controlling for composite IS only), and average NT concentration were found to be driven by NT levels in the DLPFC (Fig. 7b, 8b, 9b). Furthermore, as shown in Figure 10, while there were no significant findings regarding the NT concentration ratio averaged across voxels, the DLPFC NT concentration ratio demonstrated a positive relationship with Age of Initiation of Alcohol Consumption that was not present in the VLPFC alone. The same DLPFC-driven finding held true for the positive relationship between GABA+-specific concentration and composite IS (Fig. 11). This consistent contribution of the DLPFC over the VLPFC to our significant findings raises an interesting question about the differential roles that the DLPFC and VLPFC play in disinhibition and cognitive control as they relate to adolescent substance use. The DLPFC has been found to be more involved in goal maintenance than the VLPFC, which instead plays a larger role in mechanisms for goal-relevant selection (Milham et al., 2002; Snyder et al., 2015). The results of this study suggest that neurochemical concentrations in the DLPFC are more related to the onset of alcohol use than those in the VLPFC, which may indicate that resistance to the reward of substance use has more to do with maintenance of impulse control in the face of distractors than the selection of the goal of impulse control in the first place.

4.2. Limitations

One of the limitations of the data set in this study was the sample size. As discussed above, in the *Group Analyses,* the Drinkers (n = 69) and Non-Drinkers (n = 13) groups were of widely varying sizes, detracting from the statistical power of the findings of the t-tests conducted between the groups. The following linear regressions were also carried out with a relatively small sample size of 69 Drinkers. Due to the small size of the whole sample, we were unable to carry out any meaningful analyses comparing Genders, as partitioning the sample further would result in even smaller sample sizes.

Another limitation is the time-intensive, expensive, and relatively cutting-edge nature of the MRS method used in this study. Several of the analyses conducted with the NT levels had further reduced degrees of freedom due to the absence of spectroscopy data on several subjects.

Finally, the entirety of this study's dependent variable of interest was reliant on data collected via self-report surveys on a fairly sensitive topic. Self-report measures are inherently limited due to factors like the social desirability bias and participants' tendencies to exaggerate or obscure private details. In this study in particular, the majority of participants were under the age of 21 when they filled out the SUQ and AUDIT, and therefore may have been more susceptible to these limiting factors. The discrepancies between the Age of First Drink data reported at TP1 and TP2 further demonstrate the limitation of self-report questionnaires on substance use. Future studies that examine Age of Initiation of Alcohol Consumption in adolescents might benefit from a stricter definition of a "drink" to differentiate it from a "sip" (e.g. 12oz beer, 1oz hard liquor, etc…).

4.3. Future Directions

This was preliminary and exploratory study, conducted as a sub-study of the larger CoNiFER research project in order to answer a research question for which the larger study was

not explicitly designed. Although there were significant limitations in the data set, the findings of this study provide a fruitful basis for future research into the relationship between individual differences in lPFC GABA+ and Glx and adolescent substance use.

A repeat study with a larger sample size would provide a more robust look at the preliminary significant relationships discovered here. Additionally, some of the non-significant relationships in this study, such as the negative correlation between composite IS and Glx-specific concentration, may be found to be more robust in a larger sample.

Given the fact that some of the significant findings in this study were confined to the DLPFC, future research into the comparative relationships of the DLPFC and VLPFC with adolescent substance use might provide insight into the regional neurochemical mechanisms of impulse control during development. This distinction can be further examined in the context of potential gender differences between male and female development of the lPFC.

Given the possibility that higher lPFC GABA levels may indicate development that is further along its trajectory, our *a priori* hypotheses might be better suited for a study conducted in adults to understand the relationship between lPFC NT levels and substance use without the potential confounding variable of developmental NT changes. Additionally, another study could attempt to control further for developmental age rather than just chronological age. One way to do this might be to administer a behavioral reward task as a measure of the adolescents' sensitivity to temptation and examine whether reward processing plays a role in the relationship between lPFC NT levels and the onset and frequency of substance use. As the TP1 substance use data was found to be invalid in the course of this study, we were unable to examine the change over time in these variables throughout adolescence. A future study could also investigate the changing relationships

between neural correlates of disinhibition and substance use as cognitive development progresses to better understand the contribution of the developmental age.

Once this topic has been explored in greater depth and a stronger scientific understanding of the relationship between inhibitory-excitatory signaling control in the brain and substance use has been established, this research could be extended into clinical populations in order to understand how neural correlates of disinhibition may predispose individuals to substance use and substance addiction. Ultimately, the results of this research can lend clarity to our understanding of the development of substance misuse during and following adolescence. This has significant implications for our abilities to treat and even prevent the formation of SUDs.

The results of this study provide preliminary evidence that disinhibition and inhibitory-excitatory signaling in the lPFC have a different relationship with one another than expected in adolescents. Further research into this area will help to elucidate the nature of this relationship in the developing brain.

References

Abé, C., Mon, A., Durazzo, T.C., Pennington, D.L., Schmidt, T.P., & Meyerhoff, D.J. (2013). Polysubstance and alcohol dependence: Unique abnormalities of magnetic resonance-derived brain metabolite levels. *Drug and Alcohol Dependence 130*(1): 30-37.

Bak, L.K., Schousboe, A., & Waagepeterson, H.S. (2006). The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *Journal of Neurochemistry 98*(3): 641-653.

Bertin, L., Benca-Bachman, C., Kogan, S., Palmer, R. (2021). Examining the differential effects of latent impulsivity factors on substance use outcomes in African American men. *Addictive Behaviors 117*.

Blakemore, S. & Robbins, T. (2012). Decision-making in the adolescent brain. *Nature: Neuroscience 15*(9): 1184-1191.

Brook, J., Kessler, R., & Cohen, P. (1999). The onset of marijuana use from preadolescence and early adolescence to young adulthood. *Development and Psychopathology 11*: 901-914.

Carver, C. & White, T. (1994). Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS scales. *Journal of Personality and Social Psychology 67*(2): 319-333.

Chen, T., Tan, H., Lei, H., Su, H., & Zhao, M. Proton magnetic resonance spectroscopy in substance use disorder: recent advances and future clinical applications. *Science China: Information Sciences 63*: 1-14.

de la Vega, A., Brown, M., Snyder, H., Singel, D., Munakata, Y., & Banich, M. (2014). Individual differences in the balance of GABA to glutamate in pFC predict the ability to select among competing options. *Journal of Cognitive Neuroscience 26*(11): 2490-2502.

de la Vega, A., Chang, L., Banich, M., Wager, T., & Yarkoni, T. (2016). Large-scale meta-analysis of human medical frontal cortex reveals tripartite functional organization. *The Journal of Neuroscience 36*(24): 6553-6562.

de la Vega, A., Yarkoni, T., Wager, T., & Banich, M. (2017). Large-scale meta-analysis suggests low regional modularity in lateral frontal cortex. *Cerebral Cortex*: 1-15.

Epperson, C.N., O'Malley, S., Czarkowski, K.A., Gueorguieva, R., Jatlow, P., Sanacora, G., Rothman, D.L., Krystal, J.H., & Mason, G.F. (2005). Sex, GABA, and nicotine: The impact of smoking on cortical GABA levels across the menstrual cycle as measured with proton magnetic resonance spectroscopy. *Biological Psychiatry 57*(1): 44-48.

Euston, D.R., Gruber, A.J., & McNaughton, B.L. (2012). The role of medial prefrontal cortex in memory and decision making. *Neuron 76*(6): 1057-1070.

Gullo, M.J. & Dawe, S. (2008). Impulsivity and adolescent substance use: Rashly dismissed as "all-bad"? *Neuroscience and Biobehavioral Reviews 32*(8): 1507-1518.

Hermann, D., Weber-Fahr, W., Sartorius, A., Mann, K., Ende, G., & Sommer, W.H. (2011). Translational magnetic resonance spectroscopy reveals excessive central glutamate levels during alcohol withdrawal in humans and rats. *Biological Psychiatry 71*(11): 1015-1021.

Ivanov, I., Schulz, K.P., London, E.D., & Newcorn, J.H. (2009). Inhibitory control deficits in childhood and risk for substance use disorders: A review. *The American Journal of Drug and Alcohol Abuse 34*(3): 239-258.

Larsen, B. & Luna, B. (2018). Adolescence as a neurobiological critical period for the development of higher-order cognition. *Neuroscience & Biobehavioral Reviews 94*: 179-195.

Liu, M., Argyriou, E., & Cyders, M. (2020). Developmental considerations for assessment and treatment of impulsivity in older adults. *Recent Advances in Research on Impulsivity and Impulsive Behaviors*: 165-177.

Luna, B., Marek, S., Larsen, B., Tervo-Clemmens, B., & Chahal, R. (2017). An integrative model of the maturation of cognitive control. *Annual Review of Neuroscience 38*: 151-170.

Lynskey, M., Fergusson, D., & Horwood, L. (1998). The origins of the correlations between tobacco, alcohol, and cannabis use during adolescence. *Journal of Child Psychology and Psychiatry and Allied Disciplines 39*: 995-1005.

Milham, M., Erikson, K., Banich, M., Kramer, A., Webb, A., Wszalek, T., & Cohen, N. (2002). Attentional control in the aging brain: insights from an fMRI study of the Stroop task. *Brain Cognition 49*(3): 277-296.

Miller, E.K. (2000). The prefrontal cortex and cognitive control. *Nature Reviews Neuroscience 1*: 59-65.

Molina, B.G. & Pelham, W.E. (2003). Childhood predictors of adolescent substance use in a longitudinal study of children with ADHD. *Journal of Abnormal Psychology 112*(3): 497-507.

Patton, J., Stanford, M., & Barratt, E. (1995). Factor structure of the Barratt Impulsiveness Scale. *Journal of Clinical Psychology 51*: 768-774.

Reubi, J.C., Van Der Berg, D., & Cuénod, M. (1978). Glutamine as precursor for the GABA and glutamate transmitter pools. *Neuroscience Letters 10*(1-2): 171-174.

Rubia, K., Russell, T., Overmeyer, S., Brammer, M., Bullmore, E., Sharma, T., Simmons, A., Williams, S., Giampietro, V., Andrew C., & Taylor, E. (2001). Mapping motor inhibition: conjunctive brain activations across different versions of go/no-go and stop tasks. *Neuroimage 13*(2): 250-261.

Saunders, J.B., Aasland, O.G., Babor, T.F., de la Fuente, J.R., and Grant, M. (1993). Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption: II. *Addiction, 88*(6): 791-804.

Shin, S., Chung, Y., & Jeon, S. (2013). Impulsivity and substance use in young adulthood. *The American Journal on Addictions 22*: 39-45.

Shulman, E., Smith, A., Silva, K., Icenogle, G., Duell, N., Chein, J., & Steinberg, L. (2016). The dual systems model: Review, reappraisal, and reaffirmation. *Developmental Cognitive Neuroscience 17*: 103-117.

Siddiqui, S.V., Chatterjee, U., Kumar, D., Siddiqui, A., & Goyal, N. (2008). Neuropsychology of prefrontal cortex. *Indian Journal of Psychiatry 50*(3): 202-208.

Silveri, M.M., Sneider, J.T., Crowley, D.J., Covell, M.J., Acharya, D., Rosso, I.M., & Jensen, J.E. (2013). Frontal lobe gamma-aminobutyric acid levels during adolescence: Associations with impulsivity and response inhibition. *Biological Psychiatry 74*(4): 296-304.

Snyder, H., Banich, M., & Munakata, Y. (2015). All competition is not alike: Neural mechanisms for resolving underdetermined and prepotent competition. *Journal of Cognitive Neuroscience 26*(11): 2608-2623.

Soloff, P.H., Meltzer, C.C., Greer, P.J., Constantine, D., & Kelly, T.M. (2000). A fenfluramine-activated FDG-PET study of borderline personality disorder. *Biological Psychiatry 47*(6): 540-547.

Steinberg, L. & Monahan, K.C. (2007). Age differences in resistance to peer influence. *Developmental Psychology 43*(6): 1531-1543.

Steinberg, L., Albert, D., Cauffman, E., Banich, M., & Woolard, J. (2008). Age differences in sensation seeking and impulsivity as indexed by behavior and self-report: evidence for a dual systems model. *Developmental Psychology 44*(6): 1764-1778.

Substance Abuse and Mental Health Services Association (SAMHSA). (n.d.). *Substance use disorders*.

Torrubia, R., Ávila, C., Moltó, J., & Caseras, X. (2001). The Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ) as a measure of Gray's anxiety and impulsivity dimensions. *Personality and Individual Differences 31*(6): 837-862.

Varney, N.R., Pinkston, J.B., & Wu, J.C. (2001). Quantitative PET findings in patients with posttraumatic anosmia. *Journal of Head Trauma Rehabilitation 16*(3): 253-259.

Von Diemen, L., Bassani, D.G., Fuchs, S.C., Szobot, C.M., & Pechansky, F. (2008). Impulsivity, age of first alcohol use and substance use disorders among male adolescents: a population-based case-control study. *Addiction 103*(7): n.p.

Wang, G., Eijk, J.V., Demirakca, T., Sack, M., Krause-Utz, A., Cackowski, S., Schmahl, C., & Ende, G. (2017). ACC GABA levels are associated with functional activation and connectivity in the fronto-striatal network during interference inhibition in patients with borderline personality disorder. *NeuroImage 147*(15): 164-174.

Weinberger, D.A. & Schwartz, G.E. (1990). Distress and restraint as superordinate dimensions of self-reported adjustment: A typological perspective. *Journal of Personality 58*(2): 381-417.

Whiteside, S. & Lynam, D. (2001). The Five Factor model and impulsivity: Using a structural model of personality to understand impulsivity. *Personality and Individual Differences 30*(4): 669-689.

Zuckerman, M., Eysenck, S.B.J., & Eysenck, H.J. (1978). Sensation seeking in England and America: Cross-cultural, age, and sex comparisons. *Journal of Consulting and Clinical Psychology 46*(1): 139-149.

Appendix A: Composite Impulsivity Score Measures

Scale	Items	Response Scale
Sensation Seeking Scale (Zuckerman et al., 1978) *reverse-coded	1. I tend to begin a new project without much planning on how I will do it. 2. I usually think about what I am going to do before doing it. * 3. I often act without thinking. 4. I hardly spend much time on the details of planning ahead. 5. I like to have new and exciting experiencing and feelings even if they are a little frightening. 6. Before I begin a difficult project, I make careful plans about how I would complete it. * 7. I would like to take a trip without planning where I would go or what I would do. 8. I enjoy getting into new things that are a little frightening. 9. I like doing things just for the thrill of it. 10. I tend to change the things I like to do often. 11. I sometimes like to do things that are a little frightening. 12. I'll try anything once. 13. I would like the kind of day where there is lots of change and excitement. 14. I sometimes do "crazy" things just for fun. 15. I like to explore a new neighborhood by myself, even if it means getting lost. 16. I think friends who do things you don't expect are exciting. 17. I often get carried away by new and exciting things and ideas that I never think of possible problems. 18. I usually act before I think about what I want to do. 19. I like "wild and crazy" parties.	True **(1)** – False **(2)**
Impulse Control Subscale of the Weinberger Adjustment Inventory (Weinberger & Schwartz, 1990) *reverse-coded	1. I'm the kind of person who will try anything once, even if it is not safe. * 2. I should try harder to control myself when I'm having fun. * 3. I do things without giving them enough thought. * 4. I become "wild and crazy" and do things other people might not like. * 5. When I am doing something for fun (for example, partying, acting silly), I tend to get carried away and go too far. * 6. I like to do new and different things that many people would consider weird or not really safe. * 7. I say the first thing that comes to my mind without thinking enough about it. * 8. I stop and think things through before I act.	Disagree **(1)** – Not Sure **(3)** – Agree **(5)**
Resistance to Peer Influence Scale (Steinberg & Monahan, 2007) *reverse-coded	For each question, select the statement that best describes you. For the statement you selected ONLY, how true is it for you? 1. Some people go along with their friends just to keep their friends happy BUT… Other people refuse to go along with what their friends want to do, even though they know it will make their friends unhappy. 2. Some people think it is more important to be an individual than to fit in with the crowd BUT… Other people think it is more important to fit in with the crowd than to stand out as an individual. * 3. Some people would do something that they knew was wrong just to stay on their friends' good side BUT… Other people would not do something they knew was wrong just to stay on their friends' good side. 4. Some people hide their true opinion from their friends if they think their friends will make fun of them because of it BUT… Other people will say their true opinion in front of their friends, even if they know their friends will make fun of them because of it. 5. Some people will not break the law just because their friends say that they would BUT… Other people would break the law if their friends said they would break it. * 6. Some people change the way they act so much when they are with their friends that they wonder who they "really are" BUT… Other people act the same way when they are alone as they do when they are with their friends. 7. Some people take more risks when they are with their friends than they do when they are alone BUT… Other people act just as risky when they are alone as when they are with their friends. 8. Some people say things they don't really believe because they think it will make their friends respect them more BUT… Other people would not say things they didn't really believe just to get their friends to respect them more. 9. Some people think it's better to be an individual even if people will be angry at you for going against the crowd BUT… Other people think it's better to go along with the crowd than to make people angry at you. *	Statement #1 is Really true for me **(1)** – Statement #1 is Sort of true for me **(2)** – Statement #2 is Sort of true for me **(3)** – Statement #2 is Really true for me **(4)**