HHS Public Access

Author manuscript

Brain Imaging Behav. Author manuscript; available in PMC 2021 October 01.

Published in final edited form as:

Brain Imaging Behav. 2020 October ; 14(5): 2062–2071. doi:10.1007/s11682-019-00165-w.

An investigation of the relationship between glutamate and resting state connectivity in chronic cannabis users

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Abstract

Human and animal studies have shown that heavy cannabis (CB) use interacts with glutamatergic signaling. Additionally, recent studies have suggested that glutamate (Glu) may drive resting state functional connectivity (RSfc). The aims of the current preliminary study were to: 1) determine whether dorsal anterior cingulate cortex (dACC) Glu is related to RSfc between the dACC and two nodes of the reward network, the nucleus accumbens (NAc) and hippocampus (Hp); and 2) determine whether CB use interacts with the relationship between dACC Glu and RSfc. A group of 23 chronic CB users and 23 healthy controls participated in this multimodal MRI study. Glu levels were assessed in the dACC using magnetic resonance spectroscopy (MRS). Linear regression models were used to determine whether dACC Glu and CB use predicts RSfc between the dACC and the NAc and Hp. While the effect size is small, the results showed that the connectivity between the dACC and right NAc was predicted by the interaction between dACC Glu levels and monthly CB use. Additionally, while there is some suggestion that dACC Glu is correlated with dACC-hippocampal connectivity, unlike for dACC/NAc connectivity the relationship between them does not appear to be affected by CB use. These preliminary findings are significant in that they demonstrate the need for future studies with larger sample sizes to better characterize the relationship between resting state connectivity and neurochemistry as well as to characterize how CB use interacts with that relationship.

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Conflicts of Interest: All authors reported no biomedical financial interests or potential conflicts of interest.

Informed consent: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, and the applicable revisions at the time of the investigation. Informed consent was obtained from all patients for being included in the study.

Keywords

cannabis; MRS; Dorsal anterior cingulate; resting state connectivity

Introduction

The use of cannabis has increased over the past decade in the United States, and past-year prevalence of cannabis (CB) use exceeds 10% (Grucza et al., 2016) with few users seeking treatment (Brown et al., 2003). The primary psychoactive component of cannabis (CB) is Δ9-tetrahydrocannabinol (THC). THC has been found in animal models to modulate glutamatergic (Glu) neurotransmission and concentrations. The CB1 receptor is highly expressed in axons and terminals of glutamatergic neurons of the CNS and acts to inhibit synaptic transmission or reduce neuronal excitability through depolarization induced suppression of inhibition or excitation. Glutamate in the extracellular space is tightly controlled due to its potential toxic properties. Brown and colleagues (2003) reported that THC reduces the release and uptake of Glu in a dose-dependent manner in rat striatal slices. Additionally, Straiker and Mackie (2005) showed CB receptor-mediated reduction in Glu transmission in mice. Exposure to THC in utero has also been found to result in reductions in basal extracellular Glu levels in adolescent rats (Castaldo et al., 2007). In a recent review, Colizzi and colleagues (2016) concluded that THC depresses endocannabinoid-mediated Glu synaptic transmission and that it affects Glu release, enzyme activity and the expression and activity of receptors and transporters.

While the evidence supporting a relationship between Glu and CB is evident in preclinical cellular studies, there are relatively few studies examining this relationship in humans using magnetic resonance spectroscopy (MRS). Several MRS studies have associated CB use with a reduction in Glu levels in specific brain regions (Colizzi et al., 2016; Sneider et al., 2013). For example, Prescot and colleagues (2011, 2013) found lower anterior cingulate (ACC) Glu levels in adolescent CB users compared to controls. Additionally, Chang et al (2006) and Muetzel et al. (2013) found lower Glu levels in the basal ganglia in CB users. Not all studies have found a reduction in Glu, however. For example, van de Giessen et al (2017) used PET to measure dopamine release and MRS to measure Glu in the striatum and hippocampus of controls and CB dependent participants. CB use was associated with decreased dopamine in the striatum; however, Glu did not differ between groups in either the striatum or the hippocampus. Additionally, while Muetzel et al (2013) did show Glu decreases, they were only observed in female users not male users. Thus, while reduced Glu or Glx in CB users is one of the most consistent findings, variations in findings may be related to differences in frequency or duration of CB use, age group examined, sex and the anatomical location of the measurement.

Glutamatergic efferent projections from the prefrontal cortex to the nucleus accumbens (NAc) appear to be a key pathway in drug addiction (Koob & Volkow, 2009) and are likely directly affected by cannabinoids. For example, a recent study found that THC affects dopamine release in the reward system indirectly via a CB1-dependent inhibition of Glu release onto gamma-aminobutyric acid (GABA) neurons in the NAc and the ventral

tegmental area (Pertwee, 2008). The NAc plays a primary role in addiction and has been strongly associated with mediating the expression of learned behaviors in response to a motivationally relevant stimulus (Kalivas, 2005). The ACC, like the NAc, is an important region due to its role in reward processing and motivation as well as its interconnections with other prefrontal regions and the dopaminergic areas of the basal ganglia that have been linked to addiction. The ACC has also been found to have high CB1 receptor density (Glass et al., 1997; Tsou et al., 1998) suggesting that CB is likely to have an impact on the processing and neurochemistry of the region. The dorsal ACC (dACC) has been linked to inhibitory control and appraisal (Botvinick et al., 2001; Venkatraman & Huettel, 2012) and projects to the core of the NAc via a glutamatergic signaling pathway. It should also be noted that the ACC is a heterogeneous region with a number of subregions that are histologically distinct (Palomero-Gallagher et al., 2009) and that have different cytoarchitecture and connectivity patterns. The current study focuses on dACC which has shown previously to have Glu concentration differences in CB users (Prescot et al., 2011; 2013).

The process of addiction involves the reorganization of neural connectivity in the brain as well as altered neurochemistry. Resting state functional connectivity (RSfc) is a measure of intrinsic brain connectivity which has been used to characterize large-scale neural networks including the default-mode (DMN), the salience and sensorimotor networks (Smith et al., 2009) and the reward network (Barnes et al., 2010; Di Martino et al., 2008). Additionally, these resting state networks have been found to be disrupted in substance abuse disorders (Fedota & Stein, 2015; Muller-Oehring et al, 2014; Sutherland et al., 2012). Previous studies examining RSfc have shown that in healthy individuals the NAc has positive connectivity with reward and emotion-related regions while showing negative connectivity with regions implicated in cognitive control and inhibition like the dACC (Barnes et al., 2010; Di Martino et al., 2008). Functional connectivity studies of CB use have reported alterations in both RSfc and cognitive task connectivity which includes changes in interhemispheric connectivity in adolescent CB users (Orr et al., 2013); task-based functional connectivity in the inhibitory control network (Filbey & Yezhuvath, 2013); and RSfc changes spanning from the cerebellum to the prefrontal cortex (Cheng et al., 2014).

Even though an association has been observed between glutamatergic neurotransmission, neuronal firing rate, and blood oxygen level dependent signals (BOLD) in the rat brain (Hyder et al., 2006; Smith et al., 2002), there have been very few studies examining this relationship in the human brain and no study examining this relationship in CB users. Recent studies have suggested that Glu may drive the neuronal mechanisms that underlie the sustained resting state in normal functioning adults (Duncan et al., 2014; Moeller et al., 2016). For example, it has been shown that the higher the Glu concentration in the posterior cingulate cortex (PCC; a primary hub in the DMN) the higher was the connectivity between the PCC and the pregenual ACC (members of the DMN; Duncan et al., 2013; Hu et al., 2013; Kapogiannis et al., 2013). Wagner et al. (2016) demonstrated that the connectivity between the Hp and the perigenual anterior cingulate cortex (pACC) which is located anterior to the dACC was negatively correlated with Glu in healthy adults. Also, resting state connectivity patterns using the pACC as a seed have been found to be associated with Glu levels in the pACC (Duncan et al., 2013; Enzi et al., 2012). Falkenberg and colleagues

(2012) reported a significant relationship between Glu levels in the dACC and BOLD responses in the mPFC-striatal regions. Moeller and colleagues (2016) argue that drug-addicted individuals exhibit both abnormal Glu neurotransmission in core regions of the reward network and disruptions in RSfc in those same regions, leading to the interpretation that deficits in Glu may be responsible for differences in RSfc that have been observed in drug users. It should also be noted that these studies focused on different sub-regions of the ACC but all showed similar results – ACC Glu was related to RSfc between the ACC and other regions of the brain.

The goal of the current study was to examine whether CB use interacts with the relationship between Glu and RSfc. While there are studies that have examined the relationship between Glu and RSfc in healthy adults, there have been none directly examining this relationship in chronic CB users. This is important because if Glu drives connectivity and CB use disrupts the relationship between Glu and connectivity it may highlight a potential mechanism by which CB impacts brain functioning. In this preliminary study Glu levels in the dACC were measured and the RSfc between the dACC and two regions of interest, the NAc and the Hp, were assessed in chronic CB users and nonusers. We hypothesized that dACC Glu levels would predict the connectivity strength between the dACC and NAc. Additionally, because CB has been shown previously to interact with Glu in the ACC and with RSfc between the ACC and NAc it was predicted that the relationship between them would be impacted by chronic CB use. A recent finding reported by Wagner and colleagues (2016) showed that Glu levels in the Hp was correlated with connectivity between the Hp and pregenual ACC. Therefore, we predicted that Glu would be associated with dACC-Hp connectivity. However, van de Giessen et al (2017) failed to show CB related effects of Glu in the Hp even though the Hp also has a high density of CB1 receptors (Moldrich & Wenger, 2000), we, therefore, made no prediction regarding the interaction between CB use and the connectivity between the dACC and Hp.

Methods

Participants.

A total of 79 participants completed the MRS and resting state scans. For the MRS dataset 8 participants' data were too noisy due to bad shimming, 6 were removed due to exceeding the CRLD threshold and 10 of the remaining participants were former users leaving 55 usable MRS datasets. Of those 55, 3 were removed due to a probable history of alcohol use disorder, 3 were removed due to age (> 30), and 3 due to excessive censored scans due to head motion (see below). After data cleaning there were 23 current cannabis (CB) users and 23 healthy non-user controls whose data are analyzed in the current study (see Table 1). Subjects were recruited by local advertisements. After detailed description of the study, written and verbal informed consent was obtained from each participant. All subjects were required to be 18 years or older, and free of any neurological disorder, head trauma with loss of consciousness greater than ten minutes, learning disability, and contraindication to MRI. Subjects were asked to refrain from alcohol or CB use the day prior to the MRI scan. The research protocol was approved by Indiana University's Institutional Review Board for the protection of human subjects.

Participants completed a battery of assessments including the Structured Clinical Interview for DSMIV-TR (SCID-IV-TR), Research Version (First et al., 2002); a written drug use questionnaire; a six-month time line follow back assessment to estimate current and past use of CB and alcohol; the short Michigan alcohol screening test (SMAST); and the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999). The control subjects had no history of substance dependence, a negative urine screen for CB and other substances, and no use of CB in the past three months. Participants who reported other illicit drug use were excluded from the study. Participants whose SMAST score indicated probable alcohol use disorder were eliminated from the study. Groups did not significantly differ in age, IQ score, sex, days since last alcohol use at the time of screening, or drinks per week ($p > 0.1$). Additionally, when examining just the CB group, there were no sex differences in age, age of CB use onset, monthly CB use, or lifetime CB use (p's > 0.4); females were similar to males.

MRI Acquisition.

Image acquisition was performed on a 3T Siemens Tim-Trio MRI scanner. Foam pads were used to minimize head motion for all participants. Functional scans were acquired using a single-shot echo-planar-imaging (SS-EPI) sequence [repetition time (TR) = 813 ms; echo time (TE) = 28 ms; flip angle = 60°; 42 transverse slices; slice thickness 3.4 mm; field of view (FOV) = 220×220 mm^2; imaging matrix = 64×64; in-plane voxel size = 3.44×3.44 mm^2]. Subjects were instructed to rest in the scanner looking at a fixation-cross on a screen via an LCD projector. Scans for the first 10 sec were discarded to allow the T1-magnetisation equilibrium, resulting in a total of 1000 volumes (= 14 min). Subsequently, high-resolution T1-weighted anatomical images were acquired in the sagittal plane using an MP-RAGE sequence [TR = 1.8 s; TE = 2. 67 ms; inversion time = 0.9 s; flip angle 9°; imaging matrix = 256×256; 192 slices; voxel size = 1×1×1 mm^3].

The MRS was performed using a single-voxel PRESS sequence [TR/TE = 2000/30 ms, bandwidth = 2000 Hz, 2048 data points, number of measurements = 120, scan time = 4 min], followed by a water reference scan (8 averages). Each voxel measurement began with the FASTMAP shimming method twice (Gruetter, 1993; Gruetter & Tkac, 2000). Manual shimming was performed only if FASTMAP did not give a good shimming result (< 15 Hz). The full width at half maximum (FWHM) of shimming was all below 14 Hz after these procedures. All scans were visually checked to ensure acceptable MRI quality.

Voxel Placement.

The MR spectroscopy voxel was positioned in the dorsal ACC using the T1-weighted image. The voxel was positioned in the following way: scroll the sagittal slices to find the mid-slice of the corpus callosum, then place the voxel right above the superior and posterior genu of the corpus callosum with the long axis aligned with them (see Figure S1; see supplementary materials for overlap information). The voxel size was 15 × 20 × 25 mm^3.

MRS Analysis.

There is some debate regarding whether Glu can be quantified at 3 Tesla with some studies reporting the concentration of the glutamate/glutamine complex (Glx). Henry et al., (2011)

argues that the error of Glu quantified using LCModel is relatively small (coefficient of variation = 7 – 10%) for PRESS MRS with TE 30 ms at 4 Tesla, but the glutamine (Gln) is underestimated (coefficient of variation = 16 – 141%). We, therefore, report Glu concentrations in the current study (Glx results are presented in the supplementary materials). The MRS data were processed with LCModel (http://www.s-provencher.com/, version 6.2–0R) using default settings for water attenuation, estimated water concentration and baseline modeling. LCModel was used to fit each spectrum as a weighted linear combination of a basis set of in vitro spectra from individual metabolite solutions. The water reference signal was used for Eddy current correction and scaling the metabolite concentrations. The Glu concentration was expressed in institutional units. LCModel also reports an estimated relative standard deviation (%SD) for each fitted component. Subjects were excluded if their fitting results with the Cramér-Rao lower bounds (CRLB) value was less than 17% resulting in the exclusion of 5 participants.

The Glu concentrations were normalized using a method described by Gussew and colleagues (2012). This method controls for MRS signal differences in tissue composition within the measured voxel across subjects. The high-resolution structural scan acquired to position the voxel during data acquisition was used to determine the tissue composition. The T1-weighted image was segmented for gray matter, white matter, and CSF with SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). The corresponding fraction of tissue volumes in the MRS voxel was calculated and used to correct for glutamate concentration with respect to heterogeneous tissue compositions according to equation 2 in the paper by Gussew and colleagues (2012). Additional parameters for the correction included the T1 and T2 relaxation time of GM (1.82/0.10 s), WM (1.08/0.07 s), and CSF (4.16/0.50 s) (Stanisz et al., 2005; Lin et al., 2004; Piechnik et al., 2009), relative water contents in GM (0.78), WM (0.65) and CSF (1.0) (Ernst et al., 1993), and T1 and T2 of glutamate in the GM (1.27/0.16 s) and WM (1.17/0.17) (Mlynárik et al., 2001; Choi et al., 2010) respectively.

fMRI Preprocessing.

Resting-state fMRI was preprocessed similar to standard functional connectivity preprocessing (Smith *et al.*, 2013) using AFNI (http://afni.nimh.nih.gov): de-spiking, slice timing correction, motion correction, normalization to a Talairach template, within-run intensity normalization to a whole-brain mode value of 1000, removal of nuisance time series [6 motions, white matter and ventricular signals (eroded by one voxel), with their derivatives] using linear regression, temporal band-pass filtering (0.009–0.08 Hz), spatial smoothing only in the gray matter mask (6-mm full width at half maximum). A whole brain signal was not included in nuisance covariates given on-going controversy regarding its value (Liu et al., 2017; Saad et al., 2012). Motion estimates were calculated prior to preprocessing as suggested by Power et al (2017). Volumes with high motion were censored to decrease potential motion-induced bias of functional connectivity; participants with greater than 100 censored frames were removed from the analysis. We used thresholds with a frame-wise displacement (FD) of 0.5 and a percentage of BOLD signal changes over the whole brain of 0.5, above which scans (including 1 backward and 2 forward volumes) were removed (Power et al., 2012).

Functional Connectivity Analysis.

The dACC seed used during MRS acquisition was transformed to the resting-state fMRI space, Talairach space, using nonlinear transformation parameters. Due to participant anatomical variability and variability of the placement of the dACC MRS voxel, the overlapping region across participants (>75%, resulting in 63 voxels) was defined as a common seed region for the ACC (see Figure 2). RSfc was estimated using Pearson's correlation coefficient (r) from the common dACC seed to the rest of brain and converted to z-scores using Fisher's r-to-z transformation. To determine whether whole brain RSfc varied across groups a voxel-wise t-test was performed, in which multiple comparison in the group level inference was applied with the following parameters: 10,000 Monte Carlo simulation; individual voxel level threshold, $p<0.005$; individual voxel resolution, $3\times3\times3$ mm^3; and use of a gray matter mask. According to the simulations, we obtained a corrected significance level of $p<0.05$ with an extent threshold of 68 contiguous voxels (i.e., $p<0.05$, cluster-corrected). No significant group differences were observed in the whole brain analysis (see Figure S2 in supplementary materials).

Target seed regions of interest with 5-mm radius were defined using the standard Talairach atlas of AFNI, in which the Hp (±30, 24, −9) and NAc (±12, −8, −8) were used (see Figure 2B; see supplementary materials, figure S3 for ROI overlap information). The connectivity between the dACC and each target region was extracted and entered as the dependent variable in a linear regression model to determine whether its value is predicted by dACC Glu levels and monthly CB use. A two-step linear regression analysis was performed for each target ROI using SAS version 9.4. In the first step Glu concentration, monthly CB use, and drinks per week were included in the model. The second step added the interaction between CB use and Glu. All measures were mean centered. All parameter estimate tables are in the supplementary materials along with a table containing the correlations between the independent variables.

Results.

Voxel tissue composition.

The majority of the MRS voxel was composed of grey matter in both groups. An independent samples t-test was performed and the grey matter concentration did not differ between groups (p=0.75; control group 89% grey matter; user group 85% grey matter). White matter concentration was found to be different between groups with the user group having a larger concentration of white matter (p=0.04). The tissue fractions were then used to correct the concentrations as indicated by Gussew et al. (2012).

Data quality.

Independent samples t-tests were used to examine measures of data quality. No differences were found between the user and control groups in line-width (p=0.44) or SNR (p=0.63).

RSfc.

A Pearson correlation was performed between Glu and the connectivity measures collapsing across group [right NAc: r=−0.11, p=0.47; left NAc: r=−0.12, p=0.42; right Hp: r= r=−0.19,

p=0.21; left Hp: r=−0.30, p=0.041]. After Bonferroni correction for multiple comparisons, none of the correlations reached significance; however, the left Hp did show a trend.

Regression analyses were performed with the connectivity between the dACC and right NAc as the dependent variable and Glu, CB use, and drinks per week as the predictor variables. The model failed significance testing [$F(3,42)=1.81$, $p=0.16$; $R^2=0.11$; Cohen's $f^2=0.12$]. The model that included the interaction between Glu and monthly CB use using a corrected alpha of 0.025 was not significant [$F(4,41)=3.02$, *p=0.029*; $R^2=0.23$; Cohen's $f^2=0.3$]. When examining the parameter estimates, the interaction term was significant (see Table S1) indicating that the relationship between Glu and connectivity is dependent on CB use. This relationship is demonstrated in Figure 3 and S4.

The regression model with the connectivity between the dACC and the left NAc as the dependent variable and Glu, CB use, and drinks per week as the predictor variables failed significance testing [$F(3,42)=1.1$, $p=0.36$; $R^2=0.07$; Cohen's $f^2=0.075$]. The model that included the interaction between Glu and monthly CB use also failed to reach significance [$F<1$; $R^2=0.07$; Cohen's $f^2=0.075$].

The regression model that included the connectivity between the dACC and the right Hp as the dependent variable and Glu, CB use, and drinks per week as the predictor variables failed significance testing [$F<1$; $R^2=0.05$; Cohen's $f^2=0.05$]. The model that included the interaction between Glu and monthly CB use also failed to reach significance [$F<1$; $R^2=0.07$; Cohen's $f^2=0.075$].

The regression model that included the connectivity between the dACC and the left Hp as the dependent variable and Glu, CB use, and drinks per week as the predictor variable failed significance testing [$F(3,42)=1.54$, $p=0.33$; $R^2=0.1$; Cohen's $f^2=0.11$]. The model that included the interaction between Glu and monthly CB use also failed to reach significance [$F(4,41)=1.16$, $p=0.34$; $R^2=0.1$; Cohen's $f^2=0.11$]. Interestingly, while not significant at a corrected alpha of 0.0125, there is a suggestion that Glu predicts the connectivity between the dACC and left Hp (based on the t-test for Glu in both models, p's=0.05) and that the relationship is not impacted by CB use (see Tables S4 and S8 in supplementary materials).

Discussion

This multimodal MRI study was designed to examine whether chronic CB use interacts with the relationship between Glu and the resting state functional connectivity between the dACC and two core nodes within the reward network, the NAc and Hp. The current findings will be discussed with some caution as the study may suffer from low statistical power potentially due to the large variance in CB usage in the user group. Although definitive conclusions are not drawn from the study the results do demonstrate important trends that should direct future work. For example, the results suggest that the relationship between Glu and the connectivity between the dACC and the NAc is impacted by CB use, while connectivity between the dACC and Hp is not.

The current results suggests that the relationship between Glu and dACC/NAc connectivity depends on CB use. It should be noted that the interaction between dACC Glu and CB use

was only observed for the connectivity between the dACC and the right NAc, not the left. There are at least two potential explanations for this laterality difference. One being that the study is underpowered. Given the low effect size for the dACC/left NAc connectivity, this is a distinct possibility. Another possible explanation is related to laterality differences. NAc dopamine transportor, D1-receptor and D2/3-receptor binding and DA synthesis capacity have all been observed with higher levels found in the right compared to the left (Hietala et al., 1999; Laakso et al., 2000; van Dyck et al., 2002; Vernaleken et al., 2007; Cannon et al., 2009). Additionally, it was shown by Martin-Soelch et al. (2011) that dopamine release elicited by an unexpected monetary reward was also lateralized to the right NAc. Oberlin et al (2015) also reported NAc laterality differences during a pseudo self-administration task that separately administered a flavor conditioned stimulus of either a habitually consumed beer or an appetitive control drink concomitant with the unconditioned stimulus of ethanol intoxication or saline. They found that the right NAc responded to the conditioned stimulus (flavor) while the left responded to the unconditioned stimulus (intoxication). The results implied that the left and right NAc process different information with the left associated with salient changes to interoceptive, internal states (e.g., nausea or dizziness due to intoxication) while the right is associated with salient exteroceptive, appetitive external stimulation (e.g., flavor). These two studies suggest that the right NAc responds to external rewards. The glutamatergic connection between the ACC and NAc is thought to be responsible for drug seeking behavior and is responsible for cue-induced craving (see Kalivas & Volkow, 2005 for review). The previous studies support the hypothesis that the dACC Glu interaction with dACC/R NAc is due to CB use having a greater impact on external reward processing opposed to the negative interoceptive, internal states being processed by the left NAc. Further studies with larger sample sizes as well as samples that include subjects with CB dependence are necessary to more adequately assess this hypothesis.

A relationship between Glu levels in the dACC and connectivity between the dACC and Hp was predicted based on previous research. mPFC and the Hp have been found in both animal models and humans to be connected (see Godsil et al., 2013 for review). Both the mPFC and hippocampus are members of the default-mode network and both have been implicated in cognitive reappraisal and emotion regulation (Godsil et al., 2013). The mPFC-hippocampus pathway has also been linked to symptoms of psychiatric disorders like depression, schizophrenia and anxiety disorders (Godsil et al., 2013). Interestingly, these disorders have all been associated with heavy CB use. However, the correlation between dACC Glu and connectivity with the hippocampus was not found to be significant in the current study. Nontheless, given the individuals with psychological disorders were removed from analysis future studies examining how psychiatric symptoms may contribute to this Glu/RSfc relationship are warranted.

Limitations:

As mentioned above, the results presented should be interpreted with some caution. The number of participants, while larger than some previous studies, is rather small. Also, the variance in the amount of CB consumed is quite large with only a few participants having very high use. This may result in increased variance in our measures and the appearance of outliers at the high use end. Currently there is no consistency across studies regarding the

CB use criterion within the chronic CB user group (e.g., 10 uses in past 12 months – Wright et al., 2016 – to 5 times a week in the past 12 months – Muetzal et al., 2013). Also, the current study did not use CB dependence as an inclusion criteria for the CB group. This likely contributed to the variance in our population. We argue for future studies that have a much larger sample that adequately samples a large range of CB use to better characterize how CB dose impacts Glu and the relationship between Glu and connectivity. A second limitation is the inability to properly control dose. The concentration of THC being consumed is not controlled in human studies as in preclinical studies. Together the inconsistency across studies and the inability to control THC consumption makes comparisons across human studies difficult.

MRS is a non-invasive technique that allows for the measurement of a number of molecules including Glu. MRS technology has advanced to the point that neurometabolites can be reliably measured in humans making it a powerful tool in the study of addiction, the metabolite levels measured by MRS include both intracellular and extracellular components. This is different from methods used in preclinical studies; microlysis in animal studies primarily measure extracellular concentrations. Because the extracellular concentrations of Glu are tightly controlled due to its potential toxic effects not being able to measure extracellular Glu specifically is a third limitation. This difference in measures makes the direct comparison to the animal literature difficult.

Conclusions

The results presented support previous findings and make suggestions for future research. The effect of CB use on the relationship between dACC Glu levels and its connectivity may be dependent upon the target region and therefore is not a general effect but a specific one. The current results show that CB use does not interact with the relationship between dACC Glu levels and connectivity with the hippocampus but provide some suggestion that it does interact with its connectivity with the nucleus accumbens. This is important because it may 1) extend the preclinical work showing that the dACC and NAc connection is important in substance abuse and 2) suggest that the relationship between Glu and RSfc may have some utility in characterizing the impact of CB use on specific brain systems. The work presented is a first step and studies with a larger sample that targets a range of monthly use is necessary to first confirm that CB has an impact on RSfc and then to uncover the mechanism responsible for these relationships.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

This study was supported by the National Institute on Drug Abuse (NIDA) Grant #5R21DA035493 (BFO/SDN), the National Institute of Mental Health (NIMH) Grant #2R01MH074983 (WPH), a National Science Foundation Graduate Research Fellowship Grant #1342962 (AMSM) as well as a NIDA T32 Predoctoral Fellowship Grant #T32DA024628 (AMSM). Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NIDA, NIMH, or the National Science Foundation.

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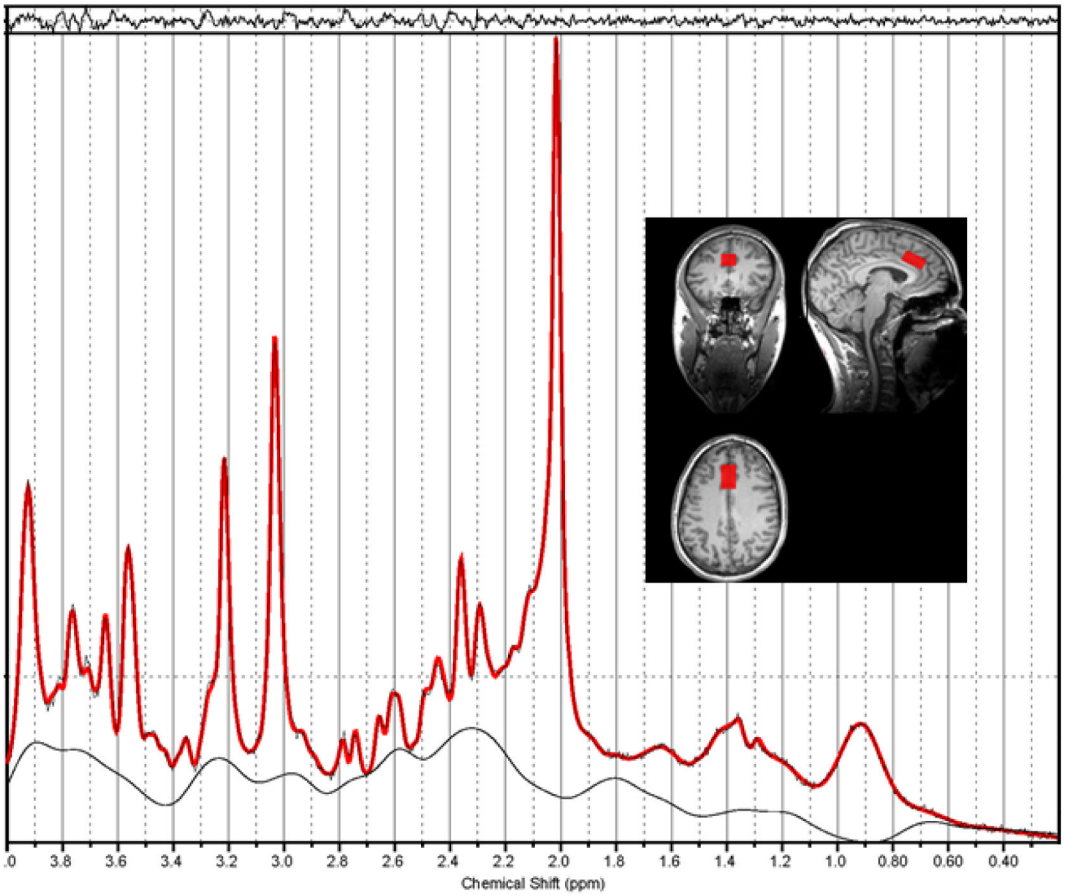

Figure 1:

An example of the location of the voxel for MRS in the dorsal anterior cingulate (left) along with the resultant spectra processed by LCModel (right). The fitted spectrum (red) is superimposed on the original spectrum (black); the residual of fitting is on the top while the baseline is at the bottom. The linewidth is 0.033 ppm.

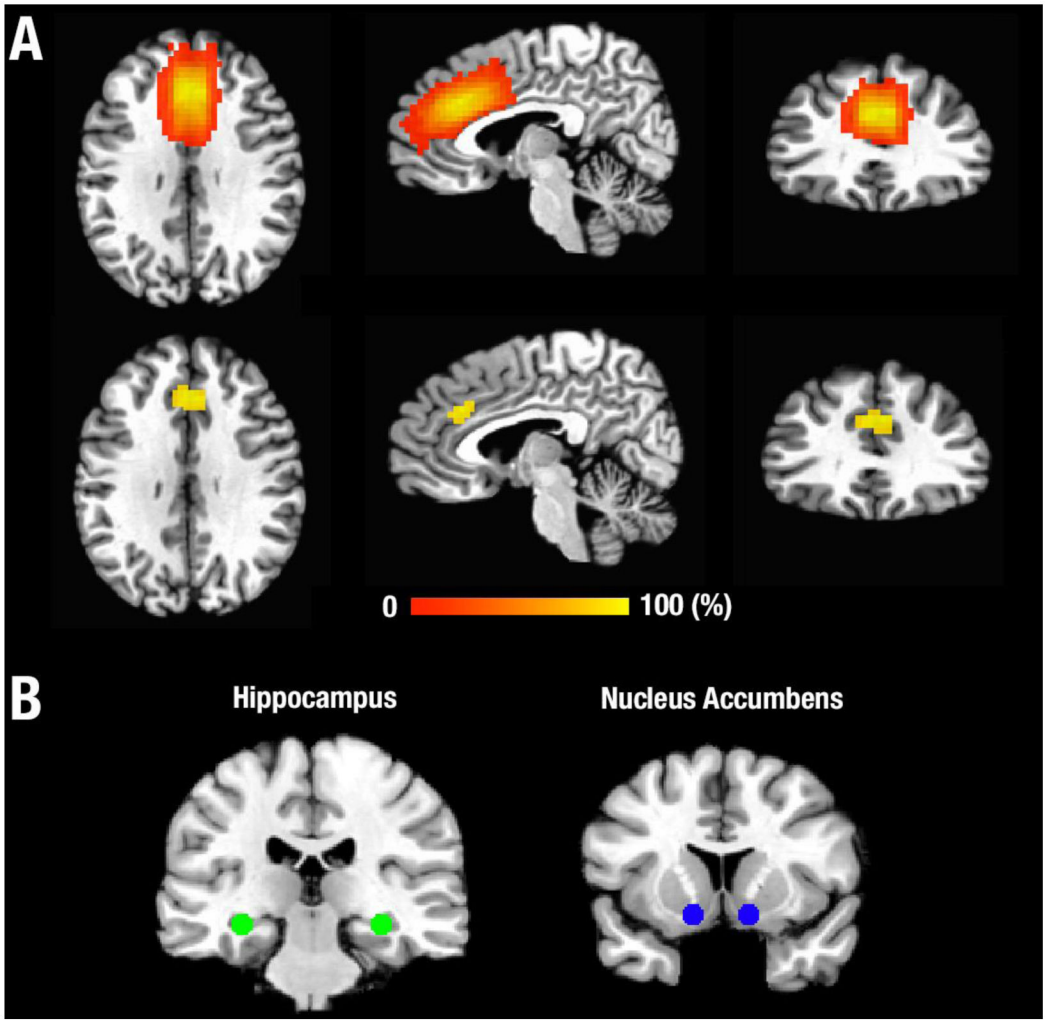

Figure 2.

Regions of interest for resting-state functional connectivity (rsFC). (A) *Upper*. Probability map of the dorsal anterior cingulate cortex (dACC) from each individual. *Lower*. Highly overlapped region (= 63 voxels) with >75% of individuals (= 40 subjects) as a seed region for FC computation. (B) Target seed spheres with 5-mm radius – hippocampus (green color; ±30, 24, −9 at Talairach atlas of AFNI) and nucleus accumbens (blue; ±12, −8, −8).

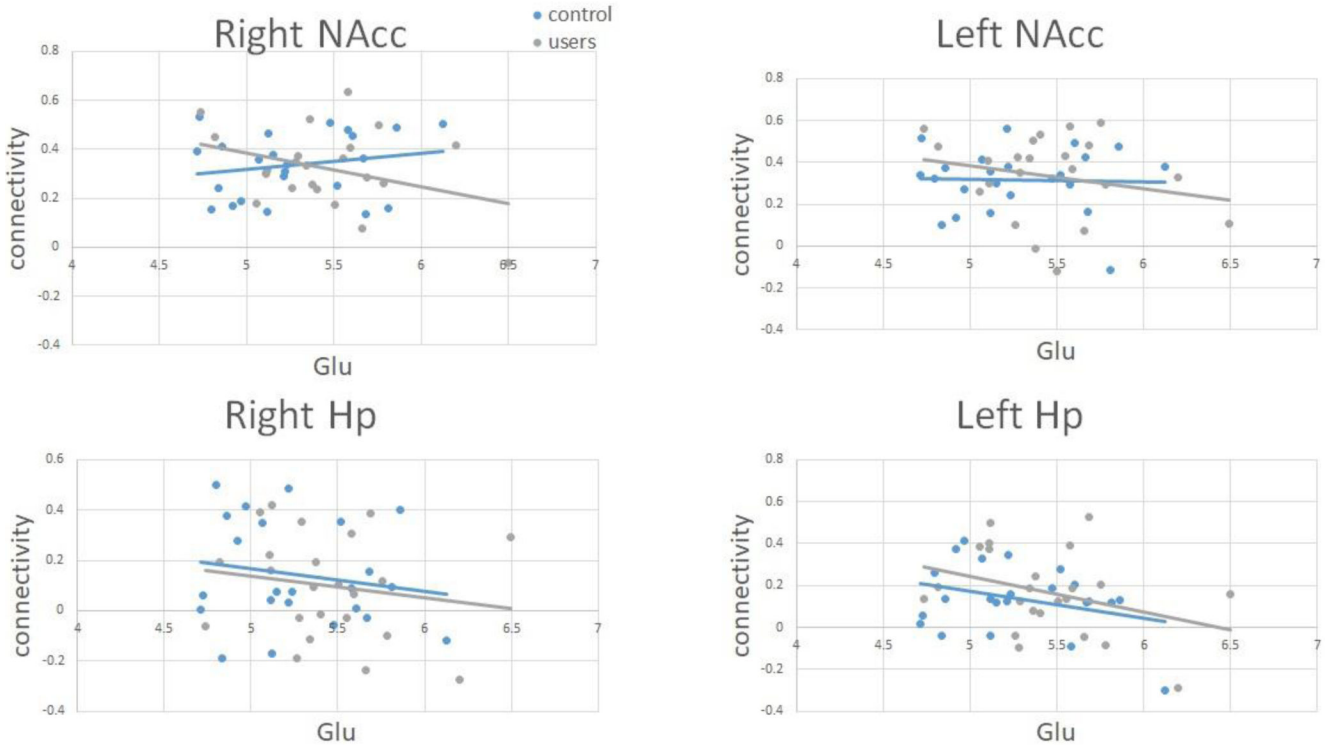

Figure 3.

Relationship between the resting-state functional connectivity (rsFC) and glutamate (Glu) at the dorsal anterior cingulate cortex (dACC. Blue denotes control participants and grey denotes users.

Table 1:

Demographics

	Controls	CB Users
n	23	23
#males	9	9
Age	21.4±2.3	20.9±2.8
Age of CB initiation	*	16.3±2.4 years
Average monthly CB use	0	30.2±24.5 instances/month
Lifetime CB use (instances)	0.55±1.3	2097.6±5229.2
Average days since last CB use (prior to scan)	*	1.4±1.3 days
Average days since last alcohol use (prior to scan)	138.9±403.2	13.6±38.2
Average drinks per week	2.3±2.9	3.7±3.2
WASI	113.7±11.1	111.1±8.7

* 5 controls have used previously