Gabapentin for Restoring GABA/glutamate Homeostasis in Co-occurring Bipolar and Cannabis Use Disorders: A Randomized, Double-blind, Placebo-controlled, Parallel-group, MRI Study

PI: James J. Prisciandaro, PhD

Protocol

3/30/2023

Specific Aims

There is an 8-fold increase in the prevalence of cannabis use disorder (CUD) in individuals with bipolar disorder (BD) relative to the general population, and individuals with co-occurring BD and CUD (BD+CUD) have substantially worse clinical outcomes (e.g., elevated rates of disability, hospitalization, and suicide) than those with either BD or CUD alone. Though it remains unclear why BD and CUD co-occur at such high rates, this phenomenon may, in part, reflect BD individuals' attempts to self-medicate persistent residual depressive and anxious symptoms with cannabis. These symptoms are particularly difficult to treat, as conventional anxiolytic and antidepressant medications are not approved and potentially dangerous in BD due to well-established safety concerns. Making things worse, response to mood-stabilizing medications that *are* indicated to treat BD is poor in individuals with BD+CUD, yet little is known about optimal treatment as there have been no randomized controlled trials (RCTs) for BD+CUD to date. Convergent evidence supports disrupted brain gamma-Aminobutyric acid (GABA)/glutamate homeostasis as a promising target for pharmacological intervention in BD+CUD, with BD potentially acting like a "multiplier" to the impact of CUD on lowering brain glutamate levels, driving them further down relative to CUD alone. Gabapentin, a safe and well-tolerated medication FDA-approved to treat other neurological diseases, has been shown to restore GABA/glutamate homeostasis in animal and human studies, with treatment studies demonstrating efficacy in treating CUD, as well as anxiety and sleep disorders that are common and impairing to both BD and CUD.

It was against this background that the study team completed an NIH/NIDA-funded (R21DA043917) preliminary, randomized, double-blind, placebo-controlled, crossover, MRI (i.e., proton magnetic resonance spectroscopy [^{1}H-MRS], functional MRI [fMRI]) study of gabapentin (1200mg/day) in BD+CUD (n = 22) which found, a) gabapentin was well-tolerated, with participants reporting fewer AEs vs. placebo and demonstrating excellent adherence to both gabapentin (≥ 94%) and study requirements (e.g., 95% completed the study), b) gabapentin increased dorsal anterior cingulate cortex (dACC) and right basal ganglia (rBG) glutamate levels, c) relative elevations of rBG glutamate levels in gabapentin-treated participants were associated with lower cannabis use, d) relative elevations of dACC GABA levels in gabapentin-treated participants were associated with lower manic/mixed and depressive symptoms, and e) gabapentin increased activation to visual cannabis cues in the posterior midcingulate (pMCC) gyrus, a region central to the recruitment of attentional-control circuitry for quick behavioral responses to stimuli, which was in turn associated with increased rBG glutamate and GABA levels, as well as lower cannabis use. Though promising in their support for gabapentin for BD+CUD, these findings should be interpreted with caution due to the study's small sample size, observed randomization order effects (e.g., on dACC glutamate levels), and post-hoc identification of statistical moderators (cigarette-smoking status, anxiety disorder), in part due to a failure of simple randomization to balance treatment orders on baseline characteristics. Further, effects of gabapentin on brain GABA levels were not as robust as anticipated.

The proposed randomized, placebo-controlled, double-blind, parallel-group, MRI study aims to overcome the limitations of our preliminary study via, a) parallel-group study design, b) larger sample size (n = 68 vs. 22), c) urn-randomization to treatment condition (e.g., by cigarette-smoking status), and d) higher dosing of gabapentin (1800mg/day) delivered over a longer period (17 days vs. 5 days/condition) to increase the likelihood of observing gabapentin effects on brain GABA levels (with both changes supported by the excellent tolerability, adherence, and retention found in our preliminary study).

With the proposed improved study design in place, the proposed study will evaluate the following hypotheses:
Hypothesis 1 (^{1}H-MRS)**:** Gabapentin will increase dACC and rBG glutamate and GABA levels, relative to placebo, in individuals with BD+CUD.
Hypothesis 2 (fMRI)**:** Gabapentin-related increases in dACC and rBG glutamate and GABA levels will be associated with changes in brain activity to visual cannabis cues consistent with reduced cannabis craving/use.
Exploratory Hypotheses: 1) Associations of gabapentin-induced changes in dACC and rBG glutamate and GABA levels with changes in brain activity to cannabis cues, mood symptoms (including anxiety and sleep), and cannabis craving and use will be explored. 2) Cigarette-smoking status and anxiety disorder diagnosis will moderate effects of gabapentin on dACC and rBG glutamate and GABA levels, as well as on brain activation to cannabis cues.

The proposed study provides the next logical step, bridging our NIH/NIDA-funded preliminary study to larger, more-costly, randomized controlled efficacy trials of adjuvant gabapentin for BD+CUD. The proposed study may also provide successful demonstration of a multimodal neuroimaging platform for evaluating the promise of glutamatergic/GABAergic drugs (e.g., the mGlur5 negative allosteric modulator, GET73) for BD+CUD and other conditions marked by GABA/glutamate dysfunction. Finally, the proposed study will add to the literature on associations between regional brain GABA/glutamate levels and constructs related to BD and CUD, including cannabis cue reactivity, cannabis craving and use, and mood and anxiety symptomatology.

A. SIGNIFICANCE

A.1. Overview. There is an 8-fold increase in the prevalence of cannabis use disorder (CUD) in individuals with bipolar disorder (BD; Pinto, 2019) relative to the general population (Hasin, 2018), and co-occurring BD and CUD (BD+CUD; relative to BD alone) is associated with more frequent mood cycling, mixed manic and depressive symptoms, poorer quality of life, elevated risk of cigarette-smoking and psychosis, and greater rates of disability, hospitalization, and suicide (Lev-Ran, 2013; Weinstock, 2016; Bartoli, 2019; Pinto, 2019), even in individuals receiving state-of-the-art pharmacotherapy (Kvitland, 2015). Although it remains unclear why BD and CUD co-occur at such high rates, this phenomenon may partly reflect BD individuals' attempts to self-medicate impairing depressive and anxious symptoms that persist between mood episodes with cannabis (Judd, 2002; 2003; Farris, 2016; Sarvet, 2018). These symptoms are particularly difficult to treat, because conventional anxiolytic and antidepressant medications are not approved and are potentially dangerous in BD due to well-established safety concerns (Ghaemi, 2008; Bobo, 2015). Unfortunately, response to mood-stabilizing medications that *are* approved to treat BD is poor in individuals with BD+CUD (van Rossum, 2009; Kim, 2015), due in part to poor treatment adherence (Bally, 2014). Despite the dire need for safe and efficacious treatments for BD+CUD, little is known about optimal treatment, as there have been no published randomized controlled trials (RCTs) in this population. Convergent evidence supports disrupted brain gamma-Aminobutyric acid (GABA)/glutamate homeostasis as a promising target for pharmacological intervention, and gabapentin as a candidate adjuvant medication to normalize frontal and striatal brain GABA and glutamate levels, in BD+CUD.

A.2. GABA/glutamate Dysregulation in CUD and BD. GABA and glutamate (the main inhibitory and excitatory neurotransmitters in mammals, respectively) are principally involved in the coordination of cortical activity, synaptic plasticity, and modulation of most other neurotransmitter systems (Hassel, 2006; Olsen, 2006); levels of GABA and glutamate are tightly coupled via the glutamate/GABA-glutamine metabolic cycle (Bak, 2006). Dysregulated GABA/glutamate transmission is central to both CUD and BD (Cohen, 2019; Gigante, 2012).

The reorganization of reward circuitry in CUD to preferentially respond to cannabis cues, manifesting clinically as drug craving and seeking, is due to cannabis-induced neuroplasticity mediated by glutamate and GABA (Kalivas, 2009; Koob; 2016). Delta-9-tetrahydrocannabinol (Δ9-THC, the psychoactive component of cannabis) activates pre-synaptic cannabinoid type-1 (CB_1) receptors that are densely distributed in frontal and striatal brain regions, a) facilitating release, and reducing astrocytic uptake, of glutamate resulting in accumulated extracellular glutamate, and b) inhibiting release of GABA resulting in disinhibition of downstream (mesolimbic) dopaminergic cells critical to the development and maintenance of CUD (Cohen, 2019). Repeated administration of Δ9-THC induces down-regulation and internalization of CB_1 and glutamate receptors, and suppresses activity of glutamic acid decarboxylase and glutamine synthetase, resulting in reduced synaptic glutamate and GABA transmission (Colizzi, 2016). Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) and cannabis-cue functional MRI (fMRI) provide the opportunity to use MRI to better understand these issues in humans. Consistent with preclinical findings, acute administration of Δ9-THC significantly increased glutamate levels in the left caudate head (striatum) of healthy volunteers, with lower baseline glutamate levels and greater prior cannabis exposure associated with greater Δ9-THC-induced increases in striatal glutamate (Colizzi, 2019). Chronic cannabis use and CUD are, in turn, associated with, a) decreased anterior cingulate cortex (ACC; Prescot, 2011; 2013) and right basal ganglia (rBG; Chang, 2006; Muetzel, 2013) glutamate levels, b) decreased ACC GABA levels (Prescot, 2013), and c) heightened functional activation to cannabis cues (e.g., cannabis plant, paraphernalia) in key brain regions underlying reward, attention, motivation, and goal-directed behavior (e.g., medial prefrontal cortex [mPFC], striatum, ACC; Cousijn, 2013; Karoly, 2019).

In contrast, ^{1}H-MRS studies of BD have consistently demonstrated *elevated* ACC and PFC glutamate levels across mood states and medication regimens (Gigante, 2012). Investigations of GABA using the gold-standard MEGA-PRESS acquisition technique have consistently found abnormal ACC and occipital cortex GABA concentrations in BD (Bhagwagar, 2007; Brady, 2013; Wang, 2006), though the direction of GABA disturbance has not been consistent. Additional evidence for GABA/glutamate disturbances in BD is provided by studies finding links between genes coding for glutamate receptor subunits (Le-Niculescu, 2009; de Sousa, 2017) and glutamate transporters (Veldic, 2019), BD, and lithium response (Perlis, 2009), along with reduced levels of cerebrospinal fluid (CSF) and plasma GABA (Gerner, 1984; Petty, 1990), reduced expression of GABA receptor subunits (Fatemi, 2017), and differences in GABA receptor genes (Horiuchi, 2004; Massat, 2002; Otani, 2005).

Although there have been no published studies of brain glutamate and GABA levels in BD+CUD relative to BD, CUD, or healthy volunteers, CUD has been associated with reduced mPFC glutamate levels in individuals with early psychosis (including those with BD and schizoaffective disorder, bipolar type; Rigucci, 2018). We similarly demonstrated that individuals with co-occurring BD and alcohol use disorder (AUD; with and without co-occurring substance use disorders [SUD], including CUD) had significantly lower ACC levels of both GABA

and glutamate relative to individuals with BD alone, AUD alone, or healthy volunteers, and that lower ACC GABA levels were associated with elevated alcohol craving and impulsivity (Prisciandaro, 2017). Together, these studies suggest that even though BD (in the absence of CUD) is associated with *elevated* brain glutamate levels (Gigante, 2012), BD appears to act like a "multiplier" to the impact of CUD on lowering glutamate levels, driving them further down relative to CUD alone (Prisciandaro, 2017; Rigucci, 2018).

A.3. Gabapentin for Restoring GABA/glutamate Homeostasis in CUD and BD. Gabapentin, a safe and well-tolerated medication that is FDA-approved to treat post-herpetic neuralgia, partial seizures, and restless-leg syndrome, holds promise as an adjuvant medication for normalizing frontal and striatal brain GABA and glutamate levels, and thereby providing symptom relief, in individuals with BD+CUD.

Gabapentin modulates GABA and glutamate transmission via selective blockade of presynaptic voltage-gated calcium channels that contain the α2δ-1 subunit (Sills, 2006). Recently, additional GABAergic and glutamatergic mechanisms of gabapentin have been identified, including activation of potassium channels (Manville, 2018), increased expression of postsynaptic δ-subunit-containing $GABA_A$ receptors (Yu, 2019), and reduced spontaneous synaptic glutamate release that is dependent on α2δ-1-linked presynaptic N-Methyl-d-aspartate (NMDA) receptors (Taylor, 2020). ^{1}H-MRS studies in healthy volunteers and adults with epilepsy have consistently demonstrated that three GABAergic medications increase brain GABA levels in vivo: gabapentin, topiramate, and vigabatrin (van Veenendaal, 2015). While topiramate and vigabatrin are associated with significant side effects, including cognitive dysfunction (Goldberg, 2001), gabapentin is well-tolerated and safe (Molero, 2019); these considerations are particularly important for BD+CUD individuals who demonstrate high baseline levels of cognitive dysfunction along with complex medication regimens. Although preclinical studies have consistently demonstrated decreased glutamate release with gabapentin administration, the only published ^{1}H-MRS gabapentin study to measure glutamate found that a relatively-low acute dose of gabapentin (i.e., 900mg) was not associated with increased glutamate levels (Cai, 2012). In contrast, gabapentin *has* been shown to significantly increase occipital GABA levels 1-6 hours following a single dose (900-1200mg) in healthy volunteers (Cai, 2012; Kuzniecky, 2002) and epileptics (Petroff, 2000). Longer-term gabapentin dosing (i.e., ≥ 2-week) has also been shown to significantly increase occipital GABA in controls (2400mg/day; Kuzniecky, 2002) and epileptics (1200-3600mg/day; Petroff, 1996; 2000) in a dose-dependent manner. Taken together, most studies reported average GABA increases of 25-50%, with lower baseline GABA levels and relatively-higher doses of gabapentin associated with the largest increases in GABA (Cai, 2012; Petroff, 2000).

By way of these GABAergic and glutamatergic mechanisms, gabapentin has been shown to reduce cannabis use and withdrawal symptoms in cannabis-dependent adults (Mason, 2012), earning it the distinction of one of few "promising" medications "warranting further research" for the treatment of CUD in a recent Cochrane Systematic Review (Nielsen, 2019). Gabapentin substitutes for Δ9-THC discriminative stimuli in cannabis users, suggesting that it may reduce cannabis use, in part, by producing interoceptive effects that are similar to, and therefore able to replace those of, Δ9-THC (Lile, 2016). The gabapentinoid, pregabalin blocks motor signs and anxiety behaviors associated with cannabis withdrawal in animal research (Aracil-Fernandez, 2013). Preclinical studies of gabapentin that have focused on other substances of abuse (e.g., ethanol, cocaine) have demonstrated decreased, a) self-administration (de Guglielmo, 2013; Roberto, 2008), b) stressor/cue-induced reinstatement (de Guglielmo, 2013), c) expression/development of stimulant sensitization (Filip, 2006; Kurokawa, 2011), d) drug-induced place preference (Kurokawa, 2011), and e) anxiogenic effects of withdrawal (Roberto, 2008). Effects of gabapentin in these studies appear to be mediated, in part, by normalization of GABAergic transmission in central amygdala and elevation of α2δ-1 subunit of voltage-gated calcium channels (Kurokawa, 2011; Roberto, 2008). The efficacy of gabapentin may be moderated by variation in $GABA_A$ receptor subunits α 1 and 3, along with genetic variation in genes that code for NMDA and AMPA receptors, as these receptors appear to be impacted by gabapentin treatment (Rose, 2002; Patel, 2016); many of these same genetic variations have been shown to differentiate individuals with and without mood disorders (Brambilla, 2003).

Gabapentin may also reduce cannabis use indirectly, by providing relief to symptoms of anxiety and sleep disturbance that appear to drive persistent cannabis use in BD+CUD (Farris, 2016; Sarvet, 2018). In individuals without BD, gabapentin has demonstrated efficacy in treating most anxiety disorders (AD), including social AD, obsessive-compulsive disorder, and general AD (Greenblatt, 2018), which are particularly prevalent and impairing in individuals with BD+SUD (Prisciandaro, 2011, 2019). Gabapentin also efficaciously treats sleep disturbances that occur in the context of medical illness (Liu, 2017), important because sleep disturbance is a central symptom of, and potential trigger of, BD mood episodes (Pancheri, 2019), as well as an impairing symptom of cannabis withdrawal (Gates, 2016). Similarly, though RCTs in treatment-refractory individuals with BD alone failed to support the efficacy of gabapentin for resolving acute mood episodes (c.f., Vieta, 2006), a long history of positive reports from open-label studies in BD support the off-label use of gabapentin for patients

suffering from anxiety and sleep disturbance (Houghton, 2017). In sum, gabapentin has been shown to restore GABA/glutamate homeostasis, with treatment studies demonstrating efficacy in treating CUD, as well as anxiety and sleep disorders that are common and impairing to both BD and CUD.

A.4. Preliminary Data Supporting Gabapentin for Restoring GABA/glutamate Levels in BD+CUD.

We recently completed a NIDA-funded (DA043917), randomized, double-blind, placebo-controlled, cross-over (1-week/ condition), MRI study of gabapentin (1200mg/day) in BD+CUD that evaluated the following hypotheses: 1) gabapentin will increase dorsal ACC (dACC) and rBG GABA and glutamate levels, relative to placebo (using ^{1}H-MRS), and 2) gabapentin will decrease functional brain activation to visual cannabis cues relative to matched neutral cues (fMRI). Please see *C. RESEARCH PLAN* below for details concerning ^{1}H-MRS and fMRI acquisition (*C.3.b*) and analysis (*C.4.b*) methods, which are the same between the preliminary and proposed studies.

Table 1

Baseline Participant Characteristics by Randomization Order

	Randomization Order #1 (n = 11)	Randomization Order #2 (n = 11)	p
Age (in years), *M* (*SD*)	38.55(11.41)	36.63(12.86)	0.717
Sex, *n* (%) female	5(45.5%)	6(54.5%)	0.670
Smoking status, *n* (%)	4(36.4%)	9(81.8%)	0.080†
Anxiety Disorder, *n* (%)	0(0.0%)	7(63.6%)	0.004*
BD subtype, *n* (%) I	6(54.5%)	6(54.5%)	1.000
YMRS, *M* (*SD*)	2.91(3.33)	5.09(4.41)	0.206
MADRS, *M* (*SD*)	5.36(4.43)	11.18(9.56)	0.082†
Cannabis Withdrawal Scale, *M* (*SD*)	36.45(37.37)	37.00(29.81)	0.970
Marijuana Craving Quest., *M* (*SD*)	36.18(15.38)	38.64(19.29)	0.745
Cannabis use (grams/day), *M* (*SD*)	0.82(0.89)	4.52(3.72)	0.007*
Medication, *n* (%)			
Lithium	2(18.2%)	2(18.2%)	1.000
Antipsychotic	8(72.7%)	10(90.9%)	0.586
Anticonvulsant	4(36.4%)	5(45.5%)	1.000
Antidepressant	4(36.4%)	5(45.5%)	1.000

χ^2 tests (categorical variables), using Fisher's Exact when applicable, and independent samples t-tests (continuous variables) were performed. BD = bipolar disorder; YMRS = Young Mania Rating Scale; MADRS = Montgomery-Asberg Depression Rating Scale; Quest. = Questionnaire. †p < 0.10; *p < 0.01.

Twenty-two individuals with BD+CUD were enrolled over approximately 18 months (see *Table 1* for participant characteristics); twenty-one (i.e., 95.5%) of these individuals completed the study. Medication adherence, determined via pill counts, was ≥ 94%. Gabapentin was very well tolerated, with placebo-treated participants reporting more AEs than gabapentin-treated participants (11 vs. 7 adverse events; AEs). Evaluation of baseline participant characteristics by Randomization Order revealed that participants in Order #2 had higher prevalence of AD (p < 0.01) and cigarette-smoking (defined as ≥ 10 cigarettes/day, Lipari, 2013; p = 0.08), along with elevated Montgomery-Asberg Depression Rating Scale (MADRS; Montgomery, 1979) scores (p = 0.08) and cannabis use (in the 90-days preceding baseline) (p < 0.01) relative to participants in Order #1 (*Table 1*). As a result, these baseline variables were tested as potential moderators of associations between treatment condition and MRI variables of interest. Unfortunately, condition orders were so unbalanced by AD, with Order #2 containing 100% of diagnosed individuals, that evaluating this variable as a moderator was not possible; though, AD was associated with, and may have been responsible for, the elevated mood symptoms (ps = 0.07-0.09) and cannabis use (p = 0.05) observed in Order #2. Age, sex, and psychiatric medications (both overall and by class) were also evaluated as potential moderators, but none were statistically significant (ps > 0.20). Statistical (i.e., mixed) models accounted for the potential effects of condition order (i.e., Order #1 – gabapentin first vs. Order #2 – placebo first) via the interaction between treatment condition (gabapentin vs. placebo) and scan number (1st scan vs. 2nd scan; Liu, 2016). Probability values provided within Figures were derived from post-hoc, within-group testing. Findings are summarized below:

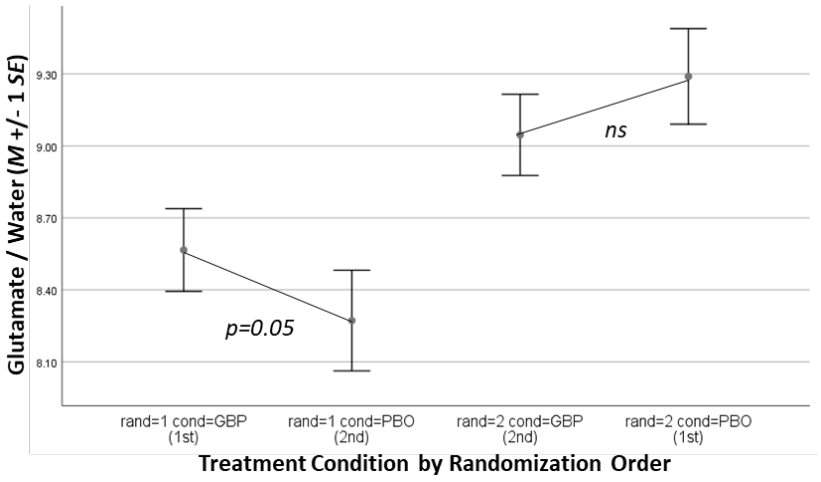

Figure 1. dACC glutamate levels by treatment condition (GBP=gabapentin, PBO = placebo) and randomization order (rand).

1) Gabapentin *increased* dACC glutamate levels, but only in *Order #1* (*Figure 1*). A significant interaction of treatment condition with scan number was found for dACC glutamate levels (p < 0.01).
2) Gabapentin *increased* rBG glutamate levels, but only in *cigarette-smoking participants (Figure 2)*. A

significant interaction between treatment condition and cigarette smoking status was found (p = 0.05). This interaction may be due to the substantially lower glutamate levels observed in placebo-treated non-cigarette-smoking participants (n = 7) relative to placebo-treated cigarette-smoking participants (n = 11; p = 0.13, Cohen's d = 0.75). Relative *elevations* of rBG glutamate levels in gabapentin-treated participants were associated with *lower* cannabis use during the study, but only in *Order #2* (i.e., there was a 3-way interaction between treatment condition, scan number, and cannabis use, p = 0.02).

3) Gabapentin failed to increase brain GABA levels across participants, as there was no significant main effect of treatment condition, nor interaction of condition with scan number, found for dACC or rBG GABA levels. However, relative *elevations* of dACC GABA in gabapentin-treated participants were associated with *lower* manic/mixed symptom scores during the study, and vice versa (*Figure 3*). A significant interaction of treatment condition with Young Mania Rating Scale (YMRS; Young, 1978) scores (p < 0.01) was found. Relative *elevations* of dACC GABA levels in gabapentin-treated participants were also associated with *lower* depressive symptom scores during the study, but only in *Order #2* (i.e., there was a 3-way interaction between condition, scan number, and MADRS, p = 0.03).

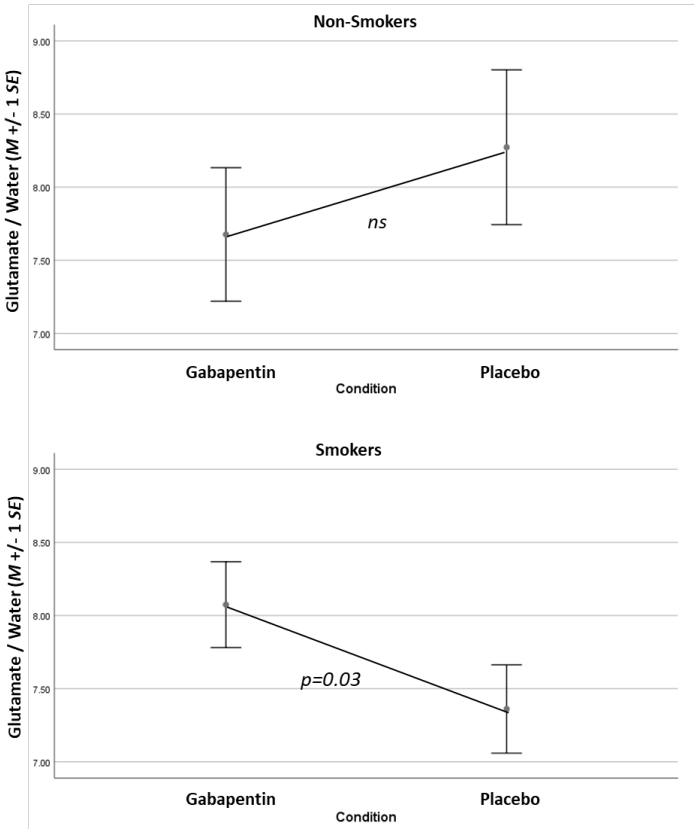

Figure 2. rBG glutamate levels by treatment condition by smoking status (top = non-cigarette-smokers, bottom = cigarette-smokers).

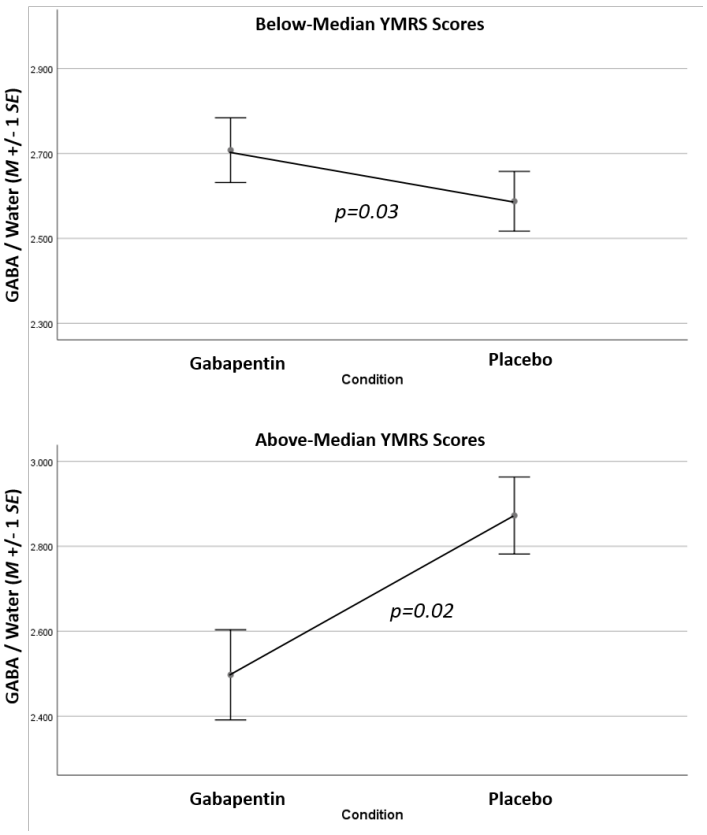

Figure 3. dACC GABA levels by treatment condition by YMRS scores during the study (top = < median YMRS, bottom = > median YMRS).

4) Gabapentin *increased* activation to visual cannabis cues in the posterior midcingulate (pMCC) gyrus, but only in *cigarette-smoking participants* (*Figure 4*). There was a significant interaction between treatment condition with cigarette smoking status in pMCC (z > 2.58, FWE-corrected p < 0.05). *Higher* pMCC activation to cannabis cues was, in turn, significantly assocaited with *lower* self-reported cannabis use during the study (r = -0.38, p = 0.03). This observed cluster has not been shown to be part of the general cannabis cue activation network by this study (i.e., in placebo-treated participants, *Figure 8b* below) or others (Karoly, 2019). However, given that the pMCC is central to recruitment of attentional control circuitry to guide body orientation and reflexive movements in response to sensory stimuli, pMCC activation could be critical to an individual's motor response to drug cues in the environment (Vogt, 2016). Since gabapentin was also found to increase rBG glutamate levels in cigarette-smoking participants, we evaluated the correlation of pMCC activation to cannabis cues with rBG glutamate and GABA levels by

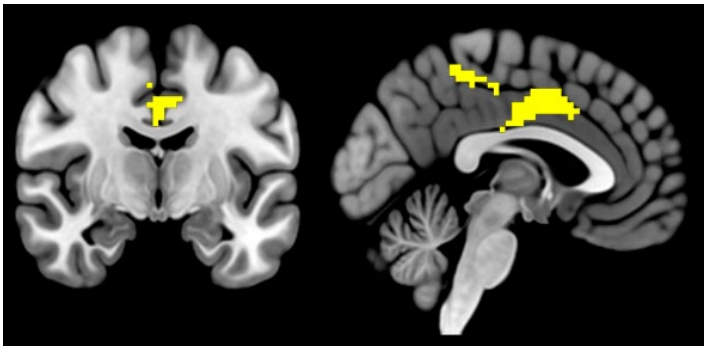

Figure 4. Posterior midcingulate cluster in which gabapentin increased activation to cannabis cues, but only in cigarette-smoking participants ($z > 2.58$, FWE < 0.05, k = 640 voxels, center [x,y,z] = 1.9, -16.6, 42.2).

cigarette-smoking status, and found a *positive association* of pMCC cue activation with both rBG glutamate ($r = 0.44$, $p = 0.08$) and GABA ($r = 0.66$, $p < 0.01$) levels in cigarette-smoking, but not in non-cigarette-smoking ($ps > 0.20$), participants.

In summary, results from this preliminary study demonstrate that, a) gabapentin increased rBG glutamate levels and pMCC activation to cannabis cues in cigarette-smoking individuals with BD+CUD, b) gabapentin increased dACC glutamate levels (depending on randomization order), and c) elevated glutamate and GABA levels in gabapentin-treated participants were associated with decreased cannabis use (depending on randomization order) and mood symptoms, respectively. These results add support to gabapentin as a candidate adjuvant medication to normalize brain GABA/glutamate levels in BD+CUD that deserves further investigation.

Though promising, these findings must be interpreted with caution due to the study's small sample size, observed randomization-order effects, and post-hoc identification of statistical moderators (e.g., cigarette-smoking status). Although order effects may have genuinely reflected the effect of receiving gabapentin 1st versus 2nd on study outcomes, they more likely reflected the failure of simple randomization to balance condition orders on highly-impactful baseline characteristics. Cigarette-smoking status and AD diagnosis were identified post-hoc as statistical moderators, however their impact on gabapentin-induced changes in brain GABA/glutamate is perhaps unsurprising given that both have been reliably associated with, a) disturbances in GABA and glutamate transmission that are believed to be central to their phenomenology (Bandelow, 2017; Alasmari, 2016), and b) worse clinical outcomes in individuals with BD+CUD relative to those who do not smoke cigarettes (Heffner, 2013) and do not have AD (Prisciandaro, 2019). Going forward, a more complicated randomization scheme (i.e., urn randomization by smoking status and AD diagnosis) in a larger sample, in tandem with a parallel-group (i.e., between subject) study design to rule out potential condition-order effects, will be critical to overcoming the potential interpretational pitfalls presented by the results of this preliminary study.

Finally, although elevated GABA levels were associated with decreased mood symptoms in gabapentin-treated participants, we did not find an overall effect of gabapentin on brain GABA levels. Past studies that have demonstrated an effect of chronic gabapentin dosing on brain GABA levels have generally evaluated a higher dose of gabapentin (≥ 1800mg/day vs. 1200mg/day) over a longer period of time (≥ 2-weeks vs. 5 days) relative to the present study. Further, 1800mg/day gabapentin dosing has been shown to confer greater efficacy in treating AUD relative to 1200mg/day dosing (Mason, 2014). The excellent tolerability of gabapentin in the present study suggests that we could safely increase gabapentin dosing to 1800mg/day, and our high participant retention rate (95.5%), combined with a parallel-group study design, suggests that we could increase the dosing duration from 5 (including 2-day titration) to 17 (including 3-day titration) days. These changes, together, would significantly increase our chances of observing a gabapentin effect on brain GABA levels.

Building off of these preliminary results, we designed a study to replicate and extend our results to a larger sample of participants with a more robust research design. Specifically, the proposed study will feature:

1) A parallel-group, as opposed to a crossover, study design.
2) A substantially larger sample of BD+CUD participants, determined by the preliminary study's observed effect sizes (see *C.4.a.* below).
3) Urn, as opposed to simple, randomization to treatment group (by cigarette-smoking status and AD diagnosis).
4) A higher dose of gabapentin (1800mg/day vs. 1200mg/day) delivered over a longer dosing period (17 days vs. 5 days) to increase our likelihood of observing gabapentin effects on brain GABA levels.
5) Better measurement of key constructs like anxiety, sleep, and motivation to reduce cannabis use, to guide future, more clinically-focused investigations of gabapentin and related medications for BD+CUD.

A.5. Conclusion. Despite a dire need for safe and efficacious treatments for BD+CUD, little is known about optimal treatment in this population. Convergent evidence, including results from an NIH/NIDA-funded preliminary study recently completed by the study team (R21DA043917), supports disrupted brain GABA/glutamate homeostasis as a promising interventional target, and gabapentin as a candidate adjuvant medication to normalize frontal and striatal brain GABA and glutamate levels, and thereby reduce symptoms and

sequelae, in BD+CUD. The proposed randomized, placebo-controlled, double-blind, parallel-group, multimodal MRI study aims to normalize the dysregulated brain GABA/glutamate homeostasis characteristic of individuals with BD and CUD using gabapentin and to evaluate medication-related, GABA/glutamate-driven changes in brain activation to cannabis cues, as well as mood and cannabis use, in individuals with BD+CUD.

B. INNOVATION

1) Despite the dire need for safe and efficacious treatments, no RCTs for individuals with BD+CUD have been published to date. Positive results from the proposed study may not only provide support and valuable information for the investigation of gabapentin for the adjuvant treatment of BD+CUD in larger clinical efficacy trials, but may also indicate additional interventions to affect GABA/glutamate transmission, while simultaneously validating a methodological platform for evaluating the promise of such interventions, in individuals with CUD and/or BD.
2) Though GABA/glutamate disturbances are central to the pathophysiology of co-occurring BD and CUD, no published ^{1}H-MRS studies have investigated GABA/glutamate disturbances in individuals with BD+CUD. The proposed study will investigate whether altering GABA/glutamate homeostasis in individuals with BD+CUD results in changes in cannabis cue activation and clinical variables, and will explore whether the effects of gabapentin on GABA/glutamate homeostasis and cannabis cue activation depend on clinical variables believed to influence these outcomes, like cigarette-smoking status and AD diagnosis.

C. RESEARCH PLAN

C.1. Overview. The proposed randomized, placebo-controlled, double-blind, parallel-group, multimodal MRI study will evaluate: a) the effects of gabapentin on dACC and rBG GABA and glutamate concentrations and b) the effects of gabapentin on brain activation to cannabis versus neutral images. The interaction and relationship between changes in GABA and glutamate levels, cannabis cue-reactivity, and clinical variables (i.e., cannabis use and craving, mood, anxiety, sleep) will be explored. Participants will be urn-randomized, based on smoking status (≥ 10 cigarettes/day; Lipari, 2013) and AD diagnosis, to receive gabapentin (1800mg/day) or matched placebo for 17 days (i.e., 3-day titration to maximum dose [Days 1-3] followed by 14-days of treatment [Days 4-17]). The study will take approximately 3 weeks to complete and will consist of 5 visits: 1) Baseline Evaluation (V1), 2) Pre-treatment MRI followed by randomization and dispensing of medication (V2, Day 1, to occur within 7 days of V1), 3) Mid-study evaluation (V3, Day 9, +/- 1 day for scheduling flexibility), and 4) Post-treatment MRI followed by discontinuation of medication (V4, Day 17), and 5) Safety visit (to occur within 72 hours of Day 17). See *Figure 5* below for a study design schematic.

C.2. Participants. Sixty-eight healthy, clinically-stable men and women ages 18-65 with current CUD and BD will be enrolled across a 54-month period (following a 3-month study-initiation period, accomplished in R21DA043917); the final 3-months of the funding period will be dedicated to analysis and manuscript preparation. Recruitment will occur via clinical referral and community advertising to reach an enrollment target of 68 BD+CUD participants (i.e., 1.25 participants/month). Please note, the inclusion/exclusion criteria below are identical to those of R21DA043917 (described in *A.4.*).

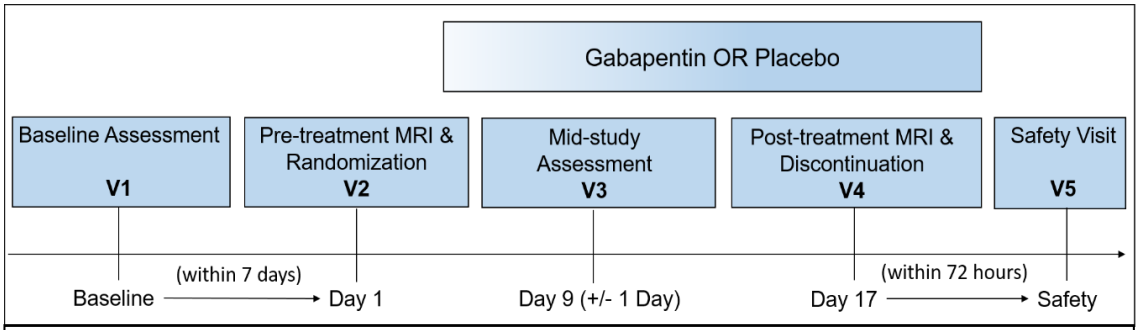

Figure 5. Study design schematic

C.2.a. Recruitment. Experience from R21DA043917 (see *Figure 6*) suggests we focus recruitment efforts for the proposed study on the following primary sources:

1) *Medical University of South Carolina (MUSC)*
    a. Inpatient - Over the past 10+ years, we have forged strong ties with the clinical enterprise of the MUSC Institute of Psychiatry. Study staff visit MUSC residents and attending physicians across 4 inpatient units, 1 of which is dedicated to dual diagnosis, 3x/week. If the inpatient treatment team believes a patient may be eligible and interested in participating in the current study, they will approach the patient and gain approval for the research study staff to speak with the patient. If they agree, patients will then be asked questions by study staff to screen for eligibility.
    b. Outpatient – We will recruit additional study participants through IRB-approved access to electronic

medical records, education and outreach to medical residents, and flyering across campus. A research data request will the submitted to indicate patients who have been diagnosed with Bipolar Disorder or Schizoaffective Disorder, Bipolar Type. Patients who have not indicated an opt-out research contact preference in their MUSC medical record will be called by study staff and screened for eligibility. Individuals who have indicated that they do wish to opt-out of research contact may be informed of the research study by their MUSC psychiatry outpatient provider if the provider feels it is appropriate. If they are interested, they will be given the study team's contact information to call or email. The study team will not cold-contact any patients who have chosen to opt-out of receiving contact about research or who have met the maximum number of contact attempts at the time of recruitment.

2) *Community Advertising*
   a. Internet – Facebook, Craigslist (1-2x/week), TrialFacts, Researchmatch.org, wesearchtogether.com, BuildClinical
   b. Radio – 1-month campaign on 2 stations (3x/year).
   c. Print ads - Flyers
3) *Other Clinical Referral Sites*
   a. The Charleston Center – The Charleston Center is an SUD treatment facility located 1 block from MUSC that screens > 2,500 patients/year and provides inpatient, outpatient, and residential treatment. Study staff visit The Center weekly. Treatment team members present the study to patients who they believe may be eligible and, if they are interested, obtain contact information for study staff.
   b. Charleston Dorchester Mental Health Center (CDMH) – The CDMH provides outpatient counseling, psychiatric treatment, and support services to adults with severe mental illness through clinics in Charleston and Dorchester Counties in SC. Study staff visit the CDMH monthly, and additionally coordinate referrals and activities via phone and e-mail between visits.

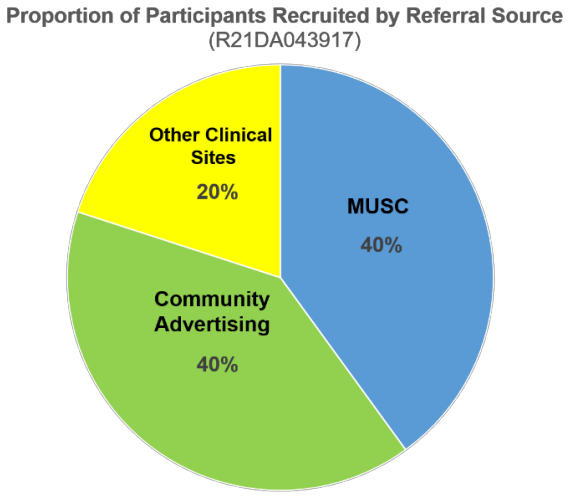

Figure 6. Proportion of participants recruited by source.

C.2.b. Inclusion Criteria. 1.) Subjects must meet DSM-5 criteria for CUD (within the past three months; APA, 2013) and provide a positive urine cannabinoid screen at baseline. While individuals may meet mild SUD criteria for other substances, they must identify cannabis as their primary substance of abuse. They must additionally self-report cannabis use within the past 30 days and report using more days than not in the month preceding abstinence. 2.) Subjects must meet DSM-5 criteria for bipolar I or II disorder, or schizoaffective disorder, bipolar type, and must be prescribed daily use of ≥ 1 FDA-approved mood-stabilizing medication for BD (lithium, lamotrigine, divalproex sodium, carbamazepine, 2nd generation antipsychotic); restricting the study to medication-naïve individuals would represent a safety hazard, severely limit recruitment (Phillips, 2008), and would be inconsistent with the stated goal of the study to evaluate gabapentin as an adjuvant medication. For these reasons, in the preliminary study presented in section *A.4* (and in Prisciandaro, 2017), FDA-approved mood-stabilizing medications were included and were not found to be associated with GABA/glutamate levels. To minimize the impact of medications on results, anticonvulsant and other medications that have been shown to change brain glutamate or GABA levels in humans (e.g., ketamine, topiramate) will be excluded. Second generation antipsychotics will not be excluded, as ^{1}H-MRS studies have failed to demonstrate associations between antipsychotic medication load and brain GABA/glutamate levels (Lindquist, 2011; Merritt, 2016; Soeiro-de-Souza, 2015). Finally, participants with medication additions, discontinuations, or dose changes of ≥ 20%, ≤ 2 weeks before testing will be excluded (Swann, 2009). 3.) Participants must be between ages 18-65 years. 4.) Women of childbearing potential must utilize birth control.

C.2.c. Exclusion Criteria. 1.) History of significant hematological, endocrine, cardiovascular, pulmonary, renal, gastrointestinal, or neurological disease. 2.) History of psychotic disorder (e.g., Schizophrenia). 3.) Current suicidal or homicidal ideation. 4.) Because neurochemical dysfunction in BD is evident across mood states (Gigante, 2012), participants with elevated mood symptoms will not be excluded, in order to maximize recruitment feasibility and generalizability to clinical populations. However, participants presenting with a severe mood disturbance that confers an acute safety risk (i.e., MADRS > 35, YMRS > 25) will be excluded. 4.) Subjects meeting DSM-5 criteria for moderate to severe SUD (other than cannabis and tobacco) within the past 90 days.

5.) Current use of opioid or benzodiazepine drugs, or other drugs hazardous if taken with gabapentin, identified via drug testing or self-report (Smith, 2016). 6.) History of allergic reaction to gabapentin. 8.) Electroconvulsive therapy in the past 3 months. 9.) History of head injury with loss of consciousness > 5 minutes. 10.) Presence of non-MRI safe materials or significant claustrophobia. 11.) Plasma creatinine levels > 2x normal range.

Table 2. Schedule of events by study visit

Study Visit	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Clinician					
Informed Consent / HIPAA / BrAC contract	X				
SCID-5 / FHS	X				
History and Physical (incl. Vitals) / Metal Screen	X				
YMRS / MADRS / HAM-A / C-SSRS	X	X	X	X	X
TLFB (90 day at V1) ALL SUBSTANCES (incl. cigarettes)	X	X	X	X	X
BDRS / BR / OAS / PANSS-7		X		X	
AEs / Vitals		X	X	X	X
Self-Report					
Demographics	X				
BIS-11 / BIS/BAS / AUDIT / ASRS / PSQI / WHODAS / DSM-5 PID-5 / SCID-5 SPQ / CTQ / STAXI-2 (Trait) / BussPerry Aggression / SHAPS (Trait) / STI (Trait) / CMMQ	X*				
BDI-II / BAI / Insomnia Severity Index / SOCRATES / MCQ / CWS / FTND / ASRM / CAPE-42 / STAXI-2 (State) / STAI (State) / CHRT-SR		X		X	
Computer Tasks					
STOP-IT / Delay Discounting		X		X	
NIH Toolbox (Post Scan)		X		X	
CNL Labs					
CMP / CBC / DNA	X				
Cannabinoid / Creatinine level / EtG / Riboflavin		X		X	
Cotinine / %dCDT	X				
In-House Labs					
UDS / Breathalyzer	X	X	X	X	X
Pregnancy Test	X	X		X	
Saliva Drug Screen		X		X	
Misc					
Actigraphy		X	X	X	
^{1}H-MRS / fMRI		X		X	
Dispense Meds		X	X		

* After eligibility confirmed via SCID-5 and H&P, whenever possible. Non-completed measures to be administered at V2, before MRI.

C.3. Procedures. See *Table 2* for a summary of study events by visit.

C.3.a. Baseline Assessment (Visit 1). Following brief screening over the phone or at a referral site, potential participants will be scheduled for formal screening at an Addiction Sciences Division research clinic. They will read and sign an IRB-approved informed consent, HIPAA, and BrAC documents and will then be assessed for eligibility using the Structured Clinical Interview for DSM-V (First, 1995). Past 90-day drug use will be assessed using the Timeline Followback method (TLFB; Sobell, 1995). Cannabis use will be recorded in times used/day as well as quantity (e.g., grams, number of blunts/joints) to standardize for different types of cannabis use. Participants will be asked to quantify cannabis use by weighing out amounts of an inert cannabis surrogate and reporting on that amount's potency through dollar value estimates. Recent methods of use will then be quantified

using this system (bowls, bongs, blunts, ingestion; Mariani, 2011). Mood symptoms will be assessed using the YMRS, MADRS, Hamilton Anxiety Scale (Hamilton, 1959). Sleep will be assessed using the Pittsburgh Sleep Quality Index (PSQI; Buysse, 1989). Safety assessments, including the Columbia Suicide Severity Rating Scale (C-SSRS; Posner, 2011), will be conducted at each visit to assess for symptoms requiring medication adjustment or hospitalization (see *Human Subjects* for full safety protocol). A Metal Screening Questionnaire will assess MRI safety. Motives for cannabis use, via the Comprehensive Marijuana Motives Questionnaire (CMMQ; Lee, 2009) will be evaluated. Family history will be assessed with the Family History Screen (FHS; Milne, 2009). Impulsivity (BIS), hedonic tone (Snaith-Hamilton Pleasure Scale [SHAPS]; Snaith et al., 1995), personality pathology (Personality Inventory for DSM-5 [PID-5; Suzuki et al., 2015]), state-trait anger (State-Trait Anger Expression Inventory [STAXI-2]; Spielberger, 1995), aggression (Aggression Questionnaire; Buss & Perry, 1992), ADHD symptoms (Adult ADHD Self-Report Scale [ASRS; Kessler et al., 2005]), childhood trauma (Childhood Trauma Questionnaire [CTQ]; Bernstein et al., 1994), functional impairment (WHO Disability Assessment Scale [WHODAS; WHO, 2010]), will be measured.

Study candidates who meet diagnostic criteria will undergo a full medical history and physical exam and will provide samples for blood chemistries (Comprehensive Metabolic Panel [CMP], Complete Blood Count [CBC]) and genetic. A disialo carbohydrate-deficient transferrin (%dCDT) will be ran to test for heavy alcohol use. Cotinine will be quantified to differentiate smokers versus non-smokers, along with 90-day timeline followback data, to determine urn-randomization. Qualitative drug screens will be performed using the Discover 12 Panel Cup® (Discover), an in vitro diagnostic test for the detection of drug or drug metabolite in urine. In addition, semi-quantitative urine cannabinoid screens (detection cut-off value=30.00 ng/ml) will be performed using the AXSSYM® system from Abbott Laboratories. Creatinine will be quantified, as creatinine normalization provides a method to differentiate new cannabis use from residual drug excretion (Schwilke, 2011). Participants will provide a saliva sample to test for recent cannabis use using SalivaConfirm® (Confirm Biosciences, Inc.). Female participants will undergo pregnancy testing.

C.3.b. MRI (Visits 2 and 4). Participants will be asked to abstain from drugs and alcohol ≥ 12 hours prior to MRI. Mood symptoms (YMRS, MADRS, HAM-A, BDI, BAI, BDRS, BR, OAS, PANSS-7, OAS), alcohol and drug consumption (Breathalyzer, TLFB, UDS, EtG, SalivaConfirm), cannabis craving (MCQ; Heishman, 2009) and withdrawal (CWS; Allsop, 2011) will be assessed upon arrival. Motivation to reduce cannabis use will be evaluated via the Stages of Change Readiness and Treatment Eagerness Scale (SOCRATES; Miller, 1996). Questionnaires evaluating cigarette and tobacco use (FTND), the presence of manic symptoms (ASRM; Altman, 1997), psychotic symptoms (CAPE-42), suicidal ideation (CHRT; Trivedi, 2008), anxiety (STAXI, STAII), and insomnia (ISI), will be evaluated.

Participants will complete STOP-IT and Delayed-Discounting computer tasks. At Visit 4, participants will take their final medication dose in front of staff, 1 hour before MRI, to ensure compliance. Participants who smoke will be allowed to have their last cigarette immediately prior to their final medication dose. Participants will provide blood and urine samples to test for gabapentin levels, riboflavin, and drug consumption. During localizing, structural, and ^{1}H-MRS scanning, participants will view scenic images via a mirror mounted to their 32-ch head coil. Then, the CCR task will be administered. Total scan time is 75-90 minutes in a Siemens 3.0T Prisma with actively-shielded magnet and high-performance gradients (80 mT/m, 200T/m-sec).

^{1}H-MRS Acquisition. A structural scan will be taken for ^{1}H-MRS voxel placement and tissue segmentation (256 sagittal slices; 1mm thick/50% gap). dACC and rBG contain markedly different concentrations of GABA (Durst, 2015) and form an important fronto-striatal reward circuit (Haber, 2010). We will acquire ^{1}H-MRS data from both regions to evaluate whether gabapentin effects, as well as associations between GABA and cannabis cue-reactivity, are region specific. The dACC voxel will be placed on midsagittal T1-weighted images, posterior to the genu of the corpus callosum, with the ventral edge of the voxel aligned with the dorsal edge of the callosum (Hermann, 2012). A right rBG voxel will be placed on an axial T1-weighted slice about 1 cm above the genu of the corpus callosum, between the Sylvian fissure and the lateral ventricles including corpus striatum (Liu, 2015).

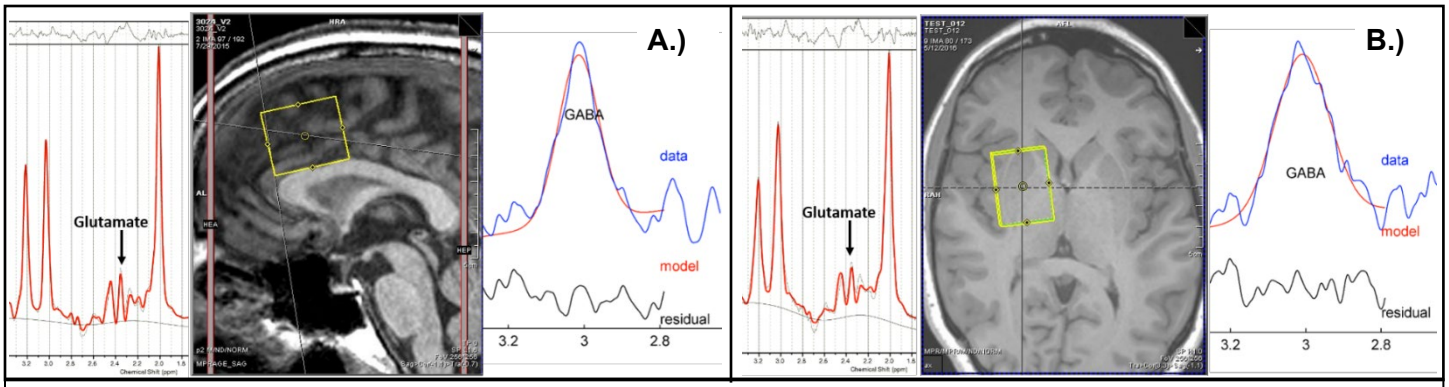

Figure 7. A.) Sample dACC voxel (center), fitted PRESS glutamate spectrum (left), fitted MEGA-PRESS GABA spectrum (right) B.) Sample rBG voxel (center), fitted PRESS glutamate spectrum (left), fitted MEGA-PRESS GABA spectrum (right).

Each voxel will be 2.5x2.5x3 cm^3 to ensure adequate signal to noise. See *Figure 7* for voxel locations and sample spectra. Following placement of saturation bands 1-cm away from voxel faces and shimming via FASTESTMAP, single-voxel water-suppressed ^{1}H-MRS spectra will be acquired using a MEGA-PRESS sequence (TR=2000ms; TE=68ms; number of averages=256) with editing-pulse frequencies symmetric with respect to water (1.9 ppm and 7.5 ppm; Mullins, 2014) and a PRESS sequence maximally sensitive to glutamate (TR=2000ms; TE=40ms; number of averages=128; Mullins, 2008). Unsuppressed water spectra will be co-acquired for each sequence.

Cannabis Cue Reactivity (CCR) Task (fMRI) Acquisition. During the CCR task (Karoly, 2019), participants are shown pseudorandomly interspersed images of cannabis (i.e., cannabis plant, paraphernalia) and neutral (e.g., pine cone, trumpet) images, and a fixation cross. The cannabis stimuli were matched to neutral images by color, hue, and complexity. Stimuli are presented in six 90-s epochs, each consisting of three 24-s blocks of an image type (one block each of cannabis, non-cannabis control, and fixation). Participants rate their "urge to use marijuana" for 6-s after each block from 0 ("none") to 4 ("severe") using an optical hand pad. See *Figure 8A* for a task schematic. A Simultaneous Multi-Slice EPI sequence will be acquired (parameters: # of simultaneously acquired slices=3; TR/TE=1200/30 ms; flip angle (FA)=65°; field of view (FOV)=213x213 mm; voxel size=2.8x2.8 mm; 51 contiguous 2.8-mm-thick slices). The main contrast of interest will be activation during cannabis vs. neutral trials. In placebo-treated BD+CUD participants (R21DA043917, see section *A.4.* for study details), this contrast was assocaited with activation in a number of brain regions associated with drug cue-reactivity, including R pallidum, superior and middle frontal gyrus, and L posterior cingulate, and caudate, and bilateral thalamus and occipital cortex (z > 2.58, FWE p < 0.05; *Figure 8B*). At Visit 2, participants will be asked to wear an actigraphy watch (Actiwatch Spectrum Plus, Philips Respironics) on their non-dominant wrist for the remainder of the study. They will be instructed on its use (e.g., not to take it off when showering) and asked to keep a brief actigraph log (e.g., sleep and wake times) delivered daily via text/e-mail using REDCap.

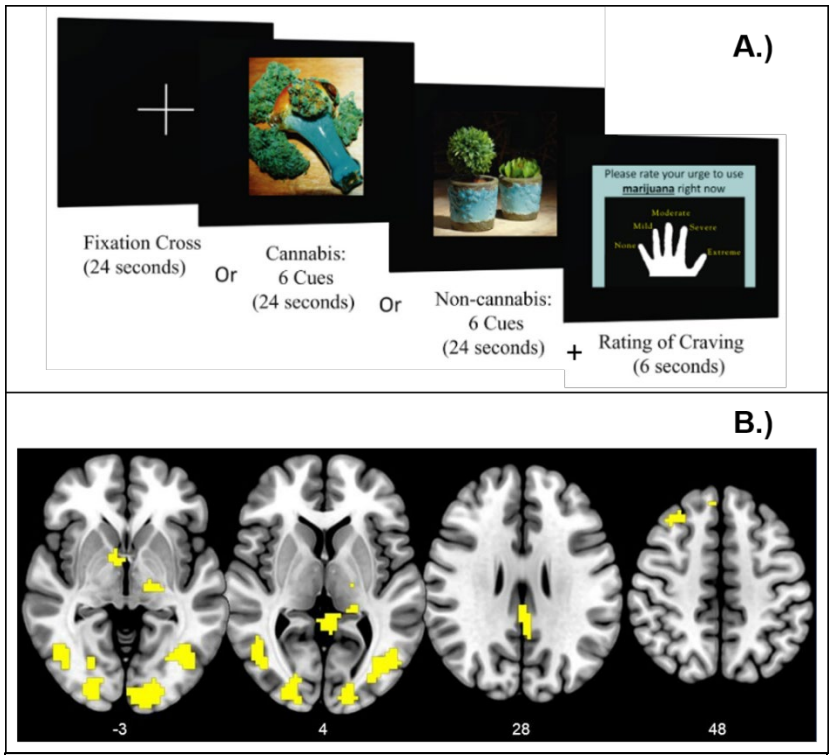

Figure 8. A.) Cannabis Cue Reactivity fMRI task schematic. Each task epoch included a fixation cross block, cannabis cue image block, and non-cannabis cue image block; after each block, participants rate their "urge to use marijuana." Adapted from *Karoly, 2019.* B) Cannabis cue minus non-cannabis cue activation (yellow) in placebo-treated BD+CUD participants (*R21DA043917*). Activated regions included, R pallidum, thalamus, superior and middle frontal gyrus, and L posterior cingulate, thalamus, and caudate.

C.3.c. Medication Dispensing (Visits 2 and 3). Medication Dispensing procedures will take place at the end of Visit 2 and will constitute the entirety of Visit 3. Preceding Dispensing procedures, mood symptoms (YMRS, MADRS, HAM-A, CSSRS, BDRS, BR, OAS, PANSS-7) and alcohol and drug consumption (Breathalyzer, TLFB, UDS,

SalivaConfirm) will also be assessed at Visit 3 (they will have already been assessed prior to MRI at Visit 2, see *C.3.b.* above). Medications will be packaged and dispensed by the MUSC Investigational Drug Service (IDS), a centralized research pharmacy that compounds medications. IDS will oversee study blinding procedures and maintain treatment assignment records. Study medication will be over-encapsulated with riboflavin (50mg/capsule, for urinary detection by fluorescence spectroscopy at each MRI), with each capsule containing either 300mg of gabapentin or matching placebo dispensed in blister packs. At each Dispensing visit, participants will take their first dose in front of study staff to ensure compliance. They will receive detailed instructions regarding the thrice daily dosing and titration schedule for days 1-17 of the study (see *Figure 9*). An 1800mg/day target dose was chosen because magnitude of clinical improvement (e.g., alcohol abstinence; Mason, 2014) and brain GABA increase (Petroff, 2000) with gabapentin is dose-dependent; given that 1200mg/day was extremely well-tolerated in our preliminary study, we anticipate that this dose increase will not lead to excessive AEs. A 17-day dosing period was chosen (i.e., 14-days of gabapentin treatment at the 1800mg/day target dose following 3-day titration) in order to bring dosing duration in line with past studies that have demonstrated GABA increases with chronic gabapentin treatment (Kuzniecky, 2002; Petroff, 1996; 2000). Gabapentin has an elimination half-life of approximately 6-7 hours (Rose, 2002). Participants should reach steady state concentrations of the 1800mg/day dose within 24-hours of initiation (Katzung, 2012). The proposed titration schedule was extrapolated from our experience studying gabapentin for AUD (Anton, 2020) and BD+CUD (R21DA043917), along with our clinical experience with dual diagnosis patients. Unused study medications will be returned for pill counts at MRI visits. Post-hoc gabapentin blood levels will be obtained for all participants randomized to gabapentin treatment, using a validated immunoassay (Juenke, 2011). Medication adherence will be evaluated as a covariate.

Dose	Day 1	Day 2	Day 3	Days 4-17
AM (mg)		300	600	600
PM (mg)	300	300	300	600
HS (mg)	600	600	600	600
Total (mg)	**900**	**1200**	**1500**	**1800**

Figure 9. Gabapentin thrice-daily dosing and titration schedule. HS = hour of sleep.

C.3.d. Safety Visit (Visit 5).

All participants will return for a final safety visit within 72 hours of day 17 to discuss adverse events, alcohol and drug consumption, and mood stability since discontinuing study medication with clinically-trained staff. Treatment referrals will be offered to all participants at this visit.

C.4. Statistical Considerations.

C.4.a Sample Size Determination. Data from R21DA043917, presented in section *A.4.* above, formed the basis of power calculations and sample size determination for the proposed study. Specifically, Cohen's *d* (i.e., standardized mean difference) was calculated for, 1) dACC glutamate levels in gabapentin- vs. placebo-treated participants in Randomization Order #1 (*d* = 0.70), 2) rBG glutamate levels in gabapentin- vs. placebo-treated cigarette-smoking participants (*d* = 0.99), and 3) pMCC cannabis cue activation in gabapentin- vs. placebo-treated cigarette-smoking participants (*d* = 1.08). Assuming condition order effects would be rendered inconsequential to the proposed parallel-group study design, we would need ≥ 52 study completers (i.e., 26 per treatment group) to achieve > 80% power to detect significantly higher dACC glutamate levels in gabapentin- relative to placebo-treated participants. Assuming that ≥ 50% of participants are cigarette-smokers (which we will ensure; 60% of the preliminary sample were cigarette-smokers), the proposed study would need ≥ 54 study completers (i.e., 28 cigarette-smoking participants divided equally between treatment groups via urn-randomization) to achieve > 80% power to detect significantly higher rBG glutamate levels or pMCC cannabis-cue activation in gabapentin-treated, versus placebo-treated, cigarette-smoking participants. In sum, a total of ≥ 54 study completers would provide > 80% power to detect all effects of interest (see *Figure 10*).

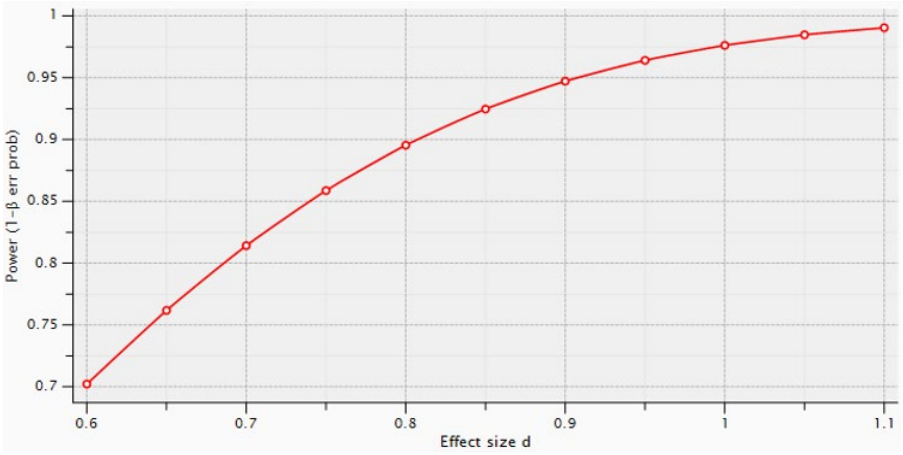

Figure 10. Statistical power (y-axis) by Cohen's d effect size (x-axis) for an independent-sample t-test (gabapentin vs. placebo in 54 BD+CUD participants.

To acquire 54 study completers, we would need to randomize approximately 68 participants, in order to

provide a 20% data-loss cushion. Although dropout in the preliminary study was 5%, and the proposed study duration is only +3-days relative to the preliminary study, up to 10% of ^{1}H-MRS scans were discarded in the preliminary study due to quality-control failure (i.e., poor linewidth). This degree of data loss is common in MRI, particularly in psychiatric participants or ^{1}H-MRS in difficult to image brain regions, like the basal ganglia. Given data loss from both participant dropout and quality-control failure, we believe a 20% data-loss cushion is reasonable and achievable and will ensure the rigor and reproducibility of the proposed study. To randomize 68 participants, we would need to accrue approximately 1.25 participants per month over a 4.5-year enrollment period. This accrual rate was achieved in our preliminary study (which had identical inclusion/exclusion criteria and the same combined start-up/wind-down time of 6-months), and we are therefore confident we could achieve this rate for the projected study.

C.4.b. Data Analysis.

^{1}H-MRS & fMRI Processing. MEGA-PRESS data will be processed using the Gannet MATLAB toolbox (Edden, 2014). PRESS data will be processed using LCModel 6.3 (Provencher, 1993). Metabolites with fitting uncertainties < 20% will be retained. Water will be quantified from a Gaussian-Lorentzian fit to the non-water-suppressed data. Within-voxel tissue fractions of gray and white matter and CSF will be calculated based on automated segmentation in Statistical Parametric Mapping 12 (SPM12, Wellcome Department of Cognitive Neurology) using a volume mask generated in Gannet (Harris, 2015). Metabolite concentrations will be normalized to the unsuppressed water signal and corrected for within-voxel CSF fraction. fMRI analysis will be completed in SPM12. Standard fMRI preprocessing including realignment, normalization, and smoothing will be performed. Preprocessed data will be analyzed within a general linear model mixed effects framework. Cannabis vs. neutral cues will be the primary contrast. Following 1st-level analysis, subject-specific spatially-normalized contrast maps will be entered into 2nd-level, random-effects analyses. Condition parameter maps will be thresholded at $p < 0.05$, corrected for multiple comparisons.

Primary Analyses. Generalized linear mixed effects models will be employed to assess the effect of gabapentin on dACC and rBG glutamate and GABA levels (*Hypothesis 1*). Whole-brain random-effects analyses will be conducted to assess the direct effect of gabapentin, as well as the direct effect of gabapentin-induced changes in glutamate and GABA, on brain activation to cannabis cues (*Hypothesis 2*). This analysis will be supplemented with a Region of Interest (ROI) approach. We will use the MarsBaR SPM toolbox to extract the percent-signal change associated with the cannabis vs. neutral cues contrast from participants' ^{1}H-MRS voxels as well as the pMCC cluster identified in preliminary analyses (*A.4.*, *Figure 4*). To minimize the impact of missing data, we will employ previously successful methods to minimize attrition. Where missing data cannot be avoided, mixed effects models yield valid inferences assuming data are missing at random (Little, 2002).

Exploratory Analyses.

1) *Mood, anxiety, sleep and cannabis use and craving.* Gabapentin may provide therapeutic benefit to individuals with BD+CUD across a number of symptom domains. Knowing the specific nature and magnitude of therapeutic effects of gabapentin in BD+CUD will be necessary to powering future, larger randomized controlled efficacy trials. We will therefore examine associations between treatment-related changes in mood symptoms (YMRS, MADRS), including anxiety (HAM-A) and sleep disturbance (PSQI), cannabis use (TLFB, UDS, SalivaConfirm) and craving (MCQ), and changes in GABA and glutamate levels by treatment group using generalized linear mixed effects models (*Exploratory Hypothesis*).
2) Cigarette-*smoking status and AD diagnosis.* Both variables were identified post-hoc as potential statistical moderators of gabapentin effects in our preliminary study. In the proposed study, participants will be urn-randomized to treatment group based on these variables (to ensure balanced representation between experimental groups), and we will evaluate both variables as potential moderators of gabapentin effects across all planned analyses (*Exploratory Hypothesis*).
3) *Motivation to reduce/quit, and reasons for, cannabis use.* Most people with CUD do not seek treatment, with lack of motivation given as the predominant reason for not getting help (Khan, 2013), and high rates of co-occurrence between BD and CUD may partly reflect efforts of BD individuals to self-medicate BD symptomatology (Judd, 2002; 2003; Farris, 2016; Sarvet, 2018). However, motivation to reduce/quit, and reasons for, cannabis use in BD+CUD have never been reported. In the proposed study, these constructs will be assessed using state of the art measures (i.e., SOCRATES and CMMQ), and their association with baseline clinical characteristics, as well as their impact on gabapentin effects, will be explored.
4) *Age, sex, and mood-stabilizing medication.* Age and sex have been associated with brain GABA and glutamate levels (Chang, 2009; O'Gorman, 2011; Gao, 2013), and participants must be prescribed daily use of ≥1 FDA-approved mood-stabilizing medication for BD (though medications with demonstrated effects on brain GABA or glutamate levels in ^{1}H-MRS studies [e.g., topiramate] will be excluded).

Although these variables were not associated with GABA/glutamate levels in our preliminary study, we will evaluate them as moderators across analyses to facilitate rigor and reproducibility. Although we will not be powered to detect sex differences, sex will be considered in all analyses and potential indications of sex differences will be considered in future, larger studies.

C.5. DISCUSSION

C.5.a. Overview. Despite the dire need for safe and efficacious treatments for BD+CUD, little is known about optimal treatment in this population. Convergent evidence, including results from an NIH/NIDA-funded preliminary study completed by the study team (R21DA043917), supports disrupted brain GABA/glutamate homeostasis as a promising interventional target, and gabapentin as a candidate adjuvant medication to normalize frontal and striatal brain GABA and glutamate levels, and thereby reduce cannabis use, anxiety, and sleep disturbance, in individuals with BD+CUD. The proposed randomized, placebo-controlled, double-blind, parallel-group, multimodal MRI study aims to normalize the dysregulated brain GABA/glutamate homeostasis characteristic of individuals with BD and CUD using gabapentin and to evaluate medication-related changes in brain activation to cannabis cues, as well as mood symptoms and cannabis use, in individuals with BD+CUD. The proposed study will overcome interpretational challenges presented by existing research by employing a more robust research design (parallel-treatment groups, urn-randomization), in a substantially larger sample of BD+CUD participants, with a higher target dose of gabapentin delivered over a longer duration.

C.5.b. Strategies to Ensure a Robust and Unbiased Approach. As detailed throughout this proposal, the proposed study will achieve robust and unbiased results via explicit inclusion/exclusion criteria; urn-randomization to treatment condition; placebo control; double blinding; sophisticated compliance monitoring; use of validated MRI, laboratory, and interview/self-report measures/methods; explicit hypotheses and planned statistical analyses; power estimates; planned handling of attrition and missing data; and careful consideration of potential confounds. Methodology is reported in a detailed and fully transparent manner to support replication.

C.5.c. Potential Limitations. 1) Although we discuss measuring brain "GABA" levels throughout the proposal, this entity is sometimes referred to as "GABA+" in the methodological literature, as it contains contributions from co-edited macromolecules (Mullins, 2014). Although suppression of macromolecules is possible, it requires a magnitude of frequency-stability not presently available on commercial MRI machines, and so the standard in the field is to acquire GABA+ data (Mullins, 2014). This potential limitation is mitigated by the fact that our preliminary study, along with all studies that have reported GABA increases with gabapentin, have acquired GABA+ levels. 2) Although previous studies have demonstrated gabapentin-induced increases in brain GABA, these studies have been conducted in healthy controls and epileptics. Whether these findings will generalize to individuals with CUD+BD remains unknown. The proposed study's larger sample size, higher dose of gabapentin, and longer dosing period relative to our preliminary study will inspire more confidence in concluding that, if we find no GABA increase in the proposed study, gabapentin may not increase GABA levels in BD+CUD (e.g., due to ongoing cannabis use), and neurobehavioral effects of gabapentin in BD+CUD may be better explained by gabapentin-induced changes in glutamate transmission. 3) A potential challenge to completing the study will be recruiting/retaining a sufficient number of BD+CUD participants. Experience with our preliminary study (R21DA043917) inspires confidence. However, if recruitment/retention goals are consistently not met, the study team will meet to discuss strategies for meeting goals without compromising the integrity of the study.

C.5.d. Future Directions. The proposed study provides the next logical step, bridging our NIH/NIDA-funded preliminary study to larger, more-costly, randomized controlled efficacy trials of gabapentin for BD+CUD (Ray, 2018). Exploratory analyses from the proposed study will provide invaluable information towards this objective including, a) in which domains, and to what extent, gabapentin is associated with reduced symptomatology (mood, anxiety, sleep, cannabis use/craving), b) specificity of gabapentin effects to cigarette-smoking or AD individuals, and c) reasons for, and motivation to reduce, cannabis use. The proposed study may also provide successful demonstration of an MRI platform for evaluating the promise of glutamatergic/GABAergic drugs (e.g., the mGlur5 negative allosteric modulator, GET73, which we are evaluating in AUD; NCT03418623) for BD+CUD and other conditions marked by GABA/glutamate dysfunction (e.g., psychotic disorders) (Grodin, 2019). Finally, the proposed study will add to the literature on associations between brain GABA/glutamate levels and constructs related to BD and CUD, including cannabis cue reactivity, craving and use, and mood and anxiety symptomology.

Protection of Human Subjects

1. **Risk to the Subjects**

a. Human Subject Involvement and Characteristics. A total of 68 individuals in stable medical condition will be enrolled in the study. Women and minorities will be recruited for this study. Children and adolescents under the age of 18 will not be enrolled.

Inclusion Criteria:

1. Ages 18-65 years
2. Meet DSM-5 criteria for cannabis use disorder (CUD; within the past 3 months), provide a positive urine cannabinoid screen at baseline, and identify cannabis as the primary substance of abuse. Self-reports cannabis use within the past 30 days and using cannabis more days than not in the month preceding abstinence.
3. Meet DSM-5 criteria for bipolar I or II disorder (BD) or Schizoaffective Disorder, Bipolar Type
4. Able to provide informed consent and read, understand, and accurately complete assessment instruments
5. Willing to commit to medication treatment and follow-up assessments
6. Prescribed daily use of at least one mood stabilizing medication (i.e., lithium, divalproex sodium, lamotrigine, carbamazepine, 2nd generation antipsychotic)

Exclusion Criteria:

1. A primary psychiatric diagnosis other than BD (e.g., Schizophrenia)
2. Meet DSM-5 criteria for moderate or severe substance use disorder (other than cannabis or tobacco) within the past 90 days
3. Any uncontrolled neurological condition (e.g., epilepsy) that could confound the results of the study
4. Any history of brain injury with loss of consciousness greater than 5 minutes
5. Any history of mental retardation, dementia, or recent electroconvulsive therapy (in the past 3 months)
6. Any uncontrolled medical condition that may adversely affect the conduct of the study or jeopardize the safety of the participant
7. Hepatocellular disease as indicated by plasma levels of liver transaminases (aspartate transaminase, alanine transaminase) greater than 3 times the normal range
8. Renal insufficiency as indicated by plasma levels of creatinine greater than 2 times the normal range
9. Concomitant use of medications that could interfere with glutamatergic/GABAergic transmission (e.g., benzodiazepines, ceftriaxone, riluzole, memantine, ketamine, topiramate, vigabatrin), due to potential confounding effects
10. Concomitant use of opioid medications, benzodiazepines, barbiturates, chloral hydrate, sodium oxybate, or any other medication deemed to be hazardous if taken with gabapentin
11. Azelastine, orphenadrine, oxomemazine, paraldehyde, and thalidomide are generally contraindicated in patients taking gabapentin; as such, individuals taking these medications will be excluded
12. Women of childbearing potential who are pregnant, lactating, or refuse adequate forms of contraception
13. Current suicidal or homicidal risk
14. Baseline scores greater than 35 on the Montgomery-Asberg Depression Rating Scale or greater than 25 on the Young Mania Rating Scale
15. Has taken gabapentin in the last month or experienced adverse effects/allergic reaction (e.g., angioedema) from it at any time
16. Significant claustrophobia and/or past negative experiences with MRI
17. Presence of non-MRI safe materials in the body (e.g., ferrous metal implants, pacemaker)

b. Source of Materials. Data collected from participants will include breathalyzer readings, urine drug screens, urine biomarkers (e.g., riboflavin), blood chemistries, structural, functional, and neurochemical MRI brain images, and interviews and self-reports regarding substance use, psychiatric diagnoses, concomitant medications, and adverse events (AEs). To ensure confidentiality, all participant data will be number-coded, and only the investigators will have access to the master list of codes. A federal Certificate of Confidentiality, protecting participants against disclosure of sensitive information (e.g., drug use), will be obtained for the study.

c. Compensation. To maximize participant retention, contingency management will be applied to compensation such that participants will be compensated significantly more for each subsequent MRI visit they attend (i.e., scan 1 = $100, scan 2 = $150), and will be given an additional $50 bonus for completing both MRI visits and returning study-medication blister packs. Participants will be compensated $50 for the baseline appointment. Finally, participants will be compensated $25 for each additional non-MRI appointment (i.e., 2 total).

Compensation will thus be $400 per participant in the form of cash. Participants will be eligible to receive a referral bonus, totaling $25 cash, for each individual they refer to the study. The referred participant(s) must attend the screening visit and meet criteria for bipolar disorder in order for the referring participant to receive the $25 cash bonus. There is no limit to how many individuals can be referred to the study. In our experience, this level of compensation is fair for the time commitment required without unduly coercing participants to enroll in the study despite potential concerns.

d. Potential Risks

1. Medication side effects. Gabapentin is generally well-tolerated, with sedation and dizziness being the most commonly reported side effects (Carta, 2003; Peng, 2007). Although the FDA has issued a class warning for antiepileptic drugs and suicidal thoughts and behavior, available data do not support an association between gabapentin, specifically, and increased suicidal ideation or behavior in individuals with BD or other psychiatric populations (Carta, 2003; Gibbons, 2010). The proposed study will minimize potential medication-related risks by implementing low starting doses and titration of study medications and by carefully monitoring potential medication-related risks via biweekly scheduled visits and assessment of AEs (including assessment of suicidal ideation and behavior at each and every study visit). The study physician will determine if the participant should be discontinued from the medication due to adverse drug reactions and will treat clinically as needed. Any confirmed incidence of serious AEs that are deemed probably or definitely due to the study medication will result in immediate discontinuation of the study medication and follow-up assessments will be conducted until resolution. Subjects will be referred for treatment as necessary.
2. Drug interactions. Due to potential interactions with gabapentin, participants may not take opioid medications (e.g., morphine, hydrocodone, buprenorphine), benzodiazepines, or sodium oxybate at any time during participation in the study (Smith, 2016). As noted earlier, participants will be required to be taking a stable pre-existing regimen of at least one FDA-approved mood-stabilizing medication treatment for BD. There are no known drug interactions with gabapentin and mood-stabilizing medications (i.e., lithium, divalproex sodium, lamotrigine, carbamazepine, 2nd generation antipsychotic).
3. MRI-related risks. Individuals with non-MRI-safe medical implants or ferrous objects would be at risk for injury, if allowed to enter the MRI scanner. Several precautions will be taken to ensure that individuals with ferrous implants or objects are not allowed to enter the MRI scanner. First, all potential participants will meet with a study physician to discuss any possible history of ferrous implants or other MRI-unsafe objects. Participants with any suspected history of ferrous implants or exposure to shrapnel will be excluded from the study. Second, participants who are deemed MRI-safe by the study physician will be screened for metal objects at the Center for Biomedical Imaging (CBI) using a handheld metal detector and a second metal detector built into the threshold of the doorway to the scanner. Participants who screen positive for metal will be asked to remove all metal objects from their person and will be rescreened. If participants continue to screen positive for metal after removing all metal objects from their person, they will be excluded from the study. Although not dangerous, participants who are claustrophobic could experience significant discomfort in the MRI scanner. As such, all participants will be assessed in terms of claustrophobia as well as past experience with MRI. Additionally, all eligible participants will be entered into a “mock scanner” at the CBI human imaging center, which features the same dimensions of the real MRI scanner but without any of the internal machinery. Participants who report claustrophobia, past negative experience with MRI, or signification discomfort in the mock scanner will be excluded from participation. These procedures have been successfully used by our staff in previous and ongoing research studies of similar participant populations. For those participants allowed to participate in the MRI study, if abnormalities in collected brain images are found, participants will immediately be referred to an appropriate clinical care provider.
4. Cue-elicited cannabis craving. It is possible that the cannabis cue exposure functional MRI paradigm could induce cannabis craving. Participants will be asked to rate their craving from one to ten both immediately preceding and following the neuroimaging protocol. Post-scan craving ratings twenty percent above baseline will require study-approved clinicians to come and speak with the patient before they are discharged. Should any craving fail to subside within 3-4 hours, participants will be provided with counseling by clinicians and appropriate referrals will be made as needed. These procedures have been successfully used by our staff in previous and ongoing research studies of similar participant populations.
5. Cannabis consumption and withdrawal. Subjects will not be required to establish abstinence at any time during the study. However, they may voluntarily abstain during the course of the study. Participants who voluntarily attempt abstinence may experience cannabis withdrawal symptoms. Study participants will be monitored for cannabis withdrawal symptoms. Conversely, participants may continue to consume cannabis. The risks of continued cannabis use may include but are not limited to psychiatric morbidity, increased risk

of traumatic injury, and other medical consequences. If in the PI's opinion a participant has significant worsening of cannabis use problems or consumption of cannabis as a result of participating in the study, the participant will be withdrawn from the study and appropriately referred.

6. Mood destabilization. Exacerbation of depressive or manic symptoms during the course of the study is a risk for all participants regardless of treatment condition. We will minimize this risk by assessing mood symptoms, including suicidal ideation and behavior using the Columbia Suicide Severity Rating Scale (C-SSRS), at each and every study visit. Participants experiencing sufficient deterioration of mood stability to result in clinically significant impairment in functional capacity will be appropriately referred. Any participant exhibiting mood destabilization that is sufficient to pose an imminent danger to self or others will be hospitalized immediately and removed from the study.

2. Adequacy of Protection Against Risks

a. Recruitment and Informed Consent. Recruitment will occur by clinical referral,response to advertisements and flyers, and clinical chart review with cold-contact methods. The principal investigator, the study coordinator, and co-investigators with completed masters-or-higher-level clinical training will obtain informed consent. At the screening visit, potential participants will be provided a copy of the IRB-approved consent document to review. After providing the participant with time to read the consent, the principal investigator, the study coordinator, or co-investigators with completed masters-or-higher-level clinical training will review the consent document page by page with the participant and answer any questions. Only then will the participant be asked to sign the consent document. Participants will be given a copy of the signed consent document. The entire informed consent process will be documented in the research progress notes. The signed, original consent document will be maintained in the participant source record with a copy of the consent binder located at the Addiction Sciences Division, Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina (MUSC).

b. Safety Assessments. At every visit, clinically-trained staff will evaluate subjects' manic and/or depressive symptoms, including suicidal ideation. Mood symptoms will also be quantified using standardized instruments including MADRS, YMRS, BDI-II, and BAI. AEs will be assessed at each and every study visit.

c. Protection Against Risk.

Psychiatric Risks. The investigative team has a great deal of experience working with the proposed study population and have the resources to manage psychiatric emergencies and/or make referrals as needed. Psychiatric symptoms will be assessed on a biweekly basis by standardized assessments and by clinical interview. Participants scoring >4 on the suicide item (item 4) of the MADRS; endorsing items 4 (intention), 5 (plan), or 6 (preparation) on the C-SSRS, or otherwise exhibiting potentially life-threatening decompensation in mood or other psychiatric symptoms at any study visit will be removed from the study and referred for outpatient or inpatient treatment as necessary.

Medical Risks. Gabapentin is excreted by a renal route. A blood chemistry panel will be performed and reviewed by the study physician prior to beginning the study medication. Participants with clinically significant renal (creatinine > twice normal), or hepatic (transaminases elevated > 3 times normal), insufficiency will not be eligible to participate in the study. Participants will be referred for treatment as necessary.

MRI Risks. The investigative team has a great deal of experience with human MRI research and Dr. Prisciandaro is a core faculty member of the CBI at MUSC. MRI safety and comfort will be assessed at baseline and at each MRI visit. Participants with non-MRI-safe medical implants or ferrous objects will be excluded from participation as will individuals evidencing significant discomfort with MRI.

Pregnancy. Gabapentin is rated as a *Category C* medication in terms of pregnancy. Because there are no adequate controlled studies of gabapentin in pregnant women, it is unknown whether the drug can cause fetal harm or affect reproductive capacity in humans. Therefore, women of childbearing potential must agree to pregnancy testing and use of adequate contraception in order to be eligible to participate in the study. Females will be given a urinary pregnancy test at the screening visit and weekly thereafter. Any female participant who becomes pregnant during the study will be discontinued from the study medication and removed from study participation. For all included females of childbearing potential, current forms of birth control and date of last menstruation will be assessed at each study visit.

Confidentiality. Records with identifying information (e.g., consent documents) will be stored in a locked file cabinet. All other non-MRI participant data will be collected via direct data capture (REDCap); MRI data will be automatically transferred to Linux-based servers managed by CBI. Both MRI and non-MRI data will be stored in restricted access directories on password-protected, encrypted servers managed by CBI and the Department of

Psychiatry and Behavioral Sciences at MUSC. Participants will be given an ID number for all MRI and non-MRI data files. The master list of codes will be accessible only to the investigators, and will be stored in a locked office.

Emergencies. All study participants will be instructed how to access the 24-hour on-call system available at MUSC. In the event that a participant experiences an AE after hours, s/he will be instructed to access the 24-hour on-call service. If it is determined that the participant needs immediate help, the participant may be advised to immediately go to the emergency room. In that event, proper medical treatment will be administered, per ER procedures. Dr. Prisciandaro and Dr. Tolliver will be available by pager, as needed, at all times. The Investigational Drug Service (IDS) will be available 24 hours/day, 7 days/week for emergency identification of treatment group assignment and unblinding as necessary. Dr. Tolliver has 16 years of experience managing over 200 BD+SUD individuals in clinical trials.

Substance Abuse Treatment. Participants may receive additional non-pharmacologic substance abuse treatment during study participation. Attendance at group-based recovery activities (e.g., Narcotics Anonymous) will be encouraged and monitored.

3. Potential Benefits of the Proposed Research to the Subjects and Others

Benefits to the participants include medical and psychiatric assessments provided at no cost. Participants may benefit by reduction of cannabis consumption as an effect of active treatment or through nonspecific effects of study participation (increased awareness of cannabis consumption, frequent interactions with study personnel, etc.), although this is not guaranteed for any given individual. Other individuals with co-occurring BD and CUD are likely to benefit by the knowledge gained from the study as it may help guide future treatments.

4. Importance of the Knowledge to Be Gained

There is an 8-fold increase in the prevalence of CUD in individuals with BD relative to the general population; this common comorbidity is associated with substantially elevated negative outcomes, including treatment resistance. Treatment research for co-occurring BD and CUD (BD+CUD) is extremely limited, with no randomized trials for BD+CUD published to date. The proposed study will evaluate the ability of a medication (i.e., gabapentin) that has been shown to increase cortical GABA levels in past research to manipulate a neurochemical dysfunction characteristic of individuals with BD+CUD (i.e., dysregulated brain GABA/glutamate homeostasis). Positive results may support investigation of gabapentin for the treatment of BD+CUD in large-scale, randomized clinical trials. Furthermore, the proposed study may provide successful demonstration of a neurobehavioral, multimodal neuroimaging platform for evaluating the potential promise of GABAergic drugs for CUD and/or BD, as well as other conditions marked by GABA/glutamate dysfunction. The proposed investigation's minimal risks are reasonable in relation to the importance of the knowledge to be gained from the investigation.

5. Clinicaltrials.gov Requirements

In accordance with Public Law 110-85, this project will be registered at the ClinicalTrials.gov Protocol Registration System Information Website prior to study initiation.

Data and Safety Monitoring Plan

This section is based on the recommendations in NIDA's "Guidelines for Developing a Data and Safety Monitoring Plan" (https://www.drugabuse.gov/funding/clinical-research/guidelines-developing-data-safety-monitoring-plan). A detailed DSMP will be developed and approved by NIH program staff prior to study initiation.

1) Summary of the Protocol.

This application proposes to investigate the effects of gabapentin on brain GABA and glutamate concentrations, a neurobehavioral measure of cannabis cue-reactivity, and mood and cannabis use in individuals with co-occurring bipolar disorder and cannabis use disorder. The primary outcomes of interest are brain GABA and glutamate levels and neurobehavioral measures of cannabis cue-reactivity. Inclusion/exclusion criteria are outlined in *Protection of Human Subjects*. Power calculations and sample sizes are detailed in the *Sample Size Determination* section of the *Research Strategy*.

2) Trial Management.

The study will be managed from the Addiction Sciences Division within the Department of Psychiatry and Behavioral Sciences at the Medical University of South Carolina (MUSC). The target population is described above in the inclusion/exclusion criteria.

3) Data Management and Analysis.

Non-MRI data will be collected via direct data capture (REDCap) on tablet devices and stored on MUSC centralized secured, backed-up servers. Recruitment projects are housed in REDCap and only IRB approved study team members will have access to this database. The research team will only have access to the REDCap recruitment project while actively enrolling for the study and the recruitment project will be stored separately from the project containing research data. MRI data will be automatically transferred to Linux-based servers managed by the Center for Biomedical Imaging (CBI). The data analysis plan is outlined in the *Data Analysis* section of the *Research Strategy*.

4) Quality Assurance.

Quarterly data audits will be conducted. Confidentiality protections are outlined above.

5) Regulatory Issues.

Potential conflicts of interest will be reported using the upcoming NIH rules for disclosure. Adverse Events (AEs)/Serious Adverse Events (SAEs) occurring during the course of the project will be collected, documented, and reported in accordance with protocol and Institutional Review Board (IRB) reporting requirements. All research staff involved with adverse event reporting will receive general and protocol specific AE/SAE training including identification, assessment and evaluation, and documentation and reporting. A research assistant will identify any potential adverse events during the course of the study from participant self-report and administration of the visit assessments and procedures. The research assistant will provide information to a study physician, who will be responsible for AE/SAE assessment and evaluation including a determination of seriousness and study relatedness. Any significant actions taken by the local IRB and protocol changes will be relayed to NIDA.

6) Definition of AE and SAE.

An Adverse Event (AE) is defined as any untoward medical occurrence in a study subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment (ICH GCP). Any unwanted change, physically, psychologically or behaviorally, that occurs in a study participant during the course of the trial is an adverse event. A Serious Adverse Event (SAE) is defined as an adverse event that has one of the following outcomes:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity,
- Is a congenital anomaly/birth defect OR
- Requires intervention to prevent one of the above outcomes.

7) Documentation and Reporting.

AEs/SAEs are documented and reported as per protocol and IRB requirements. Research staff will identify adverse events and obtain all available information to assess severity, seriousness, study relatedness, expectedness, outcome and the need for change or discontinuation in the study intervention. Adverse events are generally documented on AE Logs and AE Case Report Forms (CRFs). Additional relevant AE information if available should be documented in a progress note in the research record as appropriate to allow monitoring and evaluating of the AE. If the AE meets the definition for serious, appropriate SAE protocol specific reporting forms are completed and disseminated to the appropriate persons and within the designated timeframes as indicated above. For each AE/SAE recorded, the research staff will follow the AE/SAE until resolution, stabilization or until the participant is no longer in the study as stated in the protocol. When a reportable SAE is identified, the research staff will notify the MUSC IRB within 24 hours and complete the AE report form in conjunction with the PI. The MUSC IRB meets monthly and is located at 165 Cannon Street, Rm. 501, Charleston, SC 29425. Communication with the IRB is through email, memos, official IRB forms, and online reporting. A report will also be sent to the NIH program officer assigned to the project.

If complete information is not available when the initial 24-hour SAE report is disseminated, follow-up information will be gathered to enable a complete assessment and outcome of the event. This information may include hospital discharge records, autopsy reports, clinic records, etc. The research staff will attach copies of source documents to the SAE report for review by the PI and for forwarding to the NIH program officer as appropriate within 2 weeks of the initial SAE report. In addition, the PI will provide a signed, dated SAE summary report, which will be sent to the NIDA Medical Safety Officer within two weeks of the initial SAE report.

We will report adverse events to the MUSC IRB online as soon as possible, but no later than 10 working days after the investigator first learns of the event. The MUSC IRB AE reporting requirements are as follows: All deaths that occur during the study or 30 days post termination from the study are required to be reported as adverse events even if they are expected or unrelated. Other adverse events are reportable to the MUSC IRB if the AE is unexpected AND related or possibly related AND serious or more prevalent than expected. All three criteria must be met for an AE to be reported to the MUSC IRB. The IRB definition of unexpected is that the AE is not identified in nature, severity or frequency in the current protocol, informed consent, investigator brochure or with other current risk information. The definition of related is that there is a reasonable possibility that the adverse event may have been caused by the drug, device or intervention. Reportable AEs are reviewed by the IRB Chair and reported to the IRB Board at the next meeting.

8) Trial Safety.

The potential risks and benefits and methods to minimize these risks are outlined above. The research staff will report any unexpected AEs or any scores of “severe” on the side-effect symptom rating form or any FDA-defined serious AEs to the PI within 24 hrs so that the PI can decide on the appropriate action. All unexpected AEs will be monitored while they are active to determine if treatment is needed. Study procedures will follow the FDA’s Good Clinical Practice Guidelines (www.fda.gov/oc/gcp). Any outside requests for information or any breaches in confidentiality will be reported to Dr. Prisciandaro.

9) Trial Efficacy.

An interim analysis is not planned at this time.

10) DSM Plan Administration.

Drs. Prisciandaro, Tolliver, and Mellick will be responsible for monitoring the study, and will participate in weekly study meetings. A DSM report will be filed with the IRB and NIDA on a yearly basis, unless greater than expected problems occur. The report will include participant characteristics, retention and disposition of study participants, quality assurance issues and reports of AEs, significant/unexpected AEs and serious AEs. We will report outcomes at the end of the trial.

11) DSM Board.

A Data Safety and Monitoring Board will be formed to monitor both the rate and severity of adverse events. This panel will include 3 clinicians with expertise in cannabis use and mood disorders and a statistician.

12) Risk Benefit Ratio.

The assessments and questionnaires are non-invasive and have inherently minimal risks. Potential risks of concern are loss of confidentiality and adverse events to gabapentin or MRI. As discussed above, our research team will attempt to minimize these risks. Knowledge gained by the proposed study would help fill an important void in development of potential adjuvant treatments for co-occurring bipolar disorder and cannabis use disorder.

References

Alasmari, F., Al-Rejaie, S. S., AlSharari, S. D., & Sari, Y. (2016). Targeting glutamate homeostasis for potential treatment of nicotine dependence. *Brain research bulletin, 121*, 1-8. doi:10.1016/j.brainresbull.2015.11.010

Allsop, D. J., Norberg, M. M., Copeland, J., Fu, S., & Budney, A. J. (2011). The Cannabis Withdrawal Scale development: patterns and predictors of cannabis withdrawal and distress. *Drug Alcohol Depend, 119*(1-2), 123-129. doi:10.1016/j.drugalcdep.2011.06.003

Anton, R. F., Latham, P., Voronin, K., Book, S., Hoffman, M., Prisciandaro, J., & Bristol, E. (2020). Efficacy of Gabapentin for the Treatment of Alcohol Use Disorder in Patients With Alcohol Withdrawal Symptoms: A Randomized Clinical Trial. *JAMA Intern Med, 180*(5), 728-736. doi:10.1001/jamainternmed.2020.0249

Aracil-Fernández, A., Almela, P., & Manzanares, J. (2013). Pregabalin and topiramate regulate behavioural and brain gene transcription changes induced by spontaneous cannabinoid withdrawal in mice. *Addict Biol, 18*(2), 252-262. doi:10.1111/j.1369-1600.2011.00406.x

Association, A. P. (2013). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). Washington, DC.

Bak, L. K., Schousboe, A., & Waagepetersen, H. S. (2006). The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J Neurochem, 98*(3), 641-653. doi:10.1111/j.1471-4159.2006.03913.x

Bally, N., Zullino, D., & Aubry, J.-M. (2014). Cannabis use and first manic episode. *J Affect Disord, 165*, 103-108. doi:https://doi.org/10.1016/j.jad.2014.04.038

Bandelow, B., Michaelis, S., & Wedekind, D. (2017). Treatment of anxiety disorders. *Dialogues in clinical neuroscience, 19*(2), 93-107.

Bartoli, F., Crocamo, C., & Carrà, G. (2019). Cannabis use disorder and suicide attempts in bipolar disorder: A meta-analysis. *Neurosci Biobehav Rev, 103*, 14-20. doi:10.1016/j.neubiorev.2019.05.017

Beck, A., & Steer, R. A. (1983). *Beck Anxiety Inventory Manual*. San Antonio, TX: Psychological Corporation.

Beck, A. T., Steer, R. A., Ball, R., & Ranieri, W. (1996). Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *J Pers Assess, 67*(3), 588-597. doi:10.1207/s15327752jpa6703_13

Bhagwagar, Z., Wylezinska, M., Jezzard, P., Evans, J., Ashworth, F., Sule, A., Matthews, P. M., & Cowen, P. J. (2007). Reduction in occipital cortex gamma-aminobutyric acid concentrations in medication-free recovered unipolar depressed and bipolar subjects. *Biol Psychiatry, 61*(6), 806-812. doi:10.1016/j.biopsych.2006.08.048

Bobo, W. V., Reilly-Harrington, N. A., Ketter, T. A., Brody, B. D., Kinrys, G., Kemp, D. E., Shelton, R. C., McElroy, S. L., Sylvia, L. G., Kocsis, J. H., McInnis, M. G., Friedman, E. S., Singh, V., Tohen, M., Bowden, C. L., Deckersbach, T., Calabrese, J. R., Thase, M. E., Nierenberg, A. A., Rabideau, D. J., Schoenfeld, D. A., Faraone, S. V., & Kamali, M. (2015). Complexity of illness and adjunctive benzodiazepine use in outpatients with bipolar I or II disorder: results from the Bipolar CHOICE study. *J Clin Psychopharmacol, 35*(1), 68-74. doi:10.1097/jcp.0000000000000257

Brady, R. O., Jr., McCarthy, J. M., Prescot, A. P., Jensen, J. E., Cooper, A. J., Cohen, B. M., Renshaw, P. F., & Ongur, D. (2013). Brain gamma-aminobutyric acid (GABA) abnormalities in bipolar disorder. *Bipolar Disord, 15*(4), 434-439. doi:10.1111/bdi.12074

Brambilla, P., Perez, J., Barale, F., Schettini, G., & Soares, J. C. (2003). GABAergic dysfunction in mood disorders. *Mol Psychiatry, 8*(8), 721-737, 715. doi:10.1038/sj.mp.4001362

Buysse, D. J., Reynolds, C. F., 3rd, Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res, 28*(2), 193-213. doi:10.1016/0165-1781(89)90047-4

Cai, K., Nanga, R. P., Lamprou, L., Schinstine, C., Elliott, M., Hariharan, H., Reddy, R., & Epperson, C. N. (2012). The impact of gabapentin administration on brain GABA and glutamate concentrations: a 7T (1)H-MRS study. *Neuropsychopharmacology, 37*(13), 2764-2771. doi:10.1038/npp.2012.142

Carta, M. G., Hardoy, M. C., Hardoy, M. J., Grunze, H., & Carpiniello, B. (2003). The clinical use of gabapentin in bipolar spectrum disorders. *J Affect Disord, 75*(1), 83-91.

Chang, L., Cloak, C., Yakupov, R., & Ernst, T. (2006). Combined and independent effects of chronic marijuana use and HIV on brain metabolites. *J Neuroimmune Pharmacol, 1*(1), 65-76. doi:10.1007/s11481-005-9005-z

Chang, L., Jiang, C. S., & Ernst, T. (2009). Effects of age and sex on brain glutamate and other metabolites. *Magn Reson Imaging, 27*(1), 142-145. doi:10.1016/j.mri.2008.06.002

Cohen, K., Weizman, A., & Weinstein, A. (2019). Modulatory effects of cannabinoids on brain neurotransmission. *Eur J Neurosci, 50*(3), 2322-2345. doi:10.1111/ejn.14407

Colizzi, M., McGuire, P., Pertwee, R. G., & Bhattacharyya, S. (2016). Effect of cannabis on glutamate signalling in the brain: A systematic review of human and animal evidence. *Neurosci Biobehav Rev, 64*, 359-381. doi:10.1016/j.neubiorev.2016.03.010

Colizzi, M., Weltens, N., McGuire, P., Lythgoe, D., Williams, S., Van Oudenhove, L., & Bhattacharyya, S. (2019). Delta-9-tetrahydrocannabinol increases striatal glutamate levels in healthy individuals: implications for psychosis. *Mol Psychiatry*. doi:10.1038/s41380-019-0374-8

Cousijn, J., Goudriaan, A. E., Ridderinkhof, K. R., van den Brink, W., Veltman, D. J., & Wiers, R. W. (2013). Neural responses associated with cue-reactivity in frequent cannabis users. *Addict Biol, 18*(3), 570-580. doi:10.1111/j.1369-1600.2011.00417.x

de Guglielmo, G., Cippitelli, A., Somaini, L., Gerra, G., Li, H., Stopponi, S., Ubaldi, M., Kallupi, M., & Ciccocioppo, R. (2013). Pregabalin reduces cocaine self-administration and relapse to cocaine seeking in the rat. *Addict Biol, 18*(4), 644-653. doi:10.1111/j.1369-1600.2012.00468.x

de Sousa, R. T., Loch, A. A., Carvalho, A. F., Brunoni, A. R., Haddad, M. R., Henter, I. D., Zarate, C. A., & Machado-Vieira, R. (2017). Genetic Studies on the Tripartite Glutamate Synapse in the Pathophysiology and Therapeutics of Mood Disorders. *Neuropsychopharmacology, 42*(4), 787-800. doi:10.1038/npp.2016.149

Durst, C. R., Michael, N., Tustison, N. J., Patrie, J. T., Raghavan, P., Wintermark, M., & Sendhil Velan, S. (2015). Noninvasive evaluation of the regional variations of GABA using magnetic resonance spectroscopy at 3 Tesla. *Magn Reson Imaging, 33*(5), 611-617. doi:10.1016/j.mri.2015.02.015

Edden, R. A., Puts, N. A., Harris, A. D., Barker, P. B., & Evans, C. J. (2014). Gannet: A batch-processing tool for the quantitative analysis of gamma-aminobutyric acid-edited MR spectroscopy spectra. *J Magn Reson Imaging, 40*(6), 1445-1452. doi:10.1002/jmri.24478

Farris, S. G., Metrik, J., Bonn-Miller, M. O., Kahler, C. W., & Zvolensky, M. J. (2016). Anxiety Sensitivity and Distress Intolerance as Predictors of Cannabis Dependence Symptoms, Problems, and Craving: The Mediating Role of Coping Motives. *Journal of studies on alcohol and drugs, 77*(6), 889-897. doi:10.15288/jsad.2016.77.889

Fatemi, S. H., Folsom, T. D., & Thuras, P. D. (2017). GABAA and GABAB receptor dysregulation in superior frontal cortex of subjects with schizophrenia and bipolar disorder. *Synapse, 71*(7), e21973. doi:10.1002/syn.21973

Filip, M., Frankowska, M., Gołda, A., Zaniewska, M., Vetulani, J., & Przegaliński, E. (2006). Various GABA-mimetic drugs differently affect cocaine-evoked hyperlocomotion and sensitization. *European Journal of Pharmacology, 541*(3), 163-170. doi:https://doi.org/10.1016/j.ejphar.2006.05.011

First, M. B., Williams, J. B. W., Karg, R. S., & Spitzer, R. L. (2015). *Structured Clinical Interview for DSM-5*. Arlington, VA: American Psychiatric Association.

Gao, F., Edden, R. A., Li, M., Puts, N. A., Wang, G., Liu, C., Zhao, B., Wang, H., Bai, X., Zhao, C., Wang, X., & Barker, P. B. (2013). Edited magnetic resonance spectroscopy detects an age-related decline in brain GABA levels. *Neuroimage, 78*, 75-82. doi:10.1016/j.neuroimage.2013.04.012

Gates, P., Albertella, L., & Copeland, J. (2016). Cannabis withdrawal and sleep: A systematic review of human studies. *Substance Abuse, 37*(1), 255-269. doi:10.1080/08897077.2015.1023484

Gerner, R. H., Fairbanks, L., Anderson, G. M., Young, J. G., Scheinin, M., Linnoila, M., Hare, T. A., Shaywitz, B. A., & Cohen, D. J. (1984). CSF neurochemistry in depressed, manic, and schizophrenic patients compared with that of normal controls. *Am J Psychiatry, 141*(12), 1533-1540. doi:10.1176/ajp.141.12.1533

Ghaemi, S. N., Wingo, A. P., Filkowski, M. A., & Baldessarini, R. J. (2008). Long-term antidepressant treatment in bipolar disorder: meta-analyses of benefits and risks. *Acta Psychiatrica Scandinavica, 118*(5), 347-356. doi:10.1111/j.1600-0447.2008.01257.x

Gibbons, R. D., Hur, K., Brown, C. H., & Mann, J. J. (2010). Gabapentin and suicide attempts. *Pharmacoepidemiol Drug Saf, 19*(12), 1241-1247. doi:10.1002/pds.2036

Gigante, A. D., Bond, D. J., Lafer, B., Lam, R. W., Young, L. T., & Yatham, L. N. (2012). Brain glutamate levels measured by magnetic resonance spectroscopy in patients with bipolar disorder: a meta-analysis. *Bipolar Disord, 14*(5), 478-487. doi:10.1111/j.1399-5618.2012.01033.x

Goldberg, J. F., & Burdick, K. E. (2001). Cognitive side effects of anticonvulsants. *J Clin Psychiatry, 62 Suppl 14*, 27-33.

Greenblatt, H. K., & Greenblatt, D. J. (2018). Gabapentin and Pregabalin for the Treatment of Anxiety Disorders. *Clinical Pharmacology in Drug Development, 7*(3), 228-232. doi:10.1002/cpdd.446

Grodin, E. N., & Ray, L. A. (2019). The Use of Functional Magnetic Resonance Imaging to Test Pharmacotherapies for Alcohol Use Disorder: A Systematic Review. *Alcohol Clin Exp Res, 43*(10), 2038-2056. doi:10.1111/acer.14167

Haber, S. N., & Knutson, B. (2010). The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology, 35*(1), 4-26. doi:10.1038/npp.2009.129

Harris, A. D., Puts, N. A., & Edden, R. A. (2015). Tissue correction for GABA-edited MRS: Considerations of voxel composition, tissue segmentation, and tissue relaxations. *J Magn Reson Imaging, 42*(5), 1431-1440. doi:10.1002/jmri.24903

Hasin, D. S. (2018). US Epidemiology of Cannabis Use and Associated Problems. *Neuropsychopharmacology, 43*(1), 195-212. doi:10.1038/npp.2017.198

Hassel, B., & Dingledine, R. (2006). Glutamate. In G. J. Siegel, R. W. Albers, S. T. Brady, & D. L. Price (Eds.), *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects* (pp. 267-290). London, UK: Elsevier Academic Press.

Heffner, J. L., Anthenelli, R. M., Adler, C. M., Strakowski, S. M., Beavers, J., & DelBello, M. P. (2013). Prevalence and correlates of heavy smoking and nicotine dependence in adolescents with bipolar and cannabis use disorders. *Psychiatry Res, 210*(3), 857-862. doi:10.1016/j.psychres.2013.04.010

Heishman, S. J., Evans, R. J., Singleton, E. G., Levin, K. H., Copersino, M. L., & Gorelick, D. A. (2009). Reliability and validity of a short form of the Marijuana Craving Questionnaire. *Drug Alcohol Depend, 102*(1-3), 35-40. doi:10.1016/j.drugalcdep.2008.12.010

Hermann, D., Weber-Fahr, W., Sartorius, A., Hoerst, M., Frischknecht, U., Tunc-Skarka, N., Perreau-Lenz, S., Hansson, A. C., Krumm, B., Kiefer, F., Spanagel, R., Mann, K., Ende, G., & Sommer, W. H. (2012). Translational magnetic resonance spectroscopy reveals excessive central glutamate levels during alcohol withdrawal in humans and rats. *Biol Psychiatry, 71*(11), 1015-1021. doi:10.1016/j.biopsych.2011.07.034

Horiuchi, Y., Nakayama, J., Ishiguro, H., Ohtsuki, T., Detera-Wadleigh, S. D., Toyota, T., Yamada, K., Nankai, M., Shibuya, H., Yoshikawa, T., & Arinami, T. (2004). Possible association between a haplotype of the GABA-A receptor alpha 1 subunit gene (GABRA1) and mood disorders. *Biol Psychiatry, 55*(1), 40-45.

Houghton, K. T., Forrest, A., Awad, A., Atkinson, L. Z., Stockton, S., Harrison, P. J., Geddes, J. R., & Cipriani, A. (2017). Biological rationale and potential clinical use of gabapentin and pregabalin in bipolar disorder, insomnia and anxiety: protocol for a systematic review and meta-analysis. *BMJ open, 7*(3), e013433-e013433. doi:10.1136/bmjopen-2016-013433

Judd, L. L., Akiskal, H. S., Schettler, P. J., Endicott, J., Maser, J., Solomon, D. A., Leon, A. C., Rice, J. A., & Keller, M. B. (2002). The long-term natural history of the weekly symptomatic status of bipolar I disorder. *Arch Gen Psychiatry, 59*(6), 530-537. doi:10.1001/archpsyc.59.6.530

Judd, L. L., Schettler, P. J., Akiskal, H. S., Maser, J., Coryell, W., Solomon, D., Endicott, J., & Keller, M. (2003). Long-term symptomatic status of bipolar I vs. bipolar II disorders. *Int J Neuropsychopharmacol, 6*(2), 127-137. doi:10.1017/s1461145703003341

Juenke, J. M., Wienhoff, K. A., Anderson, B. L., McMillin, G. A., & Johnson-Davis, K. L. (2011). Performance characteristics of the ARK diagnostics gabapentin immunoassay. *Ther Drug Monit, 33*(4), 398-401. doi:10.1097/FTD.0b013e318220bb65

Kalivas, P. W. (2009). The glutamate homeostasis hypothesis of addiction. *Nat Rev Neurosci, 10*(8), 561-572. doi:10.1038/nrn2515

Karoly, H. C., Schacht, J. P., Meredith, L. R., Jacobus, J., Tapert, S. F., Gray, K. M., & Squeglia, L. M. (2019). Investigating a novel fMRI cannabis cue reactivity task in youth. *Addict Behav, 89*, 20-28. doi:10.1016/j.addbeh.2018.09.015

Katzung, B. G., Masters, S. B., & Trevor, A. J. (2012). *Basic & Clinical Pharmacology* (12th ed.). USA: McGraw-Hill Medical.

Khan, S. S., Secades-Villa, R., Okuda, M., Wang, S., Pérez-Fuentes, G., Kerridge, B. T., & Blanco, C. (2013). Gender differences in cannabis use disorders: results from the National Epidemiologic Survey of Alcohol and Related Conditions. *Drug Alcohol Depend, 130*(1-3), 101-108. doi:10.1016/j.drugalcdep.2012.10.015

Kim, S.-W., Dodd, S., Berk, L., Kulkarni, J., de Castella, A., Fitzgerald, P. B., Kim, J.-M., Yoon, J.-S., & Berk, M. (2015). Impact of Cannabis Use on Long-Term Remission in Bipolar I and Schizoaffective Disorder. *Psychiatry investigation, 12*(3), 349-355. doi:10.4306/pi.2015.12.3.349

Koob, G. F., & Volkow, N. D. (2016). Neurobiology of addiction: a neurocircuitry analysis. *Lancet Psychiatry, 3*(8), 760-773. doi:10.1016/s2215-0366(16)00104-8

Kurokawa, K., Shibasaki, M., Mizuno, K., & Ohkuma, S. (2011). Gabapentin blocks methamphetamine-induced sensitization and conditioned place preference via inhibition of α_2/δ-1 subunits of the voltage-gated calcium channels. *Neuroscience, 176*, 328-335. doi:10.1016/j.neuroscience.2010.11.062

Kuzniecky, R., Ho, S., Pan, J., Martin, R., Gilliam, F., Faught, E., & Hetherington, H. (2002). Modulation of cerebral GABA by topiramate, lamotrigine, and gabapentin in healthy adults. *Neurology, 58*(3), 368-372.

Kvitland, L. R., Melle, I., Aminoff, S. R., Demmo, C., Lagerberg, T. V., Andreassen, O. A., & Ringen, P. A. (2015). Continued cannabis use at one year follow up is associated with elevated mood and lower global functioning in bipolar I disorder. *BMC psychiatry, 15*, 11-11. doi:10.1186/s12888-015-0389-x

Lee, C. M., Neighbors, C., Hendershot, C. S., & Grossbard, J. R. (2009). Development and preliminary validation of a comprehensive marijuana motives questionnaire. *Journal of studies on alcohol and drugs, 70*(2), 279-287. doi:10.15288/jsad.2009.70.279

Le-Niculescu, H., Patel, S. D., Bhat, M., Kuczenski, R., Faraone, S. V., Tsuang, M. T., McMahon, F. J., Schork, N. J., Nurnberger, J. I., Jr., & Niculescu, A. B., 3rd. (2009). Convergent functional genomics of genome-wide association data for bipolar disorder: comprehensive identification of candidate genes, pathways and mechanisms. *Am J Med Genet B Neuropsychiatr Genet, 150b*(2), 155-181. doi:10.1002/ajmg.b.30887

Lev-Ran, S., Le Foll, B., McKenzie, K., George, T. P., & Rehm, J. (2013). Bipolar disorder and co-occurring cannabis use disorders: characteristics, co-morbidities and clinical correlates. *Psychiatry Res, 209*(3), 459-465. doi:10.1016/j.psychres.2012.12.014

Lile, J. A., Wesley, M. J., Kelly, T. H., & Hays, L. R. (2016). Separate and combined effects of gabapentin and [INCREMENT]9-tetrahydrocannabinol in humans discriminating [INCREMENT]9-tetrahydrocannabinol. *Behavioural pharmacology, 27*(2-3 Spec Issue), 215-224. doi:10.1097/FBP.0000000000000187

Lindquist, D. M., Dunn, R. S., & Cecil, K. M. (2011). Long term antipsychotic treatment does not alter metabolite concentrations in rat striatum: an in vivo magnetic resonance spectroscopy study. *Schizophr Res, 128*(1-3), 83-90. doi:10.1016/j.schres.2011.02.019

Lipari, R. N., & Van Horn, S. (2013). Smoking and Mental Illness Among Adults in the United States. In *The CBHSQ Report* (pp. 1-). Rockville (MD): Substance Abuse and Mental Health Services Administration (US).

Little, R. J. A., & Rubin, D. B. (2002). *Statistical Analysis with Missing Data* (2nd ed.). USA: Wiley-Interscience.

Liu, B., Wang, G., Gao, D., Gao, F., Zhao, B., Qiao, M., Yang, H., Yu, Y., Ren, F., Yang, P., Chen, W., & Rae, C. D. (2015). Alterations of GABA and glutamate-glutamine levels in premenstrual dysphoric disorder: a

3T proton magnetic resonance spectroscopy study. *Psychiatry Res, 231*(1), 64-70. doi:10.1016/j.pscychresns.2014.10.020

Liu, G. J., Karim, M. R., Xu, L. L., Wang, S. L., Yang, C., Ding, L., & Wang, Y.-F. (2017). Efficacy and Tolerability of Gabapentin in Adults with Sleep Disturbance in Medical Illness: A Systematic Review and Meta-analysis. *Frontiers in neurology, 8*, 316-316. doi:10.3389/fneur.2017.00316

Liu, K. (2016). AB/BA design in continuous data. In *Crossover Designs* (pp. 7-29).

Manville, R. W., & Abbott, G. W. (2018). Gabapentin Is a Potent Activator of KCNQ3 and KCNQ5 Potassium Channels. *Molecular pharmacology, 94*(4), 1155-1163. doi:10.1124/mol.118.112953

Mariani, J. J., Brooks, D., Haney, M., & Levin, F. R. (2011). Quantification and comparison of marijuana smoking practices: blunts, joints, and pipes. *Drug Alcohol Depend, 113*(2-3), 249-251. doi:10.1016/j.drugalcdep.2010.08.008

Mason, B. J., Crean, R., Goodell, V., Light, J. M., Quello, S., Shadan, F., Buffkins, K., Kyle, M., Adusumalli, M., Begovic, A., & Rao, S. (2012). A proof-of-concept randomized controlled study of gabapentin: effects on cannabis use, withdrawal and executive function deficits in cannabis-dependent adults. *Neuropsychopharmacology, 37*(7), 1689-1698. doi:10.1038/npp.2012.14

Mason, B. J., Quello, S., Goodell, V., Shadan, F., Kyle, M., & Begovic, A. (2014). Gabapentin treatment for alcohol dependence: a randomized clinical trial. *JAMA Intern Med, 174*(1), 70-77. doi:10.1001/jamainternmed.2013.11950

Massat, I., Souery, D., Del-Favero, J., Oruc, L., Noethen, M. M., Blackwood, D., Thomson, M., Muir, W., Papadimitriou, G. N., Dikeos, D. G., Kaneva, R., Serretti, A., Lilli, R., Smeraldi, E., Jakovljevic, M., Folnegovic, V., Rietschel, M., Milanova, V., Valente, F., Van Broeckhoven, C., & Mendlewicz, J. (2002). Excess of allele1 for alpha3 subunit GABA receptor gene (GABRA3) in bipolar patients: a multicentric association study. *Mol Psychiatry, 7*(2), 201-207. doi:10.1038/sj.mp.4000953

Merritt, K., Egerton, A., Kempton, M. J., Taylor, M. J., & McGuire, P. K. (2016). Nature of Glutamate Alterations in Schizophrenia: A Meta-analysis of Proton Magnetic Resonance Spectroscopy Studies. *JAMA Psychiatry, 73*(7), 665-674. doi:10.1001/jamapsychiatry.2016.0442

Miller, W. R., & Tonigan, J. S. (1996). Assessing drinkers' motivation for change: The Stages of Change Readiness and Treatment Eagerness Scale (SOCRATES). *Psychology of Addictive Behaviors, 10*(2), 81-89. doi:10.1037/0893-164X.10.2.81

Molero, Y., Larsson, H., D'Onofrio, B. M., Sharp, D. J., & Fazel, S. (2019). Associations between gabapentinoids and suicidal behaviour, unintentional overdoses, injuries, road traffic incidents, and violent crime: population based cohort study in Sweden. *BMJ, 365*, l2147. doi:10.1136/bmj.l2147

Montgomery, S. A., & Asberg, M. (1979). A new depression scale designed to be sensitive to change. *Br J Psychiatry, 134*, 382-389.

Muetzel, R. L., Marjanska, M., Collins, P. F., Becker, M. P., Valabregue, R., Auerbach, E. J., Lim, K. O., & Luciana, M. (2013). In vivo H magnetic resonance spectroscopy in young-adult daily marijuana users. *Neuroimage Clin, 2*, 581-589. doi:10.1016/j.nicl.2013.04.011

Mullins, P. G., Chen, H., Xu, J., Caprihan, A., & Gasparovic, C. (2008). Comparative reliability of proton spectroscopy techniques designed to improve detection of J-coupled metabolites. *Magn Reson Med, 60*(4), 964-969. doi:10.1002/mrm.21696

Mullins, P. G., McGonigle, D. J., O'Gorman, R. L., Puts, N. A., Vidyasagar, R., Evans, C. J., & Edden, R. A. (2014). Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. *Neuroimage, 86*, 43-52. doi:10.1016/j.neuroimage.2012.12.004

Nielsen, S., Gowing, L., Sabioni, P., & Le Foll, B. (2019). Pharmacotherapies for cannabis dependence. *Cochrane Database of Systematic Reviews*(1). doi:10.1002/14651858.CD008940.pub3

O'Gorman, R. L., Michels, L., Edden, R. A., Murdoch, J. B., & Martin, E. (2011). In vivo detection of GABA and glutamate with MEGA-PRESS: reproducibility and gender effects. *J Magn Reson Imaging, 33*(5), 1262-1267. doi:10.1002/jmri.22520

Olsen, R. W., & Betz, H. (2006). GABA and Glycine. In G. J. Siegel, R. W. Albers, S. T. Brady, & D. L. Price (Eds.), *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects* (pp. 291-301). London, UK: Elsevier Academic Press.

Otani, K., Ujike, H., Tanaka, Y., Morita, Y., Katsu, T., Nomura, A., Uchida, N., Hamamura, T., Fujiwara, Y., & Kuroda, S. (2005). The GABA type A receptor alpha5 subunit gene is associated with bipolar I disorder. *Neurosci Lett, 381*(1-2), 108-113. doi:10.1016/j.neulet.2005.02.010

Pancheri, C., Verdolini, N., Pacchiarotti, I., Samalin, L., Delle Chiaie, R., Biondi, M., Carvalho, A. F., Valdes, M., Ritter, P., Vieta, E., & Murru, A. (2019). A systematic review on sleep alterations anticipating the onset of bipolar disorder. *European Psychiatry, 58*, 45-53. doi:https://doi.org/10.1016/j.eurpsy.2019.02.003

Patel, R., & Dickenson, A. H. (2016). Mechanisms of the gabapentinoids and α 2 δ-1 calcium channel subunit in neuropathic pain. *Pharmacology research & perspectives, 4*(2), e00205-e00205. doi:10.1002/prp2.205

Peng, P. W., Wijeysundera, D. N., & Li, C. C. (2007). Use of gabapentin for perioperative pain control -- a meta-analysis. *Pain Res Manag, 12*(2), 85-92.

Perlis, R. H., Dennehy, E. B., Miklowitz, D. J., Delbello, M. P., Ostacher, M., Calabrese, J. R., Ametrano, R. M., Wisniewski, S. R., Bowden, C. L., Thase, M. E., Nierenberg, A. A., & Sachs, G. (2009). Retrospective age at onset of bipolar disorder and outcome during two-year follow-up: results from the STEP-BD study. *Bipolar Disord, 11*(4), 391-400. doi:10.1111/j.1399-5618.2009.00686.x

Petroff, O. A., Hyder, F., Rothman, D. L., & Mattson, R. H. (2000). Effects of gabapentin on brain GABA, homocarnosine, and pyrrolidinone in epilepsy patients. *Epilepsia, 41*(6), 675-680.

Petroff, O. A., Rothman, D. L., Behar, K. L., Lamoureux, D., & Mattson, R. H. (1996). The effect of gabapentin on brain gamma-aminobutyric acid in patients with epilepsy. *Ann Neurol, 39*(1), 95-99. doi:10.1002/ana.410390114

Petty, F., Kramer, G. L., Dunnam, D., & Rush, A. J. (1990). Plasma GABA in mood disorders. *Psychopharmacol Bull, 26*(2), 157-161.

Phillips, M. L., Travis, M. J., Fagiolini, A., & Kupfer, D. J. (2008). Medication effects in neuroimaging studies of bipolar disorder. *Am J Psychiatry, 165*(3), 313-320. doi:10.1176/appi.ajp.2007.07071066

Pinto, J. V., Medeiros, L. S., Santana da Rosa, G., Santana de Oliveira, C. E., Crippa, J. A. d. S., Passos, I. C., & Kauer-Sant'Anna, M. (2019). The prevalence and clinical correlates of cannabis use and cannabis use disorder among patients with bipolar disorder: A systematic review with meta-analysis and meta-regression. *Neuroscience & Biobehavioral Reviews, 101*, 78-84. doi:https://doi.org/10.1016/j.neubiorev.2019.04.004

Posner, K., Brown, G. K., Stanley, B., Brent, D. A., Yershova, K. V., Oquendo, M. A., Currier, G. W., Melvin, G. A., Greenhill, L., Shen, S., & Mann, J. J. (2011). The Columbia-Suicide Severity Rating Scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. *Am J Psychiatry, 168*(12), 1266-1277. doi:10.1176/appi.ajp.2011.10111704

Prescot, A. P., Locatelli, A. E., Renshaw, P. F., & Yurgelun-Todd, D. A. (2011). Neurochemical alterations in adolescent chronic marijuana smokers: a proton MRS study. *Neuroimage, 57*(1), 69-75. doi:10.1016/j.neuroimage.2011.02.044

Prescot, A. P., & Renshaw, P. F. (2013). Two-dimensional J-resolved proton MR spectroscopy and prior knowledge fitting (ProFit) in the frontal and parietal lobes of healthy volunteers: assessment of metabolite discrimination and general reproducibility. *J Magn Reson Imaging, 37*(3), 642-651. doi:10.1002/jmri.23848

Prisciandaro, J. J., Brown, D. G., Brady, K. T., & Tolliver, B. K. (2011). Comorbid anxiety disorders and baseline medication regimens predict clinical outcomes in individuals with co-occurring bipolar disorder and alcohol dependence: Results of a randomized controlled trial. *Psychiatry Res, 188*(3), 361-365. doi:10.1016/j.psychres.2011.04.030

Prisciandaro, J. J., Mellick, W., Mitaro, E., & Tolliver, B. K. (2019). An evaluation of the impact of co-occurring anxiety and substance use disorder on bipolar disorder illness outcomes in STEP-BD. *J Affect Disord, 246*, 794-799. doi:10.1016/j.jad.2018.12.070

Prisciandaro, J. J., Tolliver, B. K., Prescot, A. P., Brenner, H. M., Renshaw, P. F., Brown, T. R., & Anton, R. F. (2017). Unique prefrontal GABA and glutamate disturbances in co-occurring bipolar disorder and alcohol dependence. *Translational Psychiatry, 7*(7), e1163-e1163. doi:10.1038/tp.2017.141

Provencher, S. W. (1993). Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med, 30*(6), 672-679.

Ray, L. A., Bujarski, S., Roche, D. J. O., & Magill, M. (2018). Overcoming the "Valley of Death" in Medications Development for Alcohol Use Disorder. *Alcohol Clin Exp Res, 42*(9), 1612-1622. doi:10.1111/acer.13829

Rigucci, S., Xin, L., Klauser, P., Baumann, P. S., Alameda, L., Cleusix, M., Jenni, R., Ferrari, C., Pompili, M., Gruetter, R., Do, K. Q., & Conus, P. (2018). Cannabis use in early psychosis is associated with reduced glutamate levels in the prefrontal cortex. *Psychopharmacology (Berl), 235*(1), 13-22. doi:10.1007/s00213-017-4745-z

Roberto, M., Gilpin, N. W., O'Dell, L. E., Cruz, M. T., Morse, A. C., Siggins, G. R., & Koob, G. F. (2008). Cellular and behavioral interactions of gabapentin with alcohol dependence. *J Neurosci, 28*(22), 5762-5771. doi:10.1523/jneurosci.0575-08.2008

Rose, M. A., & Kam, P. C. (2002). Gabapentin: pharmacology and its use in pain management. *Anaesthesia, 57*(5), 451-462.

Sarvet, A. L., Wall, M. M., Keyes, K. M., Olfson, M., Cerdá, M., & Hasin, D. S. (2018). Self-medication of mood and anxiety disorders with marijuana: Higher in states with medical marijuana laws. *Drug Alcohol Depend, 186*, 10-15. doi:10.1016/j.drugalcdep.2018.01.009

Schwilke, E. W., Gullberg, R. G., Darwin, W. D., Chiang, C. N., Cadet, J. L., Gorelick, D. A., Pope, H. G., & Huestis, M. A. (2011). Differentiating new cannabis use from residual urinary cannabinoid excretion in chronic, daily cannabis users. *Addiction, 106*(3), 499-506. doi:10.1111/j.1360-0443.2010.03228.x

Sills, G. J. (2006). The mechanisms of action of gabapentin and pregabalin. *Curr Opin Pharmacol, 6*(1), 108-113. doi:10.1016/j.coph.2005.11.003

Smith, R. V., Havens, J. R., & Walsh, S. L. (2016). Gabapentin misuse, abuse and diversion: a systematic review. *Addiction, 111*(7), 1160-1174. doi:10.1111/add.13324

Sobell, L. C., & Sobell, M. B. (1995). *Alcohol Timeline Followback Users' Manual.* Toronto, Canada: Addiction Research Foundation.

Soeiro-de-Souza, M. G., Henning, A., Machado-Vieira, R., Moreno, R. A., Pastorello, B. F., da Costa Leite, C., Vallada, H., & Otaduy, M. C. (2015). Anterior cingulate Glutamate-Glutamine cycle metabolites are altered in euthymic bipolar I disorder. *Eur Neuropsychopharmacol, 25*(12), 2221-2229. doi:10.1016/j.euroneuro.2015.09.020

Swann, A. C., Lijffijt, M., Lane, S. D., Steinberg, J. L., & Moeller, F. G. (2009). Severity of bipolar disorder is associated with impairment of response inhibition. *J Affect Disord, 116*(1-2), 30-36. doi:10.1016/j.jad.2008.10.022

Taylor, C. P., & Harris, E. W. (2020). Analgesia with Gabapentin and Pregabalin May Involve NMDA Receptors, Neurexins and Thrombospondins. *Journal of Pharmacology and Experimental Therapeutics*, jpet.120.266056. doi:10.1124/jpet.120.266056

van Rossum, I., Boomsma, M., Tenback, D., Reed, C., & van Os, J. (2009). Does cannabis use affect treatment outcome in bipolar disorder? A longitudinal analysis. *J Nerv Ment Dis, 197*(1), 35-40. doi:10.1097/NMD.0b013e31819292a6

van Veenendaal, T. M., DM, I. J., Aldenkamp, A. P., Hofman, P. A., Vlooswijk, M. C., Rouhl, R. P., de Louw, A. J., Backes, W. H., & Jansen, J. F. (2015). Metabolic and functional MR biomarkers of antiepileptic drug effectiveness: A review. *Neurosci Biobehav Rev, 59*, 92-99. doi:10.1016/j.neubiorev.2015.10.004

Veldic, M., Millischer, V., Port, J. D., Ho, A. M.-C., Jia, Y.-F., Geske, J. R., Biernacka, J. M., Backlund, L., McElroy, S. L., Bond, D. J., Villaescusa, J. C., Skime, M., Choi, D.-S., Lavebratt, C., Schalling, M., & Frye, M. A. (2019). Genetic variant in SLC1A2 is associated with elevated anterior cingulate cortex glutamate and lifetime history of rapid cycling. *Translational Psychiatry, 9*(1), 149. doi:10.1038/s41398-019-0483-9

Vieta, E., Manuel Goikolea, J., Martinez-Aran, A., Comes, M., Verger, K., Masramon, X., Sanchez-Moreno, J., & Colom, F. (2006). A double-blind, randomized, placebo-controlled, prophylaxis study of adjunctive gabapentin for bipolar disorder. *J Clin Psychiatry, 67*(3), 473-477.

Vogt, B. A. (2016). Midcingulate cortex: Structure, connections, homologies, functions and diseases. *J Chem Neuroanat, 74*, 28-46. doi:10.1016/j.jchemneu.2016.01.010

Wang, P. W., Sailasuta, N., Chandler, R. A., & Ketter, T. A. (2006). Magnetic resonance spectroscopic measurement of cerebral gamma-aminobutyric acid concentrations in patients with bipolar disorders. *Acta Neuropsychiatrica, 18*(2), 120-126. doi:10.1111/j.1601-5215.2006.00132.x

Weinstock, L. M., Gaudiano, B. A., Wenze, S. J., Epstein-Lubow, G., & Miller, I. W. (2016). Demographic and clinical characteristics associated with comorbid cannabis use disorders (CUDs) in hospitalized patients with bipolar I disorder. *Compr Psychiatry, 65*, 57-62. doi:10.1016/j.comppsych.2015.10.003

Young, R. C., Biggs, J. T., Ziegler, V. E., & Meyer, D. A. (1978). A rating scale for mania: reliability, validity and sensitivity. *Br J Psychiatry, 133*, 429-435.

Yu, J., Wang, D. S., Bonin, R. P., Penna, A., Alavian-Ghavanini, A., Zurek, A. A., Rauw, G., Baker, G. B., & Orser, B. A. (2019). Gabapentin increases expression of δ subunit-containing GABA(A) receptors. *EBioMedicine, 42*, 203-213. doi:10.1016/j.ebiom.2019.03.008