PROTOCOL TITLE:

Neurobehavioral Effects of Cannabidiol in Youth Alcohol Use Disorder

PRINCIPAL INVESTIGATOR:

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1.0 Objectives / Specific Aims

Alcohol use is prevalent and problematic among youth, who are more likely than adults to initiate alcohol use, develop alcohol use disorder (AUD), and suffer lasting adverse alcohol-related consequences[1,2]. Despite the clear need for youth-targeted AUD treatments, established psychosocial and behavioral interventions offer limited efficacy, with very few youth achieving sustained alcohol abstinence or reduction[3]. Pharmacotherapies play a key role in bolstering substance use disorder treatment outcomes in adults, but to date, no medications for AUD in youth have merited FDA approval. The development of safe and effective adjunctive medications to treat adolescent AUD is needed to improve treatment outcomes and to potentially reduce the long-term consequences of adolescent use.

Cannabidiol (CBD), one of the main phytocannabinoids in the *Cannabis sativa* plant[4], is a potentially promising candidate pharmacotherapy for youth AUD. It is particularly appealing as a youth treatment option since it is non-intoxicating[4,5], appears generally well-tolerated, and demonstrates no signal of abuse liability[6]. Further, young people tend to have a more positive attitude towards natural or alternative medicine[7,8]. CBD has many potential targets within the central nervous system that may mitigate the symptoms of AUD[9,10] via modulation of the glutamatergic, GABAergic (gamma aminobutyric acid), dopaminergic, opioidergic, and endocannabinoid pathways[11-14]. Preclinical work has shown that CBD affects an array of drinking behaviors (e.g., reduces ethanol seeking and intake; mitigates symptoms of withdrawal, relapse, anxiety, and impulsivity)[9,10,15], and recent clinical work has indicated CBD's potential to reduce alcohol intake within adults who endorse alcohol and cannabis co-use[16]. CBD's safety profile, potential mechanistic targets, and preliminary effects on alcohol-related behaviors in humans warrants research to assess the neural mechanisms and acute effects of CBD among youth with AUD.

Translational medication screening studies have the potential to rigorously evaluate the acute effects of promising compounds, such as CBD, for neural and behavioral target engagement prior to initiating large-scale efficacy trials. Magnetic resonance spectroscopy (MRS) and functional MRI (fMRI) are two translational, developmentally appropriate techniques that can be used in humans to assess the neural mechanisms of pharmacotherapies[17,18]. MRS measures metabolite levels in the brain that are neural treatment targets (e.g., glutamate, GABA) and fMRI localizes and quantifies brain activity. Utilizing these neuroimaging methods will allow for investigation into the neural effects of CBD on two important aspects of AUD: modulation of 1) *in vivo* glutamate and GABA levels, two neurotransmitter systems involved in addictive behaviors[19-21] that have been proposed as therapeutic targets of CBD[13,14,22], and 2) brain reactivity to alcohol cues, which can be reliably measured with fMRI in heavy drinking youth[23] and correlates with alcohol craving[24]. Establishing the acute neurometabolic and neurobehavioral effects of CBD in youth with AUD will be a critical first step in the pharmacotherapy development pipeline before initiating larger scale trials.

Consistent with the trans-NIH initiative to identify neurally-informed novel substance use treatments for youth, the goal of this application is to test CBD as a potentially effective candidate medication for youth with AUD by leveraging developmentally informed neuroimaging methods (MRS, fMRI) and lab-based paradigm procedures. To accomplish this goal, this study will use a randomized, double-blind, within-subjects crossover design. In counterbalanced order, 50 youth (ages 16-22) who meet criteria for AUD (≥ 2 symptoms) will receive 600mg of CBD[25-30] or placebo with a standardized snack (to modulate CBD absorption rates) three hours before a neuroimaging and behavioral assessment paradigm, separated by an approximate 18-day washout period[31].

Aim 1: *Quantify neurometabolic effects of CBD using MRS in youth with AUD:* Youth with AUD will show increased levels of glutamate and GABA in the anterior cingulate after taking CBD compared to placebo.

Aim 2: *Quantify neurobehavioral effects of CBD using fMRI alcohol cue reactivity in youth with AUD:* Youth with AUD will show decreased cue reactivity in reward-related neural regions after taking CBD compared to placebo.

Aim 3: *Examine psychophysiological effects of CBD during alcohol cues: In vivo* response to olfactory alcohol cues (measured via heart rate, skin conductance, and subjective ratings) will be lower post CBD vs. placebo.

Adolescence and young adulthood are critical periods of time for intervention and treatment for emerging alcohol-related problems, and pharmacotherapies can provide significant support to evidence-based psychosocial interventions. In this proposal, we will leverage neuroimaging and behavioral data to examine the acute neurometabolic, neurobehavioral, and psychophysiological effects of CBD on youth with AUD to assess CBD's promise as a potential adjunctive medication for youth AUD. Findings will bridge a critical translational gap ("the valley of death"[32]) in pharmacotherapy development for youth AUD, advancing methodology for rigorous neural-behavioral early efficacy testing of CBD. Effects established through this study could pave the way to a larger-scale clinical trial and, ultimately, improved long-term outcomes for young people suffering from AUD.

2.0 Background

SIGNIFICANCE

Alcohol use during adolescence and emerging adulthood is problematic and has long-term consequences. Alcohol is the most used substance among young people; 55% of 12th graders[33] and 72% of 18-25 year olds[34] endorsed past year alcohol use. Youth alcohol use is related to serious psychosocial problems, including comorbid psychopathology[35-39], poorer academic success[40], and detrimental neurocognitive consequences[41,42]. Further, early alcohol initiation increases the risk of subsequent alcohol use disorder (AUD) and related problems[1,2,43]. Nearly 15% of youth meet the diagnostic criteria for AUD by age 18[44], and alarmingly, half of individuals that meet the criteria for lifetime AUD do so by the age of 21[45].

Existing youth substance use treatments are inadequate. Effective treatments during adolescence and young adulthood are critical for improving long-term outcomes. Current treatments for youth AUD are primarily psychosocial, such as cognitive behavioral therapy, family-based therapy, and motivational interviewing[3]. Psychosocial interventions have shown small to medium effects on reducing substance use during adolescence[46,47], and up to half of youth return to substance use within 12 months following treatment[48-50]. Given the modest efficacy of current psychosocial treatments, pharmacotherapy has been explored as a potential complement to the standard of care to improve outcomes[51]. However, there are limited data regarding the efficacy of pharmacotherapy in treating youth AUD, and the safety and efficacy of adult AUD medications cannot be extrapolated to youth[52]. This calls for the evaluation of treatments specifically for youth with AUD to improve treatment outcomes and to potentially reduce the long-term consequences of youth use. Natural or alternative medicine options may be particularly appealing for youth[7,8].

Cannabidiol (CBD) may be a promising pharmacotherapy for youth AUD. CBD is a non-intoxicating constituent of the *Cannabis sativa* plant that has garnered attention as an alternative therapy because of its wide range of therapeutic properties, including its effects on addictive behaviors[53]. Specifically, there is a growing preclinical literature[9] indicating that CBD reduces: alcohol consumption and motivation to drink[54-57]; alcohol relapse and withdrawal[55,58]; and alcohol-related neurotoxicity[59,60]. A study of adult alcohol and cannabis co-users found that high CBD cannabis (23% CBD, 1% THC) was related to a decrease in alcohol consumption over 5 days which was not seen in the THC (24% THC, 1% CBD) or THC plus CBD (9% THC, 10% CBD) groups[16].

CBD's effects on alcohol consumption and AUD symptoms may be due to its multiple brain targets that overlap with systems underlying AUD. Inside the endocannabinoid system (ECS), CBD can act as an inverse agonist or negative allosteric modulator of cannabinoid receptors (CB1, CB2)[12,61-63] and can change ECS signaling through inhibiting the enzymatic breakdown of anandamide (endogenous cannabinoid ligand)[64,65]. CB1 and CB2 receptors are expressed throughout the mesocorticolimbic pathway, which is highly implicated in addictive behaviors (e.g., reward, decision-making, substance intake, motivation, withdrawal, and relapse)[66-69]. Thus, CBD may exert its effects on AUD symptoms through indirect modulation of the ECS. Outside of the ECS, CBD has been shown to modulate glutamate[13,22], gamma aminobutyric acid (GABA)[14], dopamine[70], serotonin[71,72], and opioid[73] neurotransmission, all of which are crucial systems implicated in AUD and have each been proposed as treatment targets for adult AUD[15]. While it is harder to assess neural mechanisms in humans, CBD has been shown to increase glutamate levels in individuals with psychosis[25] and austim spectrum disorder[26] through specialized

neuroimaging techniques (discussed more below). Overall, CBD's mechanism of action makes it a promising potential candidate medication for youth with AUD.

In addition to the preliminary behavioral and mechanistic data, CBD has a record of general safety and tolerability[6,74] with limited adverse events[75] or abuse liability[6,76,77]. Due to the lack of data in humans, which is even more pronounced in youth, it is important to rigorously test the acute neural and alcohol-related behavioral effects of CBD as a candidate medication for youth AUD before moving forward with large scale clinical trials.

Lab-based paradigms can provide a critically important intermediary step in medication development, from preclinical to efficacy trials. There is a pressing need to accelerate the pace of medication development for youth AUD by focusing on moving candidate compounds through the drug development pipeline. Human medication screening paradigms can provide an essential bridge to test promising preclinical findings in humans before lengthy large-scale efficacy trials are initiated.

Magnetic resonance imaging (MRI) techniques provide promising non-invasive methods to test neural mechanisms of a candidate medication[78]. Two techniques that are particularly relevant for medication development and are also developmentally-appropriate to use with youth are magnetic resonance spectroscopy (MRS) and functional MRI (fMRI)[79]. **MRS** quantifies metabolites in the brain, including glutamate and GABA levels, which are affected by substance use and are therefore targets for substance use medication development[79-81]. Alcohol alters glutamate neurotransmission[82], which is related to GABAergic functioning, further supporting the use of MRS to test medications for AUD. **fMRI** localizes and quantifies brain activity, allowing for mechanistic understanding of neural substrates affected by the candidate medication. fMRI cue reactivity tasks activate incentive salience and reward circuitry[83], and these cue-related brain activation patterns have been modulated by pharmacological intervention; predicted later relapse; and are associated with alcohol craving[84]. Cue reactivity can also be assessed during an olfactory cue reactivity task (measured via heart rate, skin conductance, and subjective ratings)[85] to better understand psychophysiological effects of potential medications.

Summary. Adolescence and young adulthood are critical periods of time to intervene with emerging AUD and alcohol-related issues. Pharmacological interventions have the potential to bolster the efficacy of psychosocial treatments for youth AUD; however, they must be tailored to youth rather than extrapolating from the adult literature. CBD is a promising candidate pharmacotherapy for youth AUD due to its safety profile, proposed mechanisms, and signal for reducing alcohol-related behaviors in preclinical and clinical work. The purpose of this application is to conduct a translational pilot study to examine the neural, behavioral, and psychophysiological effects of CBD in youth with AUD for the first time. Effects established through this study could pave the way to a larger-scale clinical trial and, ultimately, improved long-term outcomes for youth suffering from AUD.

INNOVATION

MRS- and fMRI-based biomarkers and lab-based paradigms offer novel translational testing of the neural mechanisms of CBD in humans. A key element in translating candidate medications from preclinical to human trials is evaluation of common neural effects; MRS and fMRI provide low-risk, non-invasive means to safely and reliably assess the effects of CBD on youth with AUD. *In vivo* response to olfactory alcohol cues will help further illuminate CBD's effect on cue reactivity to better understand the potential mechanism of CBD's action.

To our knowledge, there are no published trials examining CBD as a potential treatment for youth with AUD or any other substance use disorder during adolescence or young adulthood, and according to clinicaltrials.gov, no trials are currently underway. This will be the first trial examining CBD as a potential candidate medication for youth with AUD. Due to its reported safety profile and tolerability, CBD is well-suited for a youth-specific pharmacotherapy translational trial in AUD.

APPROACH

Pilot Data/Feasibility

Team. The interdisciplinary research team consists of a clinical neuropsychologist/neuroimager (Squeglia), a licensed child and adolescent psychiatrist (Gray), a clinical psychologist/statistician (Tomko), and a neuroimaging postdoctoral fellow (Kirkland) with complementary expertise in AUD, youth substance use, neuroimaging, assessment and psychometrics, statistical analysis, and alcohol biomarkers. Our team recently completed five randomized placebo-controlled trials of pharmacotherapy and cognitive interventions for youth with substance use disorder (SUDs)[86-90] and currently have four studies underway. Several of the on-going studies will complete recruitment prior to the initiation of the proposed study, limiting competing enrollment across studies.

Neuroimaging and behavioral measures. Our team has extensive experience neuroimaging youth with the proposed methods. Dr. Squeglia has performed over 200 scans on substance-using youth (ages 15-21), using the proposed fMRI and MRS protocol, with over 95% usable data. In a pilot study using the same fMRI alcohol cue reactivity task proposed for this application[91], 11 heavy drinking youth displayed robust activation in several brain regions including the anterior cingulate, insula, striatum, and amygdala during alcohol vs. non-alcohol cue trials, which is highly consistent with activation patterns seen in adult studies[92]. Participants will undergo an olfactory alcohol cue exposure procedure which is consistent with published procedures utilized with adolescents at MUSC[85].

3.0 Intervention to be studied

CBD is a promising candidate pharmacotherapy for youth AUD due to its safety profile, proposed mechanisms, and signal for reducing alcohol-related behaviors in preclinical and clinical work. This double blind crossover trial will compare one dose of acute oral CBD (600 mg[25-27,29]) and placebo, followed by an approximate 18 day washout to allow for CBD clearance[31] (terminal half-life 18-32 hours after acute oral dose)[93].

Though CBD is often well-tolerated, it can cause side effects, such as dry mouth, diarrhea, reduced appetite, drowsiness, and fatigue. CBD can also interact with other medications that some people take, such as blood thinners. These side effects are usually mild and go away even with continued use of CBD.

"Increased risk of suicidal thoughts or behavior" is a CBD risk per package insert. However, a recently published meta-analysis indicates no risk for increased suicidality with CBD at this dose or over chronic dosing periods (Klein et al., 2021). Consistent with all of our youth substance use medication studies, we carefully assess participant suicidality and manage appropriately in the event of any compromise to safety.

We do not predict severe adverse events from a single 600 mg CBD dose. All participants will be evaluated by the study medical clinician. The DSMB will review adverse events every 6 months. Concurrent medications with potential interactions with CBD are included in the exclusion criteria. Medication response and tolerability/adverse events will be assessed at each visit.

The suggested maximum target daily dosing for children with epilepsy is 25 mg/kg/day; therefore, even for a low-weight adolescent, the proposed single dose is well within target range of tolerability. A previous within-subjects study in adults with autism spectrum disorder used an acute oral dose of CBD (600 mg) and successfully prevented carry-over effects with a 13-day washout period [26]. Further, a single dose of 600 mg has been shown to modulate brain metabolite levels measured with MRS[25,26] and blood oxygen level dependent (BOLD) signal measured with fMRI[27-30] as compared to placebo in clinical populations (autism spectrum disorder, clinical high risk for psychosis, and psychosis) and healthy controls.

Most studies in children, adolescents, or young adults are long-term studies examining the effects of CBD within epilepsy. CBD doses in those studies usually range between 0.5 mg/kg/day to 28.6 mg/kg/day, with 37% reporting drowsiness and 16% reporting fatigue[94]. In adult studies giving an acute dose of 600 mg, there have been either no adverse events[95-97] or low rates for sedation or tiredness (e.g., 15%[98]; 22%[99]). There are currently no studies on the effect of oral purified CBD on driving ability, but one study found no differences on driving performance when comparing higher CBD-cannabis flower (smoked) as compared

to placebo at 40 or 240 minutes after use[100]. We will carefully evaluate drowsiness and fatigue before participants are able to leave from the facility, and we will arrange safe transportation accordingly.

The metabolism and absorption of CBD can be modulated by food intake, with a 4-to-5 fold increase in bioavailability after a high-fat meal[74]; thus, all participants will eat a standardized high-fat snack with each medication administration. Neuroimaging will be conducted 3 hours after CBD/placebo administration to allow for CBD to reach its peak plasma concentration[26,101]. PI Squeglia received an FD exemption for using Epidiolex (CBD) with adolescent substance users (IND161500).

4.0 Study Endpoints

Type	Endpoint	Time Frame	Brief Description
Primary	Brain Metabolites	Visit 1 and Visit 2	MRS quantifies metabolites in the brain, including glutamate and GABA levels, which are affected by substance use and are therefore targets for substance use medication development[79-81].
Primary	fMRI Alcohol Cue-Reactivity	Visit 1 and Visit 2	fMRI localizes and quantifies brain activity, allowing for mechanistic understanding of neural substrates affected by the candidate medication. fMRI cue reactivity tasks activate incentive salience and reward circuitry[83], and these cue-related brain activation patterns have been modulated by pharmacological intervention; predicted later relapse; and are associated with alcohol craving[84]
Primary	Psychophysiol ogical cue reactivity	Visit 1 and Visit 2	Cue reactivity can also be assessed during an olfactory cue reactivity task (measured via heart rate, skin conductance, and subjective ratings)[85] to better understand psychophysiological effects of potential medications.

5.0 Inclusion and Exclusion Criteria/ Study Population

Youth (ages 16-22; 50% women) alcohol users (N=50) will be recruited for the current study. To promote consistency between human laboratory models and clinical trials, we will recruit youth who meet criteria for AUD ($\geq$ 2 symptoms)[102]. At the end of the trial, all participants will have the opportunity to meet with a trained clinician for a brief treatment session from NIAAA's brief alcohol intervention, which includes motivational interviewing and setting individual goals and action plans, consistent with the current standard-of-care treatment. We will also offer referral to additional clinical services, as well as provide all participants with a handout listing all community mental health and substance use programs.

Participants having completed a separate ongoing research study (PRO #94743) who have consented to future contact and meet study criteria will be offered participation.

Inclusion Criteria

(1) ages 16-22

(2) participants ages 16-17: a parent or legal guardian must be able to provide informed consent and youth must be able to provide assent

(3) participants ages 18-22: must be able to provide informed consent

(4) AUD in the past year and at least one current (past 30 days) continued symptom besides craving

(5) have used alcohol in the past two weeks before screening.

Exclusion Criteria

(1) significant or acutely unstable medical, psychiatric, or substance use problems (e.g., current manic episode, positive psychotic symptoms, severe eating disorder, severe opioid use disorder) that would contraindicate research procedures, interfere with safety, compromise data integrity, or preclude consistent study participation

(2) significant risk of homicide or suicide

(3) currently enrolled in or acutely seeking treatment for AUD or any other SUD

(4) pregnant, trying to become pregnant, or breastfeeding

(5) known allergy or intolerance to CBD

(6) current use of CBD or any supplement containing CBD

(7) history of a serious medical or neurological problem that could affect neural response or brain development

(8) non-correctable visual or hearing problems

(9) MRI contraindications (e.g., braces, claustrophobia, irremovable metal implants or piercings)

(10) acute drunkenness or consumption of alcohol within 12 hours of visit

(11) ≥10 on the Clinical Institute Withdrawal Assessment for Alcohol (CIWA)

(12) severe Cannabis Use Disorder (CUD)

(14) concurrent medications with potential drug-drug interactions with CBD, including CYP3A4 and CYP2C19 substrates, inhibitors, and inducers, as well as CYP2C8/9 substrates.

Age Range: This work emphasizes capturing neurobehavioral effects of CBD during early life AUD, which will require a broad age range as drinking habits and consequences can widely vary among youth, while limiting neural and AUD heterogeneity within the sample. The CDC defines adolescence as ages 10-24. While some youth initiate alcohol use before age 16, few meet criteria for AUD[103]. Therefore, we chose a lower age limit of 16 and upper limit of 22 to reduce heterogeneity, while still sampling across the period when substance use problems emerge and peak. We will complete exploratory analyses within age and education-level subgroups to probe the confounding effect of developmental heterogeneity. Creation of alcohol-focused interventions during adolescence could have substantial long-term implications by reducing acute and long-term negative social, academic, and cognitive consequences related to heavy teen drinking and by reducing the rate of youth transitioning from heavy drinking to more problematic alcohol dependence. While participants in this study are not treatment seeking, all participants will be given treatment referrals at the conclusion of the study.

Diverse Population: In this cohort, approximately 50% of the participants will be female, and we aim to recruit participants to approximate the racial and ethnic composition of Charleston County.

6.0 Number of Subjects

We propose to enroll 50 participants.

7.0 Setting

Visits will take place at MUSC in the Institute of Psychiatry, Roper Office Medical Building, and the Biomedical Imaging MRI research facility at 30 Bee St (for those visits requiring an MRI). If a participant is

unable to attend or complete a visit due to unexpected conflict (e.g., transportation issues, travel, University closings), arrangements may be made to remotely complete as much of the visit procedures as possible to maintain data collection and study engagement. This will be done via Zoom and REDCap assessments.

8.0 Recruitment Methods

All participants will be recruited from a pool of participants who have completed an ongoing Entryway Intake study (PRO #94743). Participants who have completed this protocol and who appear to meet eligibility criteria will be offered participation in this protocol. Individuals who participate in the Entryway Intake consent for their data to be carried forward into the study in which they ultimately enroll. The Entryway Intake includes, but is not limited to, a comprehensive substance use history assessment and a structured diagnostic interview for psychiatric conditions, including assessment of AUD and other substance use disorders, as well as bioassay collection. After completion of the Entryway Intake, participants who are eligible for the current study will be offered participation. They will consent to sharing data collected across both protocols for analysis. Since launching the shared Entryway Intake in September 2020, we have averaged 33 referrals per month of youth (ages 16-22) interested in participating in alcohol studies. In the past 10 months, we have had 74 youth in this age range complete our shared Intake process. Of the total intakes, 41 (55%) met criteria for AUD, of which 20 (49%; 2/month) met for moderate or severe AUD. This is consistent with our proposed recruitment rate of 1.8/month. Study staff may also use the following methods of advertising to promote recruitment: local publications, physician offices/local clinics, MUSC campus, local college and high school campuses as permissions are granted by the academic institution, internet, social media, TV commercials, and other locations (e.g., restaurants, movie theaters, malls, buses/transportation services) in the community that agree to post Brand and IRB approved recruitment materials for this study and that may reach our target population.

Our team has been successful with optimizing participant retention via several well-established strategies. We maintain active communication with participants between visits via their preferred mode of contact (e.g., text message, e-mail). We strictly maintain confidentiality, a particularly significant issue in studies focused on youth substance use interventions, and our team has extensive experience in managing communication with study participants while maintaining appropriate bounds of confidentiality and managing issues of safety; the resultant trust and rapport supports participant adherence and retention.

9.0 Consent Process

Prior to the initiation of any study procedures, the MUSC Institutional Review Board (IRB) written and approved Informed Consent (IC) and HIPAA authorization will be obtained by IRB-approved, trained, designated research staff in a private interview room. Potential participants (and parents/guardians, as appropriate) will be provided with a copy of the IRB-approved consent form prior to their visit. After the Entryway Intake and prior to initiating any specific study procedures, research staff will obtain written informed consent and provide a copy of HIPAA privacy practices to potential participants. Parents/guardians will participate in the informed consent/assent procedures for youth between the ages of 16-17. Participants 18 years and older will provide their own informed consent. The complex issues of informed consent and assent, and related limitations of confidentiality, as they apply to youth and their parents/guardians, are understood by the research team and will be communicated clearly during the visit. Trained research staff members complete online and lab-based training in HIPAA policy, human subjects' research, and management of other research issues. During the consent phase, participants and parents are informed that all information provided is confidential within ethical and legal limits to facilitate disclosure. During the consenting process, parents are told that they will not be informed about their child's substance use and that youth self-report and lab data are confidential, except for any acute safety issues (e.g., suicidality, abuse). After given the chance to answer any questions concerning the study, individuals who agree to participate will be asked to sign the informed consent form. As part of the informed consent procedures, participants will be asked to provide or decline consent to be contacted for future studies. The forms are reviewed with the interested parties and filed in a locked file cabinet in a locked office. Trained research staff members complete online and lab-based training in HIPAA policy, human subjects' research, and management of other research issues.

We may also use several different methods to complete electronic informed consent, if applicable and necessary, that include the following: 1) REDCap electronic consent (e-consent) combined with a video discussion on software approved by IT Security) or 2) via MUSC's doxy.me system (teleconsent). As a last resort, we will also email the consent document to the participant or parent/guardian and conduct the informed consent via video chat. Participants can then email or mail the signed consent back to the research team if needed. Video chat functionality will only be used if all parties have the capability and will be on software approved by the IT Security team at MUSC.

E-consent via REDCap will be saved in a separate informed consent database. All doxy.me signed consent forms will be saved as PDF files within our study records. Using these systems, signatures on the consent form may be obtained electronically via REDCap/doxy.me. These procedures for consenting remote study participants have been established in prior studies at MUSC (e.g., Pro# 19201). In the case that participants mail back hard copies of the consent (in rare instances), those will be stored in locked file cabinets in the offices of research staff.

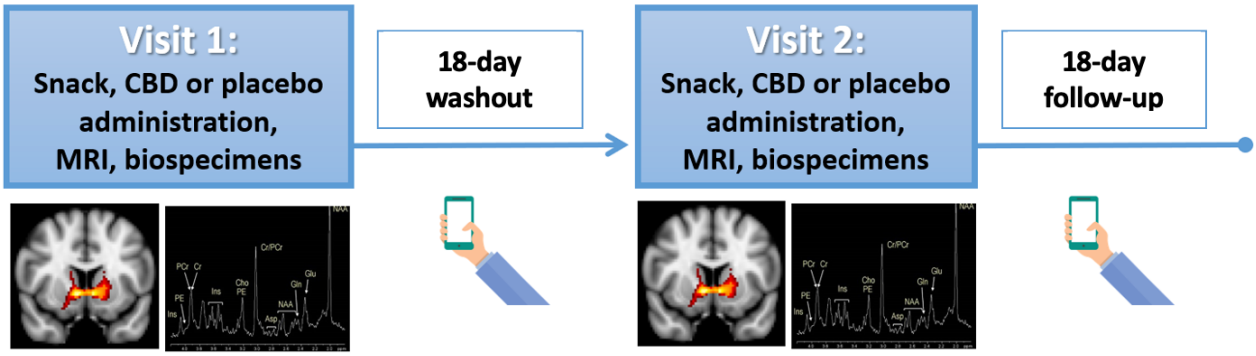
During the consent process birth certificate information will be collected from the participant (ie, first/middle/last name, birthdate, biological sex, and city/municipality of birth). This is in a separate REDCap file from data collection and is not part of our study data collection. This information is to be used solely for creating a Global Unique Identifier (GUID) for the NIMH Data Archive (NDA). This data is not transmitted to NDA but rather used in their online GUID Tool to create the GUID. The GUIDs created will be used as the participants' ID when submitting study data to the NDA. This is detailed in the consent form.

Informed consent is viewed as an ongoing process, so participants will be given the opportunity to ask questions about their participation throughout the course of the visit. The consent document will contain a thorough review of potential risks associated with participation, including breach of confidentiality and privacy concerns.

10.0 Study Design / Methods

Entryway Intake. All participants will complete a centralized Entryway Intake process for substance use studies at MUSC (i.e., the Entryway Intake (PRO #94743)) to determine eligibility before consenting for this project. This triaged approach allows for minimization of participant study shopping ("professional participants") and assurance that participants are triaged to the most appropriate study, thereby increasing the confidence and validity of study-specific results[104-106]. Eligibility criteria for all Youth Collaborative studies (including the proposed study) will be concurrently assessed during the Intake and participants will only be offered study participation in studies for which they are eligible.

Overview. Our primary goal is to test CBD as a potential candidate medication for youth with AUD, leveraging neuroimaging and behavioral data, to examine the acute neurometabolic, neurobehavioral, and psychophysiological effects. In counterbalanced order, youth (ages 16-22; 50% female) will receive 600 mg of CBD[25-30] or matched placebo with a standardized snack, separated by approximately 18 days[31] (see Figure below). We will stratify by education level (high school vs post high school) to ensure that these developmental groups are represented equally between the randomized crossover orders within our final sample. Neuroimaging will be collected after each medication trial: MRS will examine glutamate and GABA levels in the anterior cingulate cortex, and fMRI alcohol cue reactivity will examine neural response in reward networks, post-CBD and placebo. *In vivo* response to olfactory alcohol cues will be measured via heart rate, skin conductance, and subjective ratings[85].



Participant Procedures

CBD or placebo administration. This double blind crossover trial will compare acute oral CBD (600 mg[25-27,29]) and placebo, followed by an approximate 18 day washout to allow for CBD clearance[31] (terminal half-life 18-32 hours after acute oral dose)[93]. All participants will eat a standardized snack with each medication administration. Neuroimaging will be conducted 3 hours after CBD/placebo administration to allow for CBD to reach its peak plasma concentration[26,101]. Medication response and tolerability/adverse events will be assessed at each visit.

Assessments

We focused on inclusion of measures that are standardized for this age group, have good psychometric properties, and/or are part of the PhenX Toolkit or are included in the multisite Adolescent Brain and Cognitive Development study to yield comparable data to national datasets. See **Study Timetable (Table 1)**.

Clinical assessments. In general, these measures will be used to assess and track mental health and substance use-related problems over the course of study participation. Adverse events will be documented, rated for severity and relatedness, and managed/reported appropriately via established procedures. Participants will also meet with a medical clinician at each in person study visit to ensure physical and mental wellbeing throughout the study.

Primary Assessments: Entryway Intake Process (PRO #94743)

Physiological and Biological Assessments

Urine samples will be obtained from all participants to conduct pregnancy tests (female sex at birth only) and qualitative urine drug screens. For females, the pregnancy test will be completed first and a positive pregnancy test will immediately stop all subsequent procedures. No urine drug screen will be completed. An alcohol breath sample will be obtained from all participants to rule-out acute intoxication. A carbon monoxide sample will be collected via a carbon monoxide monitor. A saliva sample will be collected for profiling of microbial communities via 16S rRNA sequencing. Vital signs will be taken on all participants, including height, weight, blood pressure, and pulse.

Self-Report Measures

1. The *Demographic Form* was designed by our research team to assess basic demographics, including age, gender, race, and social history.

2. The *MacArthur Social Status Ladder* is a brief, 2-item measure with good psychometric properties (Operario, Adler, & Williams, 2004). It is designed to assess how individuals perceive their relative status in their community and nationally.

3. The *Health and Substance Use History Questionnaires* were designed by our team to assess basic health functioning and lifetime substance use exposure. Age of substance use onset will also be detailed.

4. The *Treatment Services Utilization Forms* were designed by our research team to assess history of substance use and psychiatric treatment utilization.

5. The *Pittsburgh Sleep Quality Index (PSQI; Buysee et al., 1989)* is a brief instrument designed to assess typical duration and quality of sleep.

6. The *Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q-SF; Schechter, Endicott, & Nee, 2007)* and the *Pediatric Quality of Life Enjoyment and Satisfaction Questionnaire (PQ-LES-Q-SF; Endicott, et al., 2006*) are 16-item and 15-item measures, respectively, designed to assess the degree to which one experiences joy and satisfaction. The pediatric version will be administered to youth ages 12-17, and the adult version will be administered to participants ages 18 and older.

7. *The Everyday Discrimination Scale – Short Version (Williams et al., 1997)* is a 6-item measure designed to assess experiences of discrimination due to race, ethnicity, gender, disability, physical appearance, or other attributes.

8. *The Beliefs about Medicines Questionnaire (Horne et al., 1999)* subscale related to general beliefs will be administered to assess attitudes toward medications. Three positively valanced items regarding medications were created by the study team and added to the measure, resulting in 11 total questions.

9. The *Mini International Neuropsychiatric Interview (MINI; Sheehan et al., 1998; Sheehan et al., 2010)* is a semi-structured interview designed to assess current, past, or lifetime history of major DSM 5 psychiatric and substance use disorder diagnoses. Based on the original MINI, an expanded version (MINI Plus) and a pediatric version (MINI Kid) have been developed and validated. The appropriate instrument (MINI Plus for participants ≥18 years and MINI Kid for participants <18 years) will be administered by trained staff.

10. The *Patient Health Questionnaire (PHQ-9; Kroenke et al., 2001)* is a 9-item scale used to assess DSM-IV symptoms of depression.

11. The *Generalized Anxiety Disorder scale (GAD-7; Spitzer et al., 2006)* is a 7-item measure used to assess symptoms of generalized anxiety.

12. The *UPPS-Revised-Child Impulsivity Scale (Zapolski et al., 2010; Gunn et al., 2010)* is a 40-item measure used to assess five facets of trait impulsivity among youth *(administered to participants ages 12-25)*.

13. *Difficulties in Emotion Regulation Scale Short Form (DERS-SF;* Kaufman, et al., 2015*)* is an 18-item self-report measure designed to assess six facets of emotion regulation problems among adolescents and adults: lack of emotion regulation strategies, non-acceptance of emotions, difficulties in goal-directed behavior, impulse control difficulties, lack of emotional awareness, and lack of emotional clarity.

14. *Mental Health Treatment Utilization Form*. This assessment includes a series of questions adapted from the National Survey on Drug Use and Health (a nationally representative SAMHSA-administered study) designed to assess adolescent current, past year, and lifetime mental health service utilization and reasons for service utilization.

15. *The Eating Disorder Screen for Primary Care (ESP) (Cotton et al., 2003)* is a 5-item measure to quickly screen for the presence of an eating disorder.

16. The *Timeline Follow Back* (*TLFB*; Sobell & Sobell, 1992) is a calendar-based assessment designed to enhance retrospective recall of substance use for the past 60 days. Quantity and frequency of alcohol, cannabis, tobacco/nicotine products (cigarettes, ENDS, other tobacco/nicotine products), and other drug use will be assessed. Grams of cannabis will be estimated. *Cannabis Methods*. This assessment is conducted concurrently with the TLFB and includes a series of questions to determine which routes of administration participants are employing to use cannabis.

17. The Rapid Eating Assessment for Participants- Shortened Version (REAP-S; Segal-Isaacson 2004) is a brief validated questionnaire designed to quickly assess nutrient intake.

Secondary Assessments: Entryway Intake Process

(The following measures will be administered depending on responses to TLFB):

18. The *Marijuana Assessment Problem Inventory (MAPI; Johnson & White, 1989)* is a 23-item scale assessing consequences of cannabis use. This will be administered if past 60-day cannabis use is endorsed on TLFB.

19. *Rutgers Alcohol Problem Index (RAPI; White & Labouvie, 1989)* is a 23-item questionnaire assessing consequences of alcohol use. This will be administered if past 60-day alcohol use is endorsed on TLFB.

20. The *Stages of Change Readiness and Treatment Eagerness Scale (SOCRATES; Miller & Tonigan, 1996)* is a 19-item questionnaire designed to assess readiness to change drug use. Instructions will be modified for cannabis and alcohol use. These will be administered if past 60-day cannabis or alcohol use is endorsed on TLFB, respectively.

21. *Thoughts About Abstinence Scale (e.g., Substance Use Goals; Hall et al., 1991)* assesses whether participants are interested in total abstinence, reduced use, or no change in substance use behavior for each of the following substances: alcohol, cannabis, e-cigarettes/nicotine vaping, regular cigarettes. There are 4 items in total (one for each substance).

22. *Reasons for Quitting Questionnaire-Cannabis (Steinberg et al., 2005)* is a 27-item scale designed to assess common reasons for wanting to quit using cannabis. The scale will be modified to reflect quitting or reducing cannabis use and will only be administered to participants who report an abstinence or reduced use goal for cannabis on the “Thoughts About Abstinence Scale”. Note that only items 1-27 will be administered.

23. The *Motivation to Quit Measure (Turner & Mermelstein, 2004)* is a single item assessing motivation to quit smoking. This has been adapted to additionally assess motivation to quit vaping or e-cigarette use in the current study. These will be administered if past 60-day cigarette or e-cigarette use is endorsed on TLFB, respectively.

24. The *Addiction Growth Mindset Questionnaire* (*Burnette et al., 2019*) is a 3-item questionnaire designed to assess whether individuals see their substance use and behaviors as malleable or static in nature.

25. The *Modified Fagerström Tolerance Questionnaire (mFTQ; Prokhorov et al., 2000)* is a 7-item self-rating questionnaire assessing nicotine dependence in the adolescent population and it will be given to participants that endorse tobacco use in the past 60 days on TLFB.

26. *Penn State Electronic Cigarette Dependence Index (Foulds et al., 2015)* is a 10-item questionnaire assessing electronic cigarette dependence, given to participants that endorse past 60-day e-cigarette use on TLFB.

Additional Study Specific Assessments

27. *Clinical Institute Withdrawal Assessment for Alcohol (CIWA)*: Alcohol withdrawal will be assessed at the beginning of each session using the CIWA. A score >10 will result in referral to a higher level of clinical care.

28. *Columbia Suicide Severity Rating Scale* assesses for suicidal ideation at each visit and if a higher level of clinical care is needed.

29. *Prior/concomitant medication* will assess for eligibility at each medication visit (see exclusion criteria).

30. *Adverse events* will be assessed after each medication dose.

31. *Penetration of the Blind* will be assessed after each medication dose to assess the participant's belief about whether they received the placebo or active condition.

32. *Cash Choice task* is a brief measure of motivation, inhibition, impulsivity.

33. *Alcohol Purchase Task (17 items; Murphy et al., 2006)* is a behavioral economic measure of motivation for alcohol.

34. *Alcohol Urge Questionnaire (Drobes & Thomas, 1999)* is a measure that assesses alcohol urges.

35. *Barratt Impulsiveness Scale (BIS-11)* is a 30-item self-report scale used to measure impulsiveness.

	Entryway Intake	Study Screener	Visit 1	Visit 2	Visit 3 (remote)
Study Visit Information					
Informed Consent	X	X			
Locator Form and Updates		X	X	X	X
Penetration of the Blind			X	X	
Demographics, Health, Experiences, and Functioning					
Demographics	X				
MacArthur Social Ladder	X				
Health and Substance Use History Questionnaires	X				
Treatment Services Utilization (substance use)	X		X	X	X
Treatment Services Utilization (mental health)	X				
Pittsburgh Sleep Quality Index	X		X	X	
Quality of Life Enjoyment and Satisfaction (Pediatric and Adult Versions)	X				
Everyday Discrimination Scale	X				
Beliefs About Medicines Questionnaire	X				
Biological Samples					
Pregnancy test	X		X	X	
Urine sample	X		X	X	
Alcohol breathalyzer	X		X	X	
Carbon monoxide sample	X				
Saliva sample	X		X	X	
Blood sample			X	X	
Medical Assessments					
Meet with Medical Clinician		X	X	X	X (optional)
Brief Medical History & Physical Exam		X			
Adverse Events		X	X	X	
Prior/Concomitant Meds		X	X	X	X

Clinical Institute Withdrawal Assessment for Alcohol		X	X	X	
Vitals Signs/Body Mass Index	X		X	X	
General Mental Health and Psychological Assessments					
MINI International Neuropsychiatric Interview-DSM 5	X				
MINI Alcohol Use Disorder Past 30 Day Module		X			
PHQ-9	X		X	X	
GAD-7	X		X	X	
UPPS-R-C Impulsivity Scale	X				
Difficulties in Emotion Regulation Scale Short Form (DERS-SF)	X				
Eating Disorder Screen for Primary Care (ESP)	X				
Barratt Impulsiveness Scale (BIS-11)		X			
Columbia Suicide Severity Rating Scale		X	X	X	
Cash Choice Task		X	X	X	
Substance Use					
TLFB (Past 60 Days at baseline & Past number of days since prior TLFB at visits 1, 2, & 3)	X		X	X	X
Cannabis Methods	X				
Rutgers Alcohol Problems Index (RAPI)	X				
Marijuana Assessment Problem Inventory (MAPI)	X				
SOCRATES (Alcohol)	X		X	X	
SOCRATES (Cannabis)	X				
Thoughts About Abstinence Scale (e.g., Substance Use Goals)	X				
Reasons for Quitting Questionnaire (Cannabis)	X				
Motivation to Quit (Cigarettes and E-cigarettes)	X				
Ecig Use Questionnaire	X				
Addiction Growth Mindset Questionnaire	X				
Modified Fagerström Tolerance Questionnaire	X				
Penn State Electronic Cigarette Dependence Index	X				
Alcohol Purchase Task		X	X	X	
Alcohol Urge Questionnaire			X	X	
Lab Procedures					
Alcohol Olfactory Cue Reactivity Task			X	X	
Magnetic Resonance Imaging			X	X	
Estimated Visit Length (hours)	**2-3**	**1-1.5**	**5**	**5**	**0.5**

Visits. On the day of the Entryway assessment, we will conduct an additional in-person Screening Visit, to set up and trial daily texting, ensure inclusion/exclusion criteria, and collect additional study-specific information. Visits 1 and 2 will be in-person and include neuroimaging. There will be a brief Visit 3 about two and a half weeks after Visit 2 that will be via phone or video to update the participant's information.

Daily texts. After Visits 1 and 2, participants will receive a daily text for the next 18 days with a maximum of 30 days if necessary due to scheduling. The text will have a link to an on-line REDCap survey that will ask about the prior day's use of alcohol, cannabis, tobacco, CBD, and other substances. The link can also be e-mailed if the participant prefers.

Alcohol cue exposure procedure. Consistent with published procedures utilized with youth at MUSC, all participants will undergo an olfactory alcohol cue exposure procedure[85] before the neuroimaging session. In a counterbalanced order, participants will smell water, a bottle or can of the participant's preferred alcoholic beverage, and a cup of apple juice (as this is not typically used as a mixer with alcohol[107]) for three minutes each, with three minute rest periods in between each liquid. Heart rate, skin conductance, and self-reported craving (via the Alcohol Urges Questionnaire) will be acquired based on previously used protocols[107].

Biological assessments. Combined use of ethanol metabolites and traditional biomarkers [i.e. alcohol breathalyzer, urine ethyl glucuronide (EtG), and blood phosphatidylethanol (PEth)] will be utilized to corroborate self-report alcohol use[108-111]. THC and CBD assays will be collected through urine to corroborate cannabis use. Blood samples will also be used to detect peripheral cytokines. Saliva samples will be collected for profiling of microbial communities via 16S rRNA sequencing. Urine drug tests (comprehensive panel testing for cotinine, cannabinoids, amphetamines, opioids, and benzodiazepines) will be used to monitor other substance use. Urine pregnancy tests will be conducted with participants assigned female at birth. Participants will also be asked to sniff a scented candle and report whether they can smell it and identify the scent, to check basic ability to smell.

Neuroimaging protocol (1 hour).

Mock scanner: On Visit 1, participants will be introduced to the mock scanner to help them become acclimated to the MR environment and give them the opportunity to practice remaining still while in the magnet, which will minimize motion-related confounds in the imaging data.

Participants will undergo 2 neuroimaging scans (1 scan three hours after CBD, 1 scan three hours after placebo). Breath and urine toxicology samples will be collected before every scan. Saliva samples will be collected before and after every scan. Scans will be performed with a Siemens 3T Prisma^{fit} MRI scanner. Imaging sessions (~60 minutes) consist of:

1. **T1-weighted structural:** A high-resolution anatomical scan will be acquired, to allow subsequent registration to functional images and region-of-interest (ROI) definition.
2. **The Alcohol Cue Reactivity Task:** [91,92]. During the alcohol cue reactivity task, participants are shown pseudorandomly interspersed images of alcoholic (i.e., beer, wine, and hard liquor) and non-alcoholic (e.g., soft drink, juice) beverages, visual control images (i.e., blurred images), and a fixation cross. A magnetic fieldmap will also be acquired to allow geometric unwarping and cost-function masking of EPI images induced by magnetic field inhomogeneities.
3. **Magnetic Resonance Spectroscopy:** The magnetic resonance spectroscopy protocol proposed will be utilizing previously published methods.

Participants will be compensated $25 for the Screening Visit, $300 for completing both imaging visits ($150 V1 and $150 V2), as well as an additional $1 per day for submitting their daily text questionnaire for a maximum of 60 days total. Participants will also be eligible for a $30 referral bonus for everyone they refer who successfully randomizes into this study. Mileage reimbursement will be available to participants who live >25 miles away; the current mileage reimbursement rate, which is updated each year by the state, will be offered.

11.0 Specimen Collection and Banking

Biological Assessments. Urine samples will be obtained from all participants to conduct pregnancy tests (only those assigned female at birth) and qualitative urine drug screens. Necessary pregnancy tests will be completed first and a positive pregnancy test will immediately stop all subsequent procedures. The Clinical Neurobiology Lab (CNL) at MUSC will analyze urine and blood samples (urine ethyl glucuronide (EtG)), and blood phosphatidylethanol (PEth)). CNL will also process and store blood samples for analysis or peripheral cytokines. Participants will provide saliva samples using all-in-one collection kits and the samples will be immediately stored in a freezer until analysis.

12.0 Data Management

All data will be managed via REDCap. Research staff and participants will enter data in REDCap; a secure, web-based application designed exclusively to support data capture for research studies. REDCap provides: 1) an intuitive interface for data entry (with data validation); 2) audit trails for

tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages (SPSS, SAS, R); 4) procedures for importing data from external sources; and 5) advanced features, such as branching logic and calculated fields. These procedures are effective in minimizing data entry errors (e.g., missing or errant data). Direct entry of data by study participants (self-reports) will also be conducted in this study. This is done through a front-end survey interface that allows the participant to enter data on select questionnaires/assessments, but not access any other study records or data in REDCap. Server maintenance will be conducted by Information Technology Specialists at MUSC.

Accuracy and completeness of the data collected will be ensured by weekly review in team meetings. The REDCap system does not accept outliers, illogical response patterns, etc., thus minimizing data entry errors during participant direct entry. The PI will have weekly meetings with the research coordinator and research assistants to discuss qualitative comments received during data collection and any problems in data collection or entry. Dr. Squeglia will periodically examine the database to look for irregularities. Initial data analyses will examine distributions of variable scores and comparability of baseline characteristics across conditions in case analyses need to be adjusted for these characteristics. Dr. Squeglia will be responsible for maintaining signed consent forms as source documents for quality assurance review and regulatory compliance.

Data Analysis

Crossover designs are highly cost-efficient and powerful designs[112]. Laboratory studies can enroll more participants and minimize statistical noise through greater experimental control[32]. The primary outcome measures of interest will be glutamate and GABA levels (MRS; Aim 1), alcohol cue reactivity (fMRI; Aim 2), and *in vivo* response to olfactory alcohol cues (heart rate, skin conductance, subjective rating; Aim 3). For all outcomes, a generalized linear mixed effects regression model will be developed accounting for clustering within subject. Additionally, study day (scan 1 vs. scan 2), condition (CBD vs. placebo), and condition order (CBD/placebo, placebo/CBD) will be included to ensure the crossover design and washout period were successful. During model development, random intercepts will be included to account for variations in baseline response levels for individual participants and random slopes to account for varying responses to treatment. For all models, baseline characteristics will be assessed for association with study outcomes (e.g., sex, baseline alcohol use rates, alcohol craving) and when significant, will be added in a covariate adjusted model. Model based interactions will assess any differential effect of sex on the relationship between medication condition and study outcomes.

Power and sample size. The proposed study is primarily powered to assess the relationship between treatment with CBD as compared to placebo on glutamate and GABA levels in the anterior cingulate (Aim 1) and neural response to alcohol cues (Aim 2). In a cross-over study of the effects of CBD as compared to placebo on glutamate in the hippocampus in a population of patients with schizophrenia, significant increases were found (d= 0.48; p=0.035)[25]. To our knowledge, the effects of CBD on cue reactivity have not been assessed using fMRI. However, several studies have demonstrated that CBD can modulate resting-state[28,29] or task-based[5,27,30,113-116] BOLD signal under similar study design (acute dosing, 600 mg CBD) with sample sizes smaller than our proposed sample. Given limited literature in this area, we were not able to run a power analysis for Aim 3; this pilot study will provide data to power further analyses/effect sizes. Due to the number of tests run within the fMRI and MRS aims, the study will be powered at 80% with a corrected α=.0005 (.05/100). Thus, in a crossover randomized controlled trial, **n=27 study completers** would be necessary. Although the proposed project is of relatively short duration (1.5 months), we anticipate an attrition rate of up to 7 participants (~23%) due to head motion or other artifacts, thus requiring a total of **n=50 participants enrolled and randomized**.

Rigor and Transparency. The proposed study is rigorous and will achieve robust and unbiased results by using explicit inclusion/exclusion criteria (commensurate with AUD trials for youth); use of validated measures and methods; explicit hypotheses and corresponding planned statistical analyses; power estimates; planned handling of retention/attrition and missing data; and careful consideration of potential

confounds. Key biological variables such as sex and age are considered and are incorporated into the proposed data analytic plan. All experimental details are reported in a detailed and fully transparent manner for replication.

13.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

All aspects of the study will be run through the MUSC Department of Psychiatry and Behavioral Sciences. Consistent with the guidelines of the MUSC IRB, an internal Data and Safety Monitoring Plan (DSMP) will be used. An internal DSMP is appropriate for this study because 1) it does not involve a multi-site trial and 2) it presents minimal risks to participants.

Prior to the start of the study, the protocol will be registered on the clinical trials registry (clinicaltrials.gov). Final modifications will be made to the study application with the MUSC Institutional Review Board (IRB) prior to the start of any study procedures and participant enrollment. During study enrollment, all serious adverse events (SAE; defined as any untoward medical occurrence that results in death, is life-threatening, requires or prolongs hospitalization, causes persistent or significant disability/incapacity, results in congenital anomalies/birth defects, or in the opinion of the investigators represents other significant hazards or potentially serious harm to research subjects or others) will be reported to the MUSC IRB within 24 hours of learning of the event. Follow-up of all SAEs will be reported as well. All adverse events (AEs) will be reviewed weekly by the PI and every 6 months by both the appointed Data and Safety Monitoring Board (DSMB) and the IRB. Any significant actions taken by the local IRB, including significant protocol changes, will be relayed to NIH. We anticipate the SAE rate to be low. If monitoring indicates otherwise, we will convene a special meeting of the DSMB to evaluate further.

The potential risks and benefits and methods to minimize these risks are outlined in Human Subjects Sections. Guidelines have been developed for managing and reporting of AEs, including SAEs. Dr. Squeglia will serve as the Program Manager for AEs. The Adverse Event Log will be used to document all AEs. If an AE is non-serious (self-limited with no intervention needed), no further action will be necessary. However, in the case of a serious, unresolved event, an AE follow-up log will be completed. The clinician will then call Dr. Squeglia with initial reports within 24 hours of the start of the SAE. The clinician will record the information on SAE Notification Form. The clinician will forward hard copies of the complete report (SAE Notification Form, Concomitant Medication Log, and AE Log) to Dr. Squeglia, who will, in turn notify the IRB, DSMB, and NIH about the SAE. Additionally, Dr. Squeglia will communicate summary reports of DSMB discussion of the SAE, or any deliberations of IRB regarding the review of the SAE or the trial itself, to NIH. If the event is “Serious, Unexpected and Associated” (an SAE is considered unexpected if it is not described in the Package Insert), Dr. Squeglia will complete Food and Drug Administration (FDA) Form 3500A and will forward it to the FDA. Dr. Squeglia also will inform the IRB and the study participants (and parents/guardians, as appropriate) about the SAE. In all of these reviews and reports, strict patient confidentiality will be maintained.

AEs will be coded on a weekly basis using Medical Dictionary for Regulatory Activities (MedDRA) rules and entered into a database. For each weekly study meeting, the research assistants will prepare a summary of all AEs, including their severity and presumed relation to study medication. The PI will review this at the weekly study meeting (or before if more urgent).

Study procedures will follow as much as possible the FDA’s Good Clinical Practice Guidelines. We will encourage participants (and parents/guardians as appropriate) to notify their physicians that a) they are in a randomized controlled research study evaluating CBD for adolescent AUD, and b) the physician should contact the PI directly if the physician has any questions.

The research assistants will be instructed not to reveal whether a person is a participant in the study and will report to the PI any outside requests for information about a participant or any breaches in

confidentiality. All requests by participants' physicians and other medical providers will be referred directly to the PI.

The Data and Safety Monitoring Board may request a blinded interim efficacy report (blinded to the PI and research team) for review while the trial is ongoing. Final (fully unblinded) efficacy analysis will occur after all participants have completed all visits.

Dr. Squeglia will have overall responsibility for safety and data monitoring on a day-to-day basis for this trial, including weekly checks of the AE database prepared by research assistants during study meetings to determine if particular categories are being endorsed more frequently than anticipated and to determine if severity is greater than anticipated for this trial. Dr. Gray will also serve as the primary medical monitor for this study and will provide guidance and input on a scheduled and as-needed basis throughout the trial. Co-I Dr. Tomko will examine the outcomes database twice annually for missing data, unexpected distributions or responses, and outliers. Annual data and safety monitoring reports will be written and submitted to the IRB and to NIH. This information will include, but may not be limited to, a brief description of the trial, baseline sociodemographic characteristics of participants accrued, retention of study participants, quality assurance issues, and reports of adverse events, significant/unexpected adverse events and serious adverse events.

Dr. Squeglia will create a DSMB, comprised of multidisciplinary faculty with expertise in adolescent-focused clinical trials. The DSMB will meet every 6 months (more frequently as needed for emergency situations) to review any AEs related to the study, as well as review of any data management related errors. The board may be called at any point if needed for SAEs, etc. Modification will be made in the procedures and/or the protocol, if necessary, based on the findings of the board.

Reports provided to the DSMB on an annual basis will address the following areas: 1) the progress of the research study, including assessments of data quality and participant recruitment, accrual, and retention; 2) review of outcome and adverse event data to determine whether there is any change to anticipated benefit-to-risk ratio of study participation, and whether the study should continue as originally designed, should be changed, or should be terminated; 3) assessment of external factors or relevant information; and 4) review of study procedures designed to protect the privacy of the research participants and the confidentiality of their data. Following review of the annual update on the study, the DSMB will provide a written report that will be submitted annually with the IRB renewal.

14.0 Withdrawal of Subjects

Participants will be informed that they may discontinue the study at any time during the visit without penalty and that they will be pro-rated for completed research activities. Participants may also be withdrawn from the study by the PI if it is determined that it is in the participant's best interest (e.g., safety concern, requiring intervention). Finally, participants may withdraw consent of use of their biospecimen samples for additional purposes at any time.

15.0 Risks to Subjects

The risks associated with participation in this study include adverse events related to study medication and the potential loss of confidentiality. Potential risk details are described below, and the PI will ensure that all risks are clearly defined for study participants and are thoroughly understood during the informed consent process and throughout the study period.

In the unlikely event that participants are significantly distressed by any aspect of study participation, they will be offered the opportunity to discuss these issues with one of the PIs and/or the designated medical clinician (Kevin Gray) and may withdraw from the study at any point. If the distress is more serious and requires psychiatric attention, they will meet with a designated medical clinician on the study team. If participants require longer-term intervention, they will be referred to psychiatric treatment at MUSC, where

they will pay the standard fee. Should any participant report suicidal ideation at any time during study participation, they will be immediately referred to a PI or medical clinician to determine the appropriate course of action. Suicidal ideation as measured via the PHQ-9 will automatically trigger an email alert in REDCap to go to the PIs and/or the clinicians in the study for follow-up. Events of this nature are not expected to occur at greater frequency because of the current protocol, but procedures will be in place to ensure the safety of all study participants.

Adverse Events Related to Study Medication. CBD has a generally benign adverse effect profile. It is non-intoxicating[4,5], appears generally well-tolerated, and demonstrates no signal of abuse liability[6]. Preclinical work has shown that CBD affects an array of drinking behaviors (e.g., reduces ethanol seeking and intake; mitigates symptoms of withdrawal, relapse, anxiety, and impulsivity)[9,10,15], and recent clinical work has indicated CBD's potential to reduce alcohol intake within adults who endorse alcohol and cannabis co-use[16].

Loss of Confidentiality. There is the risk of breach of confidentiality. The research team has procedures in place to minimize the risk of any confidentiality breach. Participant records are stored in locked files within locked offices, or in password-protected databases. Much of the data collected, including all neuroimaging data, is de-identified and uses a participant ID. No specific or general participant information will be left in public access areas, and no oral communication regarding participants with identifiers will be made in any public areas. Research staff members have been given extensive training in maintaining confidentiality as well as HIPAA regulations. Participants will be informed of these potential risks during the informed consent process and will have the option to leave the study at any point.

Other Minimal Risks. Risks associated with MRI are minimal for individuals who do not have metal in their body and are not claustrophobic. Some discomfort may result from lying in the scanner for up to 60 minutes. There is some psychological risk inherent in testing participants for recent substance use, psychiatric symptomatology, and pregnancy, especially if results are contrary to participants' expectations.

Protections Against Risk.

Adverse Events Related to Study Medication. The informed consent process will be used to thoroughly educate participants and parents/guardians about potential medication-related risks, including adverse events. This discussion will include thorough review of adverse events associated with CBD treatment, which are minimal. Rigorous screening procedures and strict exclusion criteria are designed to exclude potential participants at elevated risk for adverse events. The study medical clinician will conduct serial adverse events monitoring as part of medication management. Participants and parents/guardians will have access to a study medical clinician 24 hours, 7 days a week for emergencies. Co-I Gray has full hospital admitting privileges in the event of an adverse event requiring hospitalization. Urine pregnancy tests will be conducted at baseline and serially during visits for participants assigned female at birth.

Loss of Confidentiality. The research team has established procedures in place to minimize the risk of any confidentiality breach. To minimize this risk, all data will be stored in secure locations, including locked file cabinets and password-protected computers and databases. Any adverse events will be reported to the IRB, NIH, and others, as appropriate, to ensure the safety of study participants. Research staff are given extensive training in maintaining confidentiality as well as HIPAA regulations.

Policy on neuroimaging data. Participants and their family (for those under age 18) are informed that this is a research study and therefore does not include clinical imaging to confirm clinical findings. If the research staff note a structural irregularity on a scan, a neuroradiologist will review it (fully de-identified) and note if it is of clinical significance or not. If the finding has the potential of clinical significance, the participant or parents (depending on age) will be notified to follow up with his or her doctor. There is a minimal risk of undue stress or concern if the finding is determined to be benign or not clinically significant.

Since this study involves minors, particular caution will be exercised in obtaining informed adolescent

assent separately and independently from parental consent. To this end, an initial step in participant recruitment involves obtaining parental/legal guardian permission for participation by the adolescent. Once parental consent is secured, participants will be asked separately and independently for informed consent (i.e., parental consent will not be used to persuade teens to participate). This approach is considered very effective in minimizing coercion to participate.

16.0 Potential Benefits to Subjects or Others

Potential benefits of participation in this study may include a reduction in alcohol use. However, there is no guarantee or promise that participants will receive any benefit from participation in this study. Participation in this study involves minimal risk for participants yet has the potential for minimizing alcohol use.

17.0 Sharing of Results with Subjects

Aggregated study results can be shared with subjects or others in the form of peer-reviewed manuscripts, as relevant, upon request.

18.0 Drugs or Devices

Epidiolex (CBD) will be dispensed by the MUSC Investigational Drug Service. The matched placebo liquid will be compounded and dispensed by the MUSC Investigational Drug Service. PI Squeglia received an FD exemption for using Epidiolex (CBD) with adolescent substance users (IND161500).

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