Exploring the Mechanisms Underlying the Analgesic Properties of Cannabidiol using Magnetic Resonance Spectroscopy

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Objective: The overall goal of this proposal is to examine the mechanisms by which Cannabidiol (CBD) impacts brain chemistry in chronic pain by measuring *in vivo* brain metabolites using proton magnetic resonance spectroscopy (^{1}H-MRS). The cannabis plant includes hundreds of distinct chemical entities including over 110 different cannabinoids and 120 unique terpenes [1]. Although these compounds may have similar chemical structures, they differ significantly in their pharmacological actions [2, 3]. CBD is especially interesting with regard to its potential use in the treatment of a number of clinically significant conditions. Studies have demonstrated both its neuroprotective and anti-inflammatory effects as well as the lack of psychoactive effects, in stark contrast to Δ9-tetrahydrocannibinol (Δ9-THC) [4]. However, clinical research investigating the effects of CBD is limited partly due to enforcement of restrictive policies and regulations as well as quality control issues for preparations that might be used in human trials [5]. Cannabis users have long reported therapeutic properties of the plant for several medical conditions including pain relief [6]. This is consistent with results from preclinical research indicating an efficacy of cannabinoids in a number of animal pain models [7-9]. Randomized controlled studies in humans show that cannabinoid administration for the management of pain is superior to placebo in terms of efficacy and inferior in terms of tolerability [10]. To date, human studies have used cannabis extracts with varying proportions of CBD and Δ9-THC, making it difficult to disentangle the analgesic efficacy of the two compounds. Evidence suggests that CBD potentiates the analgesic effects of Δ9-THC and may reduce the psychoactive and cognitive side-effects of Δ9-THC [11]. While there is an extensive collection of evidence from animal models, the analgesic effects of CBD have not been studied extensively in humans. Importantly, CBD may potentially alter the neurochemical profile in pain processing regions of the brain, including a shift in the excitatory/inhibitory balance of the brain as well as reducing neuroinflammation both of which can be investigated using ^{1}H-MRS [12].

CBD has a complex pharmacological profile. It acts as an inverse agonist at the endocannabinoid CB_2 receptors [13]and a negative allosteric modulator of endocannabinoid CB_1 receptors [14]. Furthermore, CBD exhibits agonist activity at the transient receptor potential vanilloid type 1 (TRPV1) receptor [15], antagonistic activity at the G protein coupled receptor 55 (GPR55) [16], agonistic activity at the serotonergic 5-$HT1_A$ receptors [17], and acts as a positive allosteric modulator at gamma-amino butyric acid (GABA-A) receptors [18]. It has also been shown that CBD exhibits anti-inflammatory actions [19]. ^{1}H-MRS is a neuroimaging technique that holds promise for examining brain chemical changes associated with CBD administration and chronic pain [20].

An altered excitatory/inhibitory balance in the pain-processing regions of the brain has been demonstrated in MRS studies of chronic pain. Specifically, chronic pain has been consistently associated with higher glutamate (Glu) (major excitatory neurotransmitter) and lower GABA (major inhibitory neurotransmitter)[21-26]. An innovative aspect of this study is the 2-dimensional J-resolved (2D-J) acquisition method employed allows for the measurement of Glu as opposed to using GLX, a combined measure of Glu and glutamine (Gln). Furthermore, chronic pain is associated with increased brain and peripheral inflammation. To date there are no published MRS studies investigating the neurochemical effects of CBD in chronic pain [27].

CBD has potent anti-inflammatory activity that has been well studied in animal models. In a model of

Alzheimer's disease-related neuroinflammation, CBD reduced interleukin (IL)-1β protein expression [28]. CBD has also been shown to inhibit lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF)-α production [29]. It was also reported that CBD decreased the production and release of proinflammatory cytokines such as IL-1β, IL -6 and interferon-β, from LPS-activated microglial cells [30]. In addition, CBD inhibited microglial activation and consequent neuroinflammation [31]. Indeed, a considerable number of preclinical studies have indicated that CBD attenuated increased glial reactivity associated with pathological conditions [32-34]. The collected data have also demonstrated that increased astrocyte activity is suppressed in the presence of CBD in models of ischemia, Alzheimer-like and Multiple-Sclerosis-like neurodegenerations, sciatic nerve injury, epilepsy, and schizophrenia [35]. Moreover, CBD has been shown to decrease proinflammatory functions and signaling in astrocytes [35]. An increasing body of literature supports the idea of aberrant glial activity as a potential underlying mechanism of chronic pain. A recent human study using positron emission tomography-magnetic resonance imaging (PET/MRI) identified a pattern consistent with brain glial activation in patients with chronic low back pain, and other human cerebrospinal fluid studies support the presence of neural inflammation in chronic pain patients [36]. Given the evidence that CBD is anti-inflammatory and reduces reactive gliosis, it is plausible that CBD may be efficacious in the treatment of chronic pain via its effects on peripheral and neuroinflammation. However, studies investigating the relationship between the anti-inflammatory and analgesic effects of CBD are limited.

Chronic pain has been associated with several alterations in the brain including reduced GABA, increased glutamate, and increased neuroinflammation. Myoinositol (mI) is considered to be an astroglial marker and a proxy for neuroinflammation, often associated with activated astrocytes and microglia in brain disorders [37]. ^{1}H-MRS is an emerging technique for measurement of *in vivo* levels of brain metabolites including GABA, glutamate, and mI [20]. Importantly, the above-mentioned alterations associated with chronic pain may contribute to its etiology since several analgesics target these substrates: glutamate, GABA and neuroinflammation. For example, pharmacological agents that increase extracellular glutamate increase pain, whereas those that reduce extracellular glutamate reduce pain [38]. Furthermore, MRS studies have shown reduced glutamate after acute administration of analgesics such as oxycodone and morphine [39, 40]. Both directly acting (GABA-A and GABA-B agonists) and indirectly acting GABAergic agents (GABA uptake inhibitors and GABA-transaminase inhibitors) produce analgesia in a variety of animal test systems [41]. Pharmacological agents such as minocycline, propranolol, proprentofylline have been demonstrated to reduce neuroinflammation and exhibit efficacy in chronic pain [27]. In addition, a MRS study reported reduced mI after morphine treatment during experimental pain [40]. Overall these results suggest that interventions that reduce glutamate, increase GABA, and reduce neuroinflammation may be efficacious in treating pain. To date there are no studies investigating the effects of CBD administration on brain metabolites in chronic pain. A recent study of acute (single dose) administration of CBD in adults with autism spectrum disorder (ASD) and healthy controls showed a shift in the excitatory-inhibitory balance of the brain. In both groups, CBD increased Glx (glutamate+Gln) in the basal ganglia (BG) voxel and decreased it in the dorsomedial prefrontal cortex (DMPFC) voxel [12]. In contrast, CBD had opposite effects on GABA in each group. Specifically, both in prefrontal and subcortical regions, CBD increased GABA in the controls but decreased GABA in ASD [12]. Thus CBD can potentially alter the excitatory/inhibitory balance in a region-dependent manner. Whether pain-relevant regions such as the ACC and insula also exhibit CBD-induced changes in the excitatory/inhibitory balance needs to be investigated.

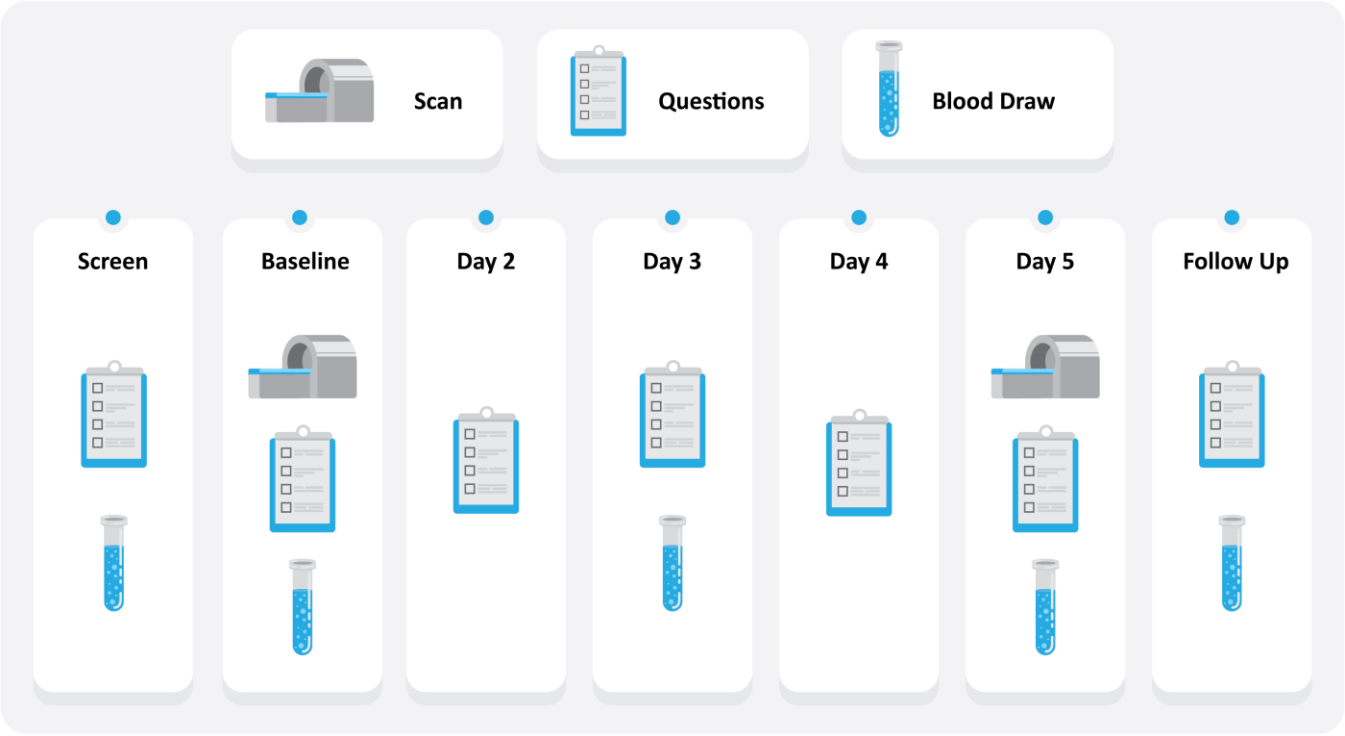
Design: This is a randomized-block designed, double-blinded, placebo-controlled study designed to evaluate the potential of CBD to modulate the alteration of the excitatory-inhibitory balance found in chronic pain. Additionally, this study will test whether short-term administration of CBD reduces peripheral inflammatory cytokine levels as well as neuroinflammation in chronic pain patients when compared to placebo. Males and females ages 18-60 who report chronic musculoskeletal or primary pain,

of at least moderate intensity (pain of at least 4 on a scale of 0-10) that is persisting beyond the usual course of acute illness or injury or more than 6 months, and which adversely affects the individual's well-being (IASP 2004), will be included in this study. Fifty-two participants will be recruited and randomly assigned to one of two groups: CBD (N = 26) or placebo (N=26). Participants will complete seven study visits: screening, baseline and the first dose, dose 2, dose 3, dose 4, dose 5, and a follow-up visit (between two and seven days after dose 5). Participants also will complete two MRS scans at baseline (before and after dosing), and one MRS scan at day 5 of administration. Total scanning time for each session will be approximately 30 minutes. Clinical measures will be administered at baseline, dosing visits, and follow-up. Blood will be collected to perform a complete blood count (CBC), complete metabolic panel (CMP), liver function panel, and to analyze peripheral cytokine levels at screening, baseline, visit 3, and visit 5.

Primary Endpoints: Primary endpoints will be measures of ^{1}H-MRS derived metabolite ratios over water in the ACC and insula to determine the effect of CBD or placebo after 2 hours of the first dose and after 5 days of once daily administration. In addition, peripheral inflammatory cytokines will be measured in the blood at the screening visit, after the first dose at baseline, on day 3 and day 5 to determine the effects of CBD or placebo on peripheral inflammatory markers.

Secondary Endpoints: Determine if the changes in brain chemistry and peripheral inflammation correlate with changes in subjective and objective pain measures after CBD or placebo administration.

Schematic of Study Design



This study is designed to be completed in two weeks by each participant. However, the screening visit may occur more than a week before the dosing week, depending on participant and equipment availability. Prospective participants will undergo a thorough clinical screening visit, including hematology samples. Potential subjects will be screened in a 10-20-minute phone interview to determine eligibility. The screening visit will involve subjects reviewing and signing the consent form prior to

engaging in any study procedures. Participants will receive an EKG; provide a urine sample for drug screening and blood sample for a comprehensive metabolic panel (sodium, potassium, chloride, carbon dioxide, urea nitrogen, creatinine, glucose, calcium, protein, inflammatory markers), liver function tests (alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total bilirubin, albumin), a complete blood count (WBC, RBC, platelet count), cytokine panel, and an Opioid quantitative blood test. Subjects who are eligible will then be scheduled for a baseline visit.

Study Visits

	Screen	Visit 1 Baseline	Visit 2	Visit 3	Visit 4	Visit 5	Follow-Up
Informed Consent	X						
Inclusion/Exclusion	X	X					
Health History and Demographics	X						
Physical Exam	X					X	
Vital Signs	X	X	X	X	X	X	X
MOSES (side effect monitoring)		X	X	X	X	X	X
Pain Intensity & Unpleasantness Ratings (Visual Analog Scale)	X	X	X	X	X	X	X
Height/Weight	X					X	
Concomitant Medications	X	X	X	X	X	X	X
Blood Draw – CBC, CMP, Liver function	X	X		X		X	
Blood Draw - Cytokines	X	X		X		X	
Urine Drug Test	X	X	X	X	X	X	X
Urine Pregnancy Test		X				X	
EKG	X					X	X
Enrollment/Randomization	X						
MRS		X				X	
Dispense Drug		X	X	X	X	X	
Monitor for Adverse Events (AE)		X	X	X	X	X	X
Pain Measures: McGill, PCS, PDI		X				X	X
Questionnaires: CSSRS (screening), OSU-TBI, TriPM, SCID, GAF	X						
CSSRS (since last visit), HAM-A, HAM-D, POMS		X				X	X

Study Population:
Participants will be required to meet the following inclusion criteria:

Inclusion criteria:
1. Age between 18-60 yrs.
2. Chronic musculoskeletal pain of at least moderate intensity for at least 6 months or longer.

Exclusion criteria:
1. Current or past neurological illness.
2. Substance abuse or dependence within the prior 3 months.
3. Contraindication to brain MRI at PI discretion.
4. Type I and type II diabetes.
5. Unstable medical conditions.
6. Consumption of more than 2 drinks of alcohol per night.
7. Current pregnancy or planning to become pregnant or breastfeeding.
8. History of seizures or head trauma at PI discretion.
9. Active or history of major mental illness.
10. LFT results 3 times greater than the upper limit of normal at screening.
11. Medical or recreational cannabis use in the past 3 months.
12. Opioid medications in the past 3 months.
13. Participants may be excluded if the PI feels they do not meet safety criteria.

Participants will be required to attend 7 in-person visits, defined as follows: At the screening visit, informed consent will be reviewed and obtained and potential study subjects will complete a medical history and physical examination. Participants will receive an EKG and provide a urine sample for drug screening and a blood sample for a comprehensive metabolic panel, liver function tests, a complete blood count, cytokine panel, and an Opioid quantitative blood test. A visual acuity test will be completed in addition to weight and height measurements. The OSU-TBI, SCID, GAF, and TriPM surveys will be administered. Participants also will be screened for concomitant medications. Following screening, subjects who are eligible will randomized to one of the two conditions (placebo or CBD) and scheduled for Visit 1. In order to participate in the study women of childbearing potential will be required to use an effective method of birth control.

Visits 1-5 will occur during five consecutive days with a single dosing each day. Participants will be queried to assess concomitant medications prior to each administration. Participants' vital signs will be taken (blood pressure, heart rate, respiratory rate) at each of these visits prior to and 30 minutes (+/- 30 minutes) after CBD/ placebo administration. Participants also will provide a urine sample for drug screening to ensure compliance to study participation criteria. Pain intensity and quality will be assessed using the 10-point visual analog scale prior to and 30 minutes (+/- 30 minutes) after the administered dose of the study drug. Participants also will complete side effect profile measures at each of the visits. Participants will be provided taxi transportation to and from the study visit in cases where a family member or friend cannot transport the participant.

At visits 1 and 5, participants will complete the baseline and follow up MRS scans, clinical measures (CSSRS (since last visit), HAM-A, HAM-D, POMS), and pain measures (McGill, PCS, PDI). At visit 1, participants will complete two MRS scans: one before and one 2 hours (+/- 30 minutes) after CBD (time for peak plasma concentration[12]) in an attempt to capture the acute effects of CBD. Females of

childbearing potential will additionally complete a urine pregnancy test at the beginning of the baseline visit and visit 5. Visit 5 will also include height and weight measurements, follow up blood work, and another EKG to monitor cardiac functioning. A blood sample will be collected at visits 1, 3 and 5 to assess levels of cytokines and TNF-α using a cytokine panel assay (ARUP laboratories).

The follow-up visit will occur within one week following the final dosing visit (visit 5). Vital signs, side effects, adverse effects, cardiac function, pain intensity ratings, pain measures, and clinical measures will be assessed. If subjects are found to be non-compliant at any time during the study they will be immediately discontinued and replaced.

Drug Management:
The Cannabis product (CBD extract) will be procured by the study team through the National Institute on Drug Abuse (NIDA) drug supply program and in accordance with federal and state laws, regulations and rules. Stability information on the CBD extract (DMF 029842) may be referenced per letters of authorization supplied to the Diagnostic Neuroimaging Lab. Only enough of each extract will be ordered to compound doses for the participants in the active treatment arm.

There are two treatment allocations on this study: CBD or placebo. The CBD will be supplied in the form of a distinct high CBD/low THC extract which will have >50% CBD and <1% THC per dose. All percentages are measured in % w/w. All CBD doses will be prepared from extracts for oral administration [42]. The extract has accompanying certificates of analysis that confirm the %w/w proportion of CBD and the %w/w proportion THC.

Participants in the CBD group will receive 500 mg of CBD and 0.08 mg of THC per 5 ml dose of a compounded suspension; due to extract composition, a total of 842.6 mg of the high CBD/low THC extract will be needed to compound each daily dose.

Subjects in the placebo group will not receive any CBD, but will receive 5 ml of placebo.

Per Dose Compounded	High CBD/Low THC extract product	Placebo
Total amount of extract	842.6 mg	0 mg
Amount of CBD	500 mg	0 mg
Amount of THC	0.08 mg	0 mg

Both groups will take doses orally once daily for five consecutive days.

The CBD extract will be stored and prepared at the University of Utah investigational drug service (IDS) pharmacy.

The CBD extract supplied by the study team will be used by the IDS pharmacy to compound the high CBD/low THC dose. Olive oil will be used as the diluting agent for the suspension as the CBD extract is not water-soluble. Olive oil (without any additional items added) will serve as the placebo. Medical supply grade olive oil (from Medisca Inc.) will be used both for compounding as well as for placebo. The olive oil and the compounding supplies will be provided by the IDS pharmacy and paid for by the research study team.

The CBD extract will be stored in the IDS -20 Celsius Freezer and compounded suspensions will be stored at controlled room temperature (15-25 Celsius). If a temperature excursion occurs, as defined by IDS standard operating procedures, the research team is to be notified and product is not to be dispensed until further information is obtained from the supplier of the extracts. The CBD extract will be stored

undiluted as it comes from the NIDA drug supply program at until their expiration date, or their use in preparing a compounded suspension for this study.

In order to maintain effective controls against diversion of controlled substances and any schedule I substance the following measures have been taken by the IDS pharmacy:

- The entrance to the pharmacy is locked and is only accessible by licensed pharmacists and licensed pharmacy technicians.
- During non-business hours, the pharmacy is electronically-armed with a security panel that triggers an alarm when the pharmacy is accessed without an appropriate disarming code being entered within 2 minutes.
- When the alarm is triggered institutional security is notified and reviews the situation in person as to why the alarm was triggered in the pharmacy. Security personnel will then contact the pharmacy manager or appropriate pharmacy director informing them that the alarm was triggered and relay their findings as to why.
- Only licensed pharmacists are allocated disarming codes for the security panel.
- A schedule I controlled substance safe is located in the IDS pharmacy located at the Huntsman Cancer Hospital.
    - The safe is a NMFC 172280 – class – 70 – safe with solid walls.
    - The safe is manufactured by Sargent and Greenleaf, model number 2218T15-G-G1R, serial number of S-54148.
    - The safe weighs approximately 800 lbs. and is located on the floor in the pharmacy. The dimensions of the safe are approximately 23 inches x 25 inches by x 27 inches.
    - This safe has been in the pharmacy since June 2015.
    - Only IDS pharmacists and the IDS pharmacy manager will have access to safe combination.
        - Upon receipt of the combination, IDS pharmacists and/or the IDS pharmacy manager will sign a document indicating they have been provided with the schedule I controlled substance safe combination and that it will not share with anyone else.
            - The appropriate individuals will sign a new copy of the above document each time the safe combination is changed.
        - If at any time an individual that has a combination to the safe terminates employment within the IDS pharmacy, the combination to the safe will be changed at the time the employee departs from employment.
            - Only the IDS Pharmacy Manager or a Pharmacy Director can modify the combination to the schedule I controlled substance safe.
            - Instructions on how to modify the safe combination are located in a locked cabinet in the IDS Pharmacy Manager's office.

There will be seven pharmacists that will collectively be responsible for inventory, handling, preparation and dispensing of the CBD extract and the compounded suspension medication in the investigational drug service pharmacy.

Upon order receipt and appropriate participant enrollment confirmation, the CBD suspension will be compounded on a participant-by-participant basis for participants randomized to the intervention group.

USP <795> Pharmaceutical Compounding – Nonsterile Preparations standards will be followed during the compounding process. Controlled substance handling and dispensing requirements will be followed according to institutional policy as well as federal and state laws, rules and regulations.

Each time a suspension is prepared, a total volume of 27.5 ml will be compounded. This will ensure that there is enough compounded suspension to provide all five doses of the investigational medication, understanding that there may be residual compounded suspension that is in the compounding equipment, as well as in the storage bottle described below.

The compounding process of the suspensions should be as follows:

- The extract must be stored in the -20 Celsius Freezer.
    - IDS will store the extract in a locked -20 C freezer in a lock box. The key to the lock box will be stored in the C1 safe.
- The product can only be thawed 2 times and still be considered equipotent.
- Once the product is compounded in oil, it is stable for 7 days at room temperature.

Compounding individual patient quantities
To limit the number of times the extract is thawed, the IDS pharmacy will divide the large bottle of extract into individual patient quantities during the first thaw and thaw these for the second time when compounding a specific patient suspension.

Instructions for dividing large bottles of extract into individual patient quantities:

1. Remove extract from freezer lock box.
2. Consider slightly warming extract in a warm water bath, it is very sticky and thick.
3. Weigh the bottle prior to removing any extract.
4. Using wax paper, weigh the appropriate quantity for a weekly dose of CBD (see below).
5. Wrap wax paper around individual patient quantity.
6. Wrap outside with colored foil (Gold = CBD)
7. Use tamper evident tape to seal foil.
8. Weigh the bottle frequently between separating patient doses.
9. Place back in lock box in IDS freezer.

Equation for determining quantity of CBD extract for this compound.

- On the Certificate of Analysis from The U of Mississippi, at the top of the chart for CBD concentration, find the results column to identify percentage for the exact extract.
- Per protocol, the dose is 500mg of CBD per dose. We are planning on preparing 5.5 doses to account for any loss of product that will occur on transfer therefore per CBD patient, each patient specific parcel should be 2750 mg of CBD (5.5 x 500 = 2750).
- Equation is:
    - _____ mg (extract) x 0.____(percent CBD) = 2750 mg CBD per patient

For the currently available products:

- 4634.3 mg (extract) x 0.5934% (percent CBD) = 2750 mg CBD per patient.

For 27.5 ml of the **high CBD/low THC** suspension:

1. Ensure the scale is calibrated appropriately
    1. Using either a weighing dish or weigh paper, weigh out 4634.3 mg of **high CBD/low THC** extract (this has been done and individual patient quantities are in the -20 degree freezer)
2. Measure out 5 ml of olive oil using a 10 ml syringe
3. Mix the 5 ml olive oil and the CBD extract in a warmed mortar with a pestle creating the compounded suspension
4. Measure out an additional 17.87 ml of olive oil
    a. use a 10 ml syringe for the 17 mL
    b. use a 1 mL syringe for the 0.87 mL

5. Add the additional 17.87 ml of olive oil to the mortar and mix with the pestle
6. Once well mixed, pour the entire volume of compounded suspension into an amber glass bottle using a rubber spatula to get as much of the suspension as possible into the glass bottle.
7. Place a lid on the glass bottle.
8. Place a tamper-indicating seal over the lid.
9. Label the bottle with a participant-specific label.

As indicated above the completed compounded product will be stored in a participant-specific labeled thirty milliliter (30 ml) amber glass bottle. From this bottle individual, participant-specific doses will be drawn-up on a daily basis prior to dispensing from the IDS pharmacy using the following process:

1. Prior to each dose being drawn up, confirm that the participant labeled bottle matches the name of the participant the dose is to be prepared for
2. Ensure the tamper-indicating seal is intact, if intact, then break/remove the seal
3. Using a 5-ml oral dosing syringe, draw up 5 mL of the compounded suspension from the glass bottle
4. Replace the lid on the glass bottle
5. On days 1 through 4, place a new tamper-indicating seal over the lid of the glass amber bottle.
    a. On day 5 once the final dose has been drawn-up for dispense, any residual suspension in the amber glass bottle will be poured into a container that has isolyser in it.
    b. The empty amber glass bottle will then be disposed of in accordance with the Medication and Medical Waste Process document to prevent diversion and reduce any potential environmental impacts.
6. Cap the syringe with a tamper evident cap
7. Label the syringe for individual participant use

All steps of preparing, dispensing and wasting of the suspensions/extracts used for compounding will be performed by an IDS staff member while under witness of another IDS staff member (all steps will be verified by two independent IDS pharmacy staff members, one of which MUST be a pharmacist). All waste will be documented on a controlled substance dispense/return log and signed by two witnesses in accordance with institutional policy.

Placebo product will be prepared using only medical supply grade olive oil using the following procedure:

For preparing one dose of **placebo:**

1. Using a 5-ml oral dosing syringe, draw up 5 mL of olive oil
2. Cap the syringe with a tamper evident cap
3. Label the syringe for individual participant use

Placebo doses will also be prepared immediately prior to dispense. Preparation of placebo does not require two independent IDS pharmacy staff witnesses.

Identical oral syringes, tamper evident caps and labels will be used for all doses of compounded suspension and placebo to maintain product blinding to the study team.

Each participant dose will be prepared for dispense on a daily basis only after the study participant has arrived to the study site. Once prepared for dispense by the IDS pharmacy, the oral syringe containing the assigned treatment will be picked up from the IDS pharmacy by appropriate personnel listed on the Delegation of Authority Log. No other individuals will be allowed to pick-up and transport the study drug to the study site. A chain of custody form and a medication pick-up log will be completed by both an IDS staff member and the research team member picking up the drug. The chain of custody form will

be maintained by the research study team, the medication pick-up log will be maintained by IDS pharmacy personnel.

The study drug will then be transported to the study site. Once at study site the tamper evident cap will be removed and the entire contents of the oral dosing syringe will be added to a 114 ml (a single prepackaged cup) of shelf-stable, snack-pack brand chocolate pudding by a research team member. The contents of the syringe will be thoroughly mixed into the chocolate pudding using a disposable spoon for a minimum of 15 seconds. Mixing of the contents of the oral syringe and the chocolate pudding will occur immediately prior to oral consumption of the mixed product by the study participant. Chocolate pudding is used to mask the unpleasant taste of the CBD extract to reduce the risk of accidental un-blinding. The chocolate pudding will be supplied by the research team and will be stored on-site at the University Neuropsychiatric Institute for up to two weeks until it is mixed with the provided study medication.

One the study medication is mixed into the pudding it will be given immediately to the study participant with the same disposable spoon that was used for mixing the study drug into the pudding. Consumption of the mixed pudding product will be witnessed by a member of the study team, and may only be administered on site at the University Neuropsychiatric Institute. Each study participant will have a maximum of 30 minutes to consume the entire contents of the mixed pudding product. At completion of consumption, the spoon and the empty pudding cup will be disposed of in accordance with the Medication and Medical Waste Process document to prevent diversion and reduce any potential environmental impacts.

IDS Pharmacy staff will destroy any extracts that reach their expiration date in accordance with institutional policy and the Medication and Medical Waste Process document. CBD extract drug accountability will be performed in duplicate by two independent IDS pharmacy staff members. Any compounded study drug not consumed by participants will be destroyed by the research staff at the University Neuropsychiatric Institute in accordance with the Medication and Medical Waste Process document to prevent diversion and reduce any potential environmental impacts.

All waste will be documented on a controlled substance dispense/return log and signed by two witnesses in accordance with institutional policy.

Clinical Protocol: A number of reliable, well validated clinical measures will be administered in order to measure changes in pain and mood state after administration of CBD/placebo. Change in these behavioral measures also will be related to the MRS measures and markers of peripheral inflammation.

Blood Collection

We will collect a blood sample for a complete metabolic panel, liver function panel, peripheral cytokine levels, and a complete blood count at the screening visit as well as visits 1, 3, and 5. The cytokine panel assay will be performed by ARUP laboratories, a national reference laboratory and a worldwide leader in innovative laboratory research and development. Serum or plasma will be separated from cells within 2 hours of collection and 1 ml of serum or plasma will be transferred to an ARUP transport tube and transported to the lab. A quantitative multiplex bead assay will be performed to quantitate a number of cytokines (for entire list of cytokines; refer to https://ltd.aruplab.com/Tests/Pub/0051394).

Pain measures

The Visual Analog Scale (VAS): is designed to measure current pain level on a set scale that ranges from 1 to 10. The scale is a single line between 0, which is an anchor for little or no pain, and 10, which is an anchor for the most pain imaginable. Participants mark along the line where their pain level is at the time

of completing the measure [43].

The McGill Pain Questionnaire (McGill): is designed to examine the subjective qualities of pain [44]. The scale consists of a body drawing for individuals to mark their pain locations as well as 80 words that describe the sensory, affective, and evaluative components of pain. Pain intensity is reported from 0-80 depending upon the number of words selected by the participant.

The Pain Catastrophizing Scale (PCS): is a 13-item self-report measure that asks participants to reflect on past pain and to indicate the degree to which they experienced the 13 catastrophizing thoughts. Participants rate each thought on a 5-point scale from zero or "not at all" to four or "all the time." The PCS yields four scores: a total score and rumination, magnification, and helplessness subscale scores [45]

The Pain Disability Index (PDI): is a self-report inventory that assesses the extent to which pain interferes with an individual's functioning across seven broad domains of life activities. These domains are family/home, recreation, social activity, sexual behavior, self-care, and life-support. Each of the domains can be rated from zero or "no interference" to 10 or "total interference" [46].

Clinical Measures

The Structured Clinical Interview for DSM-IV Non-Patient Patient Version (SCID-NP) is a widely used diagnostic instrument to reliably determine Axis I disorders in clinical populations [49]

DSM-V Global Assessment of Functioning (GAF) is used to assess global functioning using a scale from 1(worst) to 100 (best) [50].

The Profile of Mood States (POMS) is a self-report inventory in which respondents' rate how well various mood states describe them.

The Hamilton Depression Rating Scale (HAM-D) is based on the clinician's interview with the patient and probes symptoms such as depressed mood, feelings of guilt, suicidal ideation, sleep disturbances, anxiety symptoms, and weight loss [51].

The Hamilton Anxiety Scale (HAM-A) is a clinician-administered rating scale developed to quantify the severity of anxiety symptomatology, often used in psychotropic drug evaluation [52]

MOSES: Potential side effects will be documented using the Monitoring of Side Effects Scale (MOSES) two days after the final dosing visit. The MOSES is comprehensive in its assessment of potential adverse effects, covering 83 symptoms across 8 bodily areas. Another advantage of the MOSES is that it integrates self-report and observational methods, thus increasing the accuracy of ratings.

TriPM: The TriPM is a 58-item assessment of adaptive and maladaptive personality traits. It yields three scale scores: Boldness, Disinhibition, and Meanness. Boldness reflects tendencies towards fearlessness, emotional resilience, and tolerance for uncertainty. Disinhibition measures characteristics related to problematic impulsivity and irresponsibility. Meanness indexes tendencies towards lack of empathy and aggression [32,33].

The Ohio State University Traumatic Brain Injury (OSU-TBI) Identification method: The OSU-TBI is a structured interview to identify traumatic brain injury (TBI) occurrences throughout a person's lifetime. Summary indices obtained from the OSU-TBI include the number, severity, timing, and effects of lifetime TBIs [62].

Columbia-Suicide Severity Rating Scale (CSRSS): The CSRSS is a standardized rating scale consisting of a

short questionnaire to assess an individual's risk of suicide.
CSRSS: 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). [63]

Imaging Protocol: Scans will be acquired on a Siemens 3-Tesla whole-body MRI system (Siemens AG, Erlangen, Germany). Scanning is performed at the University of Utah Neuropsychiatric Institute. The MR anatomic session allows localization of the Region of Interest (ROI) grid. A manufacturer-supplied 32-channel, phased array head coil for signal reception, and a circularly polarized body coil for RF transmission. Participants may discontinue scans at any time if they experience discomfort, e.g. claustrophobia.

Anatomic MRI: Structural imaging to rule out structural abnormalities will be obtained for all subjects, and a board-certified Radiologist reviews all scans. The MR anatomic session also allows for prescription of ACC and insula ^{1}H MRS voxels as well as post-hoc whole-brain and within-voxel segmentation of grey matter (GM), white matter (WM), and CSF. High-resolution, and high-contrast T1-weighted MR images will be obtained using a volume-navigated 3D-Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence (TR/TE/TI = 2500/2.88/1060 ms, matrix = 256x256, FOV = 256x256 mm, flip angle = 8^{o}, slab thickness = 176 mm, isotropic resolution = 1 mm, bandwidth = 240 Hz/pixel, GRAPPA in-plane acceleration factor = 2).

^{1}H MRS Data Acquisition: MRS voxels for ACC and insula regions will measure 25 x 25 x 30 mm^{3} and positioned either along the midline of the ACC or within the anterior insula, with the largest dimension spanning the anterior-posterior orientation. Static magnetic field (B_0) shimming will be performed using FASTMAP to achieve ACC and insula water signal linewidths (real component) of ≤ 9 Hz. 2D *J*-resolved ^{1}H MRS data will be acquired with a maximum-echo sampled acquisition scheme with the following parameters; TR/TE range = 2000/31-229 ms, 1TE = 2 ms, NEX per TE = 4. Total measurement time for setup, imaging, and ^{1}H MRS will not exceed 60 minutes.

^{1}H MRS Data Processing: Skull stripping and whole-brain tissue-type segmentation will be applied to MPRAGE data using the BET [53] and FAST [54] tools, respectively, provided with the FMRIB Software Library (FSL) [55]. Home-written MATLAB (R2016a, The MathWorks, Natick, MA) scripts then will be used to extract the 3D volume corresponding to ACC and insula voxels to obtain within-voxel GM, WM, and CSF tissue content. 2D *J*-resolved ^{1}H MRS data will be processed using the ProFit spectral fitting software [56] as described elsewhere [57].

Statistical methods, data analysis and interpretation: This study is designed to explore whether oral treatment with CBD for 5 consecutive days will result in changes in brain chemistry and peripheral inflammation. An N of 52, ages 18-60 will be randomly assigned to take CBD or placebo, for 5 days. Data will be expressed as mean ± standard deviation, except where noted. Variables will be compared using ANOVAs with a=0.05. All statistics will be computed using SPSS version 11.0 for the Macintosh. Data will be checked using distribution parameters before hypotheses testing. Where data is normally distributed, parametric statistical tests will be applied; whereas, in cases of non-normally distributed data, nonparametric statistical tests will be utilized. The primary dependent variables of interest are the continuous measures of ^{1}H-MRS derived metabolite ratios over water in the ACC and insula. These outcome variables will be derived after applying software analysis as described in the data processing section above. Group-based analyses will be conducted using Analysis of Variance (ANOVA) between chronic pain patients randomized to placebo and chronic pain patients randomized to CBD. Appropriate posthoc testing will be performed.

Risks to Study Participants: During the screening visit, participants may become emotionally upset when asked about their psychiatric history including suicide attempts. Participants may also experience discomfort when providing urine for pregnancy tests and/or drug tests or blood samples for health monitoring. It is possible that a participant's condition could worsen during the study. This could be related or unrelated to the study. If the participant's condition worsens to the point that they are a danger to them self or others, they will be referred for appropriate care. Participants will be screened prior to drug administration to ensure there are no mental health problems that are believed to putting the participant at further risk. The safety profile of CBD has been reported to be favorable in clinical trials with minimal side-effects reported [58]. The most commonly reported side effects were tiredness, diarrhea, and changes of appetite/weight [59]. CBD is known to inhibit and induce several CYP enzymes and hence drug-drug interactions may occur [59]. Participants will be counseled about these risks, informed of the signs and symptoms of these conditions, provided with information sheets detailing the symptoms of these side effects in lay terms and describing steps to take for further evaluation if indicated, and will be monitored for all of the listed conditions at each visit after the initiation of treatment.

MRI/MRS scans do not use ionizing radiation like x-rays or CT scans. Instead, magnetic fields and radio waves are used to take the pictures. There are no known risks related to MRI scans – other than the risk of injury when metallic objects are brought into the scanning room by mistake. Serious injury can occur during an MRI scan to persons who have:

1 Cardiac (heart) pacemakers.
1 Metal clips on blood vessels (also called stents).
1 Artificial heart valves.
1 Artificial arms, hands, legs, etc.
1 Brain stimulator devices.
1 Implanted drug pumps.
1 Cochlear (ear) implants.
1 Ocular (eye) implants or known metal fragments in eyes.
1 Exposure to shrapnel or metal fillings
1 Other metallic surgical parts.
1 Orthodontic braces on the teeth.
1 Body jewelry or piercings that cannot be removed for the scan.
1 Certain tattoos with metallic ink
1 Certain transdermal (skin) patches such as NicoDerm (nicotine for tobacco dependence),
1 Transdermal scopolamine or Ortho-Evra (birth control)

If the participants have any such devices, or has had a surgery where metal devices were placed in their body, they cannot take part in the study unless cleared for MRI scanning by the surgeon who implanted the medical device(s).

Serious risks exist if ferromagnetic objects (things that stick to magnets) are brought into the scanning area. These items can become dangerous flying objects, and are not allowed near the MRI scanner. The FDA has approved the 3T scanner for routine clinical studies. The FDA has decided that MRI machines of 8T or less do not pose a risk. Although the scans we are using in this study have no known risks, there could be ill effects that are delayed, such that the FDA has not yet recognized them. The brain scans do not cause pain. Apart from the scanner noise, the participant will not know the scan is taking place. Inside the scanner, some people experience claustrophobia (fear of being in small spaces), dizziness, headaches, or a metallic taste in the mouth. Some people experience double vision or see flashing lights. These symptoms are temporary, and will stop when the participant leaves the scanner. The participant may feel cramped inside the scanner. There is a mirror placed inside the scanner so the subject can see his or her face, and look out into the scanning room. The technologist will be able to hear the participant at all times. Very rarely, someone having an MRI scan feels a tingling in his or her back. This is due to the magnetic field changing

quickly during the scan. The precautions taken will avoid all the known risks related to MRI scans. The participant can stop the scan at any time.

Administrative responsibilities:
Study Resources: This research will be conducted at the Diagnostic Neuroimaging Laboratory at the University of Utah. The Diagnostic Neuroimaging Laboratory currently occupies 5,900 square foot of office and laboratory space in a building in the University of Utah Research Park adjacent to the Health Sciences campus. It also has access to a 3Tesla MRI scanner at University Neuropsychiatric Hospital.

Confidentiality: Confidentiality of information collected will be maintained with the assignment of an identification number or code, which will be used in place of subject names in all data analyses and reports. Keys showing the assignment of identification numbers to subject names will be stored in a locked file cabinet. Only the Principal Investigator, Co-Investigators and the research assistants will have access to the data that is collected. In addition, a Certificate of Confidentiality will be obtained from the FDA for all participants.

Recruitment: Adult males and females who report chronic pain conditions will be recruited from the surrounding community through newspaper advertisements, the posting of flyers, and word of mouth. All rules for posting flyers will be followed at every institution we canvass. Potential subjects will initially be screened over the phone to ensure that they meet the most basic inclusion criteria for this study.

Reporting to the U.S. Food and Drug Administration (FDA):

(a) Terms and Definitions:

Associated with the use of the drug: There is a reasonable possibility that the experience may have been caused by the investigational drug being studied.

Disability: A substantial disruption of a person's ability to conduct normal life functions and activities of daily living (ADLs).

Life-threatening adverse drug experience: Any adverse drug experience that places the participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred.

Serious adverse drug experience: An adverse drug experience occurring at any dose that results in any of the following outcomes: Death, a life-threatening adverse drug experience, hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include: allergic bronchospasm requiring treatment in an emergency room or at home, blood dyscrasias, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Unexpected adverse drug experience: Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this

definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure only referred to “elevated hepatic enzymes” or “hepatitis.” Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure only listed “cerebral vascular accidents.” "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

(b) Review of Safety Information:

The Principal Investigator will promptly review all information relevant to the safety of the drug obtained or otherwise received from any source, foreign or domestic, including information derived from any clinical or epidemiological investigations, animal investigations, commercial marketing experience, reports in the scientific literature, and unpublished scientific papers, as well as reports from foreign regulatory authorities that have not already been previously reported to the FDA.

(c) IND Safety Reports (IND#136053):

(1) Written reports

(i) As the IND holder, Dr. Renshaw shall notify the FDA in a written IND safety report of:

(A) Any adverse experience associated with the use of the drug that is both serious and unexpected; or

(B) Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity. Each notification shall be made as soon as possible and in no event later than 15 calendar days after the sponsor's initial receipt of the information. Each written notification may be submitted on FDA Form 3500A or in a narrative format (foreign events may be submitted either on an FDA Form 3500A or, if preferred, on a CIOMS I form; reports from animal or epidemiological studies shall be submitted in a narrative format) and shall bear prominent identification of its contents, i.e., "IND Safety Report." Each written notification to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND. If FDA determines that additional data are needed, the agency may require further data to be submitted.

(ii) In each written IND safety report, Dr. Renshaw shall identify all safety reports previously filed with the IND concerning a similar adverse experience, and shall analyze the significance of the adverse experience in light of the previous, similar reports.

(iii) The study team will send an annual report 60 days within the “study may proceed” date.

(2) *Telephone and facsimile transmission safety reports.* Dr. Renshaw shall also notify FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but in no event later than seven calendar days after the event. Each telephone call or facsimile transmission to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND.

(3) *Reporting format or frequency.* FDA may request that Dr. Renshaw submit IND safety reports in a

format or at a frequency different than that required under this paragraph.

(d) Follow-Up:

(1) Dr. Renshaw shall promptly investigate all safety information received by it.

(2) Follow-up information to a safety report shall be submitted as soon as the relevant information is available.

(3) If the results of Dr. Renshaw's investigation show that an adverse drug experience not initially determined to be reportable is so reportable, he shall report such experience in a written safety report as soon as possible, but in no event later than 15 calendar days after the determination is made.

(4) Results of Dr. Renshaw's investigation of other safety information shall be submitted, as appropriate, in an information amendment or annual report to the FDA.

Reporting to the University of Utah Institutional Review Board (IRB): The study team will strictly observe the University of Utah IRB policy that requires researchers to submit reports of events that may represent unanticipated problems (UPs) involving risks to participants and others, including unexpected, research-related adverse events. Reports will be submitted to the IRB as soon as possible after the principal investigator learns of the event, but in all cases within 10 working days. Late reports will be accompanied by a written explanation from the principal investigator as to why the report is tardy.

<u>The following will be reported promptly by the principal investigator to the IRB:</u>

- Unexpected, research-related adverse events;
- Breached of confidentiality or privacy that involves real or potential risk such as unauthorized use or disclosure of protected health information (PHI);
- New information indicating a change to the risks or benefits of the research, such as:
  - Reports that indicate that frequency or magnitude of harms or benefits may be different than initially presented to the IRB;
  - Publications that show that the risks or potential benefits of the research may be different than initially presented to the IRB;
  - Changes in FDA labeling or withdrawal from IND status or marketing of a drug, device, or biologic used in the research protocol;
- Incarceration of a participant, because this study is not approved to enroll prisoners;
- Complaints from participants or others involved in the research that indicate unexpected risks; or complaints that cannot be resolved by the research team;
- Warning or determination letters issued by any funding agency or regulatory body including the Office of Human Research Protections (OHRP), the Department of Health and Human Services (DHHS), or the Food and Drug Administration (FDA).
- Protocol Deviations**,** if they are:
  - Intended to eliminate apparent immediate hazard to a research participant;
  - Harmful (i.e. caused harm to participants or others, or placed them at increased risk of harm – including physical, psychological, economic, or social harm).
  - Possible *Serious Non-Compliance* (defined as an act or omission to act that resulted in increased physical, psychological, safety, or privacy risk that compromised the rights and welfare of research participants) – such as deliberate or repeated failure to obtain prior review and approval of new protocols and on-going human participants research by the IRB, or deliberate or repeated failure to obtain or document informed consent from human participants, or deliberate or repeated omission of a description of serious risks of the experimental therapy when obtaining informed consent, or deliberate or repeated failure to limit administration of the investigational drug or

device to those participants under the investigator's supervision, or deliberate or repeated failure to maintain accurate study records, report changes to the research, or report unanticipated problems posing risk to subjects or others to the IRB, or deliberate or repeated failure to comply with the conditions placed on the study by the University, the IRB, sponsor, or the FDA.

§ Possible *Continued Non-Compliance* (defined as a pattern of repeated actions or omissions to act that suggests a future likelihood of recurrence and that indicates a deficiency in the ability or willingness to comply with Federal regulations, or the policy, requirements, and determinations of the University of Utah IRB governing human subjects research) – such as consistently late submission of continuing review or items that require prompt reporting, repeated failure to comply with IRB requirements for completion of human subjects training before initiating study procedures, repeated failure to submit the required documents to the IRB, repeated refusal to comply with IRB requests, or repeated failure to submit progress reports.

Record Retention: In keeping with 21CFR312.57, study records will be maintained for at least two years after the drug is approved by the FDA or after shipment and delivery of the drug for investigational use has ceased and the FDA has been notified.

Protocol Changes: Any amendments or administrative changes in the research protocol during the period, for which the IRB approval has already been given, will not be initiated without submission of an amendment for IRB review and approval.

All amendments will be submitted to the FDA for review.

These requirements for approval will in no way prevent any immediate action from being taken by the investigator in the interests of preserving the safety of all patients included in the trial.

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