DETAILED PROTOCOL

Investigation of Cannabidiol for Reduction of NeuroInflammation in Chronic Back Pain

NCT # NCT03891264
11/12/2020

DETAILED PROTOCOL:

Investigation of Cannabidiol for Reduction of NeuroInflammation in Chronic Back Pain

PI: Jodi Gilman, PhD
Co-PI: Marco Loggia, PhD
Co-I: A. Eden Evins, MD
Co-I: Yi Zhang, MD
Co-I: Jianren Mao, MD
Co-I: Kristina Schnitzer, MD

Date: 11/12/2020

BACKGROUND AND SIGNIFICANCE

Millions of individuals suffer from chronic pain

Chronic pain is defined as pain without apparent biological value that has persisted beyond the normal tissue healing time (usually taken to be 3 or 6 months [1,2]). Chronic pain is a widespread public health issue [3], and its prevalence is enormous. The weighted mean prevalence of chronic pain in the general population has been estimated by some at 35.5%, or 105 million, in the United States [4]. Not only does chronic pain affect both physical and mental functioning, thus compromising quality of life; it is also associated with astronomical costs. In addition to the direct costs of treating pain—including health care for diagnosis and treatment, drugs, therapies, and other medical expenses—chronic pain results in lost work time and reduced productivity [5,6]. Past estimates of the annual cost of chronic pain in the United States, including healthcare expenses, lost income and productivity, were close to $100 billion [7].

Treatment for chronic pain is unsatisfactory

Despite the enormity of the phenomenon, clinical needs for chronic pain are largely unmet. The treatment of choice for the largest majority (as many as 90% [8]) of patients seeking chronic pain management is based on opioid analgesics. However, the evidence supporting long-term effectiveness of opioid drugs in relieving pain and improving functional status is weak [9]. For instance, despite the widespread use of opioids for

palliative care, more than half of all hospitalized patients experience pain in the last days of their lives, and 50-75% of cancer patients die in moderate to severe pain [10].

The current opioid-based pharmacological approaches to treat chronic pain are not only ineffective, but they generally have multiple unpleasant side effects, including constipation, pruritus, respiratory depression, nausea, vomiting, hyperalgesia, dizziness, sedation [9], as well as abuse and dependence [8,11,12]. Taken together, the unsatisfactory treatment efficacy and the occurrence of significant side effects, clearly stress the importance of achieving a deeper understanding of the pathophysiological mechanisms underlying chronic pain, in order to eventually identify viable treatment options alternative to ones currently available.

Microglia and pain

One of the reasons for the poor efficacy of the treatment options currently available for chronic pain might be that these are primarily aimed at suppressing neuronal activity within nociceptive pathways of the nervous system. However, it is now increasingly clear that neurons are far from being the only players that drive the establishment and/or maintenance of clinical pain symptoms. Rather, evidence from animal studies now suggests a central role of glial cells in the nervous system, including microglia [13,14].

Microglia are a subpopulation of macrophages that rapidly activate in response to a variety of pathological conditions [15], including persistent pain [16-23]. Microglial activation (MA) is characterized by a stereotypic pattern of cellular responses, including specific morphological changes, proliferation, increased or de-novo expression of cell surface markers or receptors, and migration to the site of injury [24]. MA generally represents an adaptive homeostatic defense response which enables the destruction of invading micro-organisms, the removal of potentially deleterious debris as well the promotion of tissue repair. However, animal studies have now showed that the uncontrolled activation of microglial cells under pathological pain conditions induces the release of substances that can sensitize pain pathways, such as proinflammatory cytokines, complement components, and others [25]. While evidence of pain-related MA was originally observed in the spinal cord, more recently it was also discovered at the level of the brain, including in the rostral ventromedial medulla [20,26], the trigeminal nuclear complex [19,27], and the ventral posterolateral nucleus of the thalamus [28,29].

While most of the evidence on the occurrence of pain-related glial responses in the central nervous system comes from animal studies, a few important observations indicate that similar phenomena should occur also in humans [13]. First, immunohistochemical markers of microglial and astroglial activation have been detected in the spinal cord of a patient with chronic regional pain syndrome in a postmortem study [30]. Furthermore, an increase in the concentration of the glial marker s-100β was reported in the cerebrospinal fluid of patients with lumbar disc herniation and in the serum of children with recurrent headaches [31,32]. Finally, a positron emission tomography (PET) study has revealed that

human subjects with neuropathic pain secondary to peripheral nerve damage express increased thalamic binding for [^{11}C](R)-PK11195 [33], an in vivo marker of microglial cell activation [34,35].

Recently, Co-PI Dr. Marco Loggia has also shown that patients with chronic low back pain (cLBP) have increased brain levels of the 18kDa translocator protein (TSPO), a marker of glial activation[36]. In addition, preliminary data collected from a different cohort of patients with cLBP and sciatica suggest an increase in spinal cord TSPO levels. Together, these results suggest that human chronic pain conditions are likely to be associated with a glial reaction, both in the spinal cord, as well as in the brain.

Cannabidiol and pain

There is a growing body of evidence to suggest that cannabinoids are beneficial for a range of clinical conditions, including pain, inflammation, epilepsy, and sleep disorders[37]. A large body of preclinical and clinical research indicates that the cannabinoid system modulates a broad range of physiological processes and behaviors including, but not limited to, pain, mood, appetite, neuronal activity, memory, immunity, and cell development. The endocannabinoid system's contribution to the regulation of such a variety of processes makes phytocannabinoid pharmacological modulation a promising therapeutic strategy [38].

The primary cannabinoids found in the cannabis plant include delta-9 - tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN), with THC being the primary psychoactive compound. The second most abundant compound in the plant is CBD, which is minimally psychoactive [39]. Cannabinoid receptor type 1 (CB1) and type 2 (CB2) belong to a family of seven transmembrane Guanosine Binding Protein-Coupled Receptors, and are widely expressed and distinguished by their specific functions, localization and signaling mechanisms. The psychotropic effects of cannabis are principally mediated by CB1, which is widely distributed throughout the brain, while CB2 is considered the peripheral cannabinoid receptor, found mainly in immune cells, as well as in chondrocytes, osteocytes and fibroblasts. Agonists targeting CB2 receptors have been proposed as therapies for the treatment or management of a range of painful conditions, including acute pain, chronic inflammatory pain, and neuropathic pain [40]. In a preclinical model, researchers showed that stimulation of CB2 suppresses microglial activation [41].

In the current study, we will test whether CBD is a glial inhibitor in patients with chronic lower back pain (cLBP). CBD was recently FDA-approved as a liquid formulation (see EPIDIOLEX package insert) for epilepsy for children ages 2 and up as well as adults, demonstrating significant reductions in total seizure frequency with minimal side effects. It is unclear whether cannabidiol reduces glial activation in humans. We will study 20 patients with low back pain (pain duration > 6 months) longitudinally before and after 4

weeks of treatment with cannabidiol. Endpoints will be pain scores as well as brain levels of the 18kDa translocator protein (TSPO), a marker of glial activation[36].

SPECIFIC AIMS

Primary Aim: Assess whether CBD reduces neuroinflammation in patients with cLBP.

Hypothesis: Patients will demonstrate higher PET signal in the brain (thalamus, S1/M1 representation of the lumbar spine) before CBD treatment in comparison to after CBD treatment. This within-subject comparison will allow us to evaluate short-term change over time in cLBP patients.

SUBJECTS SELECTION

We plan to identify 20 patients with chronic low back pain (cLBP; i.e., with a pain duration longer than 6 months), who will complete the study.

We are not planning to enroll subjects from at-risk populations (e.g., children and minors, cognitively impaired persons, prisoners). Written informed consent form will be obtained in all cases.

Inclusion Criteria:

- age ≥ 18 and ≤ 75;
- the ability to give written, informed consent;
- ongoing pain that averaged at least 4, on a 0-10 scale of pain during a typical week, and present for at least 50% of days during a typical week;
- on a stable pain treatment (pharmacological or otherwise) for the previous four weeks.
- Chronic low back pain, ongoing for at least 6 months prior to enrollment.

Exclusion Criteria:

- outpatient surgery within 2 months and inpatient surgery within 6 months from the time of scanning;
- elevated baseline transaminase (ALT and AST) levels above 3 times the Upper Limit of Normal (ULN), accompanied by elevations in bilirubin above 2 times the ULN
- any interventional pain procedures within 6 weeks prior to scanning procedure or at any point during study enrollment;
- surgical intervention or introduction/change in opioid regimen at any point during study enrollment
- contraindications to fMRI scanning and PET scanning (including presence

of a cardiac pacemaker or pacemaker wires, metallic particles in the body, vascular clips in the head or previous neurosurgery, prosthetic heart valves, claustrophobia);

- current or past history within the last 5 years of major medical illness not affecting the central nervous system, other than chronic pain;
- implanted spinal cord stimulator (SCS) for pain treatment;
- any history of neurological illness or major medical illness affecting the central nervous system, unless clearly resolved without long-term consequences;
- current or past history of major psychiatric illness;
    - PTSD, depression, and anxiety are exclusion criteria only if the conditions were so severe as to require hospitalization in the past year.
- pregnancy or breast feeding;
- history of head trauma requiring hospitalization;
- major cardiac event within the past 10 years;
- any use of recreational drugs in the past 3 months;
- any marijuana use, medical or recreational, in the past 3 months;
- an abnormal physical exam (e.g., peripheral edema);
- use of immunosuppressive medications, such as prednisone, TNF medications within 2 weeks of the visit;
- current bacterial or viral infection;
- epilepsy or any prescription of an anti-epileptic drug
- use of the medications valproate and clobazam, which may increase risk of hepatic AEs
- use of medications with:
    - Strong and moderate CYP3A4 inhibitors including boceprevir, cobicistat, conivaptan, danoprevir, elvitegravir, ritonavir, indinavir, itraconazole, ketoconazole, lopinavir, paritaprevir and ombitasvir and/or dasabuvir, posaconazole, saquinavir and telaprevir, tipranavir, clarithromycin, diltiazem, idelalisib, nefazodone, nelfinavir, troleandomycin, voriconazole, aprepitant, cimetidine, ciprofloxacin, clotrimazole, crizotinib, cyclosporine, dronedarone, erythromycin, fluconazole, fluvoxamine, imatinib, tofisopam, disulfiram, and verapamil;
    - Strong and moderate inhibitors of CYP2C19 including fluoxetine and ticlopidine;
    - Sensitive and moderately sensitive substrates of CYP2C19 including clobazam, lansoprazole, omeprazole, S-mephenytoin, and rabeprazole;
    - Sensitive and moderately sensitive substrates of CYP1A2 including alosetron, duloxetine, melatonin, ramelteon, tasimelteon, theophylline, tizanidine, pirfenidone, and ramosetron;

    - Sensitive and moderately sensitive substrates of CYP2B6 including bupropion and efavirenz;
    - Sensitive and moderately sensitive substrates of CYP2C8 including repaglinide, montelukast, pioglitazone, and rosiglitazone;
    - Sensitive and moderately sensitive substrates of CYP2C9 including tolbutamide, celecoxib, glimepiride, and warfarin;
    - Sensitive and moderately sensitive substrates of UGT1A9 including diflunisal, propofol, and fenofibrate;
    - Sensitive and moderately sensitive substrates of UGT2B7 including, gemfibrozil, lamotrigine, and morphine;
- The following CNS depressants including all antipsychotics, benzodiazepines (except for alprazolam, clonazepam, and lorazepam), and the non-benzodiazepine sleep aids including butabarbital sodium, eszopiclone, phenobarbital, ramelteon secobarbital sodium, suvorexant, zaleplon, and zolpidem;
- regular use of opioids
- actively suicidal, history of suicide attempt or an aborted attempt within the last 5 years, or engagement in non-suicidal self-injurious behavior within the last year
- Any other contraindications to CBD administration noted by the study physician.

SUBJECT ENROLLMENT

Subjects will be recruited through advertising by flyers and printed announcements posted within as well as outside of our Partners community. In addition, email, web and bulletin board announcements posted in the community will be used. We will also use the Partners' RSVP for Health system, Partners Clinical Trials, as well as ResearchMatch, a database of research volunteers developed by Vanderbilt University and approved for use by the PHRC. In addition, methods that advertise the study to the greater community will be used such as social media posts, posting flyers on community billboards in the greater Boston area, emails to physicians and family medicine centers, and advertisements in newspapers etc. will be adopted. Advertisements will briefly describe the study and invite subjects to call if they are interested. All subjects will undergo a telephone screening to attempt to distinguish potential subjects from those not meeting eligibility criteria. Informed consent will be obtained from all subjects before initiating any study procedures at study visit #1 (Behavioral visit).

During screening, potential participants will be fully informed of the purpose and activities involved in the research study. Interested subjects will be scheduled for an in-person visit where written informed consent will be immediately obtained, prior to initiating any of the study procedures. One copy of the signed consent form will be given to the patient and one will be kept in the study files for documentation. No time limits

will be imposed on the informed consent process. Participants will be permitted to take as much time as they desire to engage in the informed consent process; any and all of their questions will be answered. It is anticipated that obtaining written informed consent will take approximately 15-25 minutes, on average. Comprehension of the consent information will be assessed via solicitation of answers to questions throughout the process. If comprehension appears to be limited, participants will be actively queried to determine whether they need further explanation.

A physician member of the staff will obtain informed consent, as in all other protocols involving [^{11}C]PBR28 PET/MR scanning (e.g., 2011P002311).

STUDY PROCEDURES

After a behavioral visit aimed at determining eligibility, subjects will be scheduled for their first imaging visit, during which they will undergo a simultaneous MR-PET scan. At this visit, subjects will receive CBD. During the drug trial period, subjects will also be sent a daily survey to assess the treatment effect of the medication on their pain. As soon as possible after the end of the 4-week drug trial period, all subjects will be scanned again.

Investigational Agent

Epidiolex, an agent within the anti-epileptic drug class, will be used. Epidiolex, Greenwich Biosciences Inc.'s CBD formulation, is a 100 mg/mL purified oral solution, dissolved in the excipients sesame oil and anhydrous ethanol with added sweetener (sucralose) and strawberry flavoring. The drug is formulated from extracts prepared from Cannabis sativa L. plants that have a defined chemical profile and contain consistent levels of CBD as the principal phytocannabinoid. Extracts from these plants are processed to yield pure (>95%) CBD that typically contains less than 0.5% (w/w) THC. Cannabidiol is the active ingredient in Epidiolex; inactive ingredients include dehydrated alcohol, sesame seed oil, strawberry flavor, and sucralose. Of note, CBD has no psychoactive properties. The empirical formula of Epidiolex is $C_{21}H_{30}O_2$ and its molecular weight is 314.46. The structure of CBD is provided in the figure below.

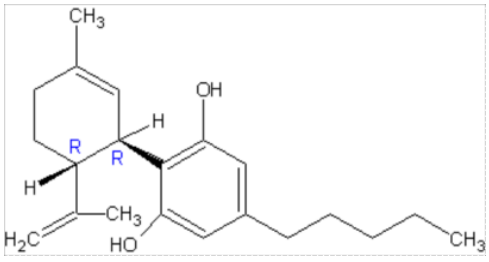

Figure 1. Cannabidiol Structure

Dose and Exposure
The recommended starting dosage is 2.5mg/kg taken twice daily (5mg/kg/day). Participants will follow a titration schedule, with 5 mg/kg/d in week 1, 10 mg/kg/d in week 2, 15 mg/kg/d in week 3, and 20 mg/kg/d in week 4. Subjects will increase to 20 mg/kg/d on the first day of the final week of the study (week 4) and take Epidiolex at this dose for the remainder of this final week. If participants report AEs (tiredness, dizziness, not tolerating the medication well), the physician will decrease the dose by 5 mg/kg. Participants will be treated for 4 weeks in total.

The 4-week duration of CBD administration is proposed because in the current study, we are investigating an endophenotype of pain- neuroinflammation- which may be detectable before verbal reports of pain reduction, which is notoriously noisy and susceptible to placebo effects. This pilot study will inform us of whether 4 weeks is enough to detect changes in TSPO binding that may precede verbal reports of pain reduction.

Screening visit: Subjects eligible to participate will be recruited to participate in a 3-hour characterization session. In this session, we will obtain a signed consent form from the subjects, explain the procedures involved in the experiment, and administer some or all of the following validated assessments. We will also collect detailed contact information (address, social security number, medical record number) as well as collect a blood sample and a urine sample. Computer-based rating scales and questionnaires will be completed on a laptop. All assessments will be performed by fully trained study staff members such as post-doctoral research fellows and Clinical Research Coordinators, under the supervision of and periodic monitoring by the Principal Investigator (PI).

Many of these assessments are already in use in one or more IRB approved protocols (e.g., 2011P002311).

Hospital Anxiety and Depression Scale (HADS): The HADS is a 14-item self-report survey designed for populations with medical illness[42]. It does not include somatic symptoms, such as fatigue and sleeplessness, which may otherwise be attributable to pain. It asks patients to rate depression and anxiety symptom over the past week on a 4-point Likert scale. It has been validated in several medical illness populations and has been used extensively in chronic pain patients. This scale will be repeated at Baseline scan, 2-week visit, and 4-week visit, and any significant increase will warrant clinical attention. If there is more than a 30% increase in symptoms of anxiety or depression, this will be immediately reported to the PI, who will consult with study clinicians. Clinicians will them determine, with the participant, whether it is in their best interest to continue the medication.

The Pain Catastrophizing Scale (PCS): It is a 13-item self-report scale which measures pain-related Rumination, Magnification and Helplessness[43].

PainDETECT[44]: The PainDETECT is a screening questionnaire used to estimate the likelihood of a neuropathic component in chronic pain.

Social Provisions Scale (SPS): The social provisions scale measures the social support provided by the patient's current relationships.[45]

Snaith-Hamilton Pleasure Scale (SHAPS)[46]*:* The Snaith-Hamilton survey is a 14-item questionnaire designed to estimate the degree to which an individual is able to experience pleasure, or the anticipation of pleasure. The questionnaire assesses four domains of hedonic experience: interest/pastimes, social interaction, sensory experience, and food/drink. All items relate to daily experiences encountered by most people.

Fibromyalgia Survey Questionnaire (FSQ)[117]*:* The Fibromyalgia Survey Questionnaire is a 5-item questionnaire that is aimed at assessing the presence of fibromyalgia, or pain centralization, subclinically in people who do not meet the fibromyalgia criteria.

History and physical examination: an MD or NP will also collect medical history and perform a formal physical examination, including the recording of vital signs (heart rate, blood pressure, and body temperature).

Suicidal Ideation. The Columbia-Suicide Severity Rating Scale (C-SSRS) (Posner et al., 2010) will be used for prospective suicidality assessment. C-SSRS is a tool used to assess the lifetime suicidality of a participant and to track suicidal events through the treatment. The structured interview prompts recollection of suicidal ideation, including the intensity of the ideation, behavior and attempts with actual/potential lethality. The scale will be administered by study staff at the screening visit, baseline, two-week visit, and four-week visit. The C-SSRS "Screening/Baseline" will be collected at Screening and Baseline and the C-SSRS "since last visit" will be collected at subsequent visits. Participants who answer "yes" to any suicidal behavior questions or to suicidal ideation questions 4 or 5 on the C-SSRS during the study should be referred for appropriate psychiatric care. If there is more than a 30% increase in symptoms of anxiety or depression, this will be immediately reported to the PI, who will consult with study clinicians. Clinicians will then determine, with the participant, whether it is in their best interest to continue the medication. The decision to discontinue the participant from the study will be made by the PI in conjunction with clinical Co-Investigators.

Safety monitoring: As participant suicidality and depression is monitored throughout the study with the C-SSRS and BDI questionnaires, any new or worsening expression of suicidal ideation or Answers of "Yes" to questions 4 or 5 on C-SSRS throughout the study will be evaluation by a licensed clinician member of study staff. The Standard Operation Procedure will be reviewed. To summarize, if any risk for self-harm or suicidality is identified at visit 1, visit 3, or visit 4, the research coordinators will immediately report this to study clinicians, who will determine whether a safety assessment is needed. If a clinician is needed to perform a safety assessment, study staff will record the date, clinician initials, and comments related to the suicidality assessment

in the Study Trax C-SSRS module. Following the initial suspicion or identification of self-harm and/or suicidality, a study clinician will follow up with the participant on the current nature of their situation, querying about any new ideation, intent, and/or plan since the last visit. These clinicians, along with the PI will then determine whether a participant can safely continue the study. If the clinicians determine that the participant cannot safely continue the study, the participant will be discontinued, and will be provided with a list of resources for follow-up care.

Blood tests: a trained member of the study staff will draw venous blood (up to 10 ml) from all subjects in order to have them genotyped for the **Ala147Thr TSPO polymorphism** in the *TSPO* gene (rs6971) (unless this genotype information is already available), and to check liver enzyme values. Additionally, during screening for eligibility, we will conduct routine chemistry and LFT/GGT. We will obtain serum transaminases (ALT and AST) and total bilirubin levels in all patients prior to starting treatment with CBD.

While [^{11}C]PBR28 has the advantage of binding to the TSPO protein with a higher ratio of specific-to-nonspecific binding than [^{11}C](R)PK11195[47], it also presents a potential limitation, in that about 10% of human subjects show no binding to PBR28[48] (whereas [^{11}C](R)-PK11195 has never been associated with non-binding[49]). A recent study has demonstrated that the rs6971 polymorphism predicts PBR28 binding affinity in human platelets[50]. Since the low-affinity binder phenotype is consistent across all tissues within the same subject [49], testing for the Ala147Thr polymorphism has been suggested to predict low affinity for [^{11}C]PBR28 in all organs, including the brain. High or Mixed affinity binders (Ala/Ala or Ala/Thr) will be considered eligible, whereas the Low affinity binders (Thr/Thr) will be considered ineligible for the research study**.** The MGH lab responsible for genotyping typically runs the genotyping assay only twice per month, requiring that we normally schedule screening and scanning visits approximately two weeks apart.

An additional 10 mL of venous blood will be collected and stored for future investigations on the roles of genetic, molecular, and cellular factors in pain disorders. This will include the future possibility to generate induced pluripotent stem cells (iPSCs) from peripheral blood mononuclear cells[51-53] to assess in-vitro alterations in patient-derived neural or glial cells[54,55].

Urine drug test: We will also perform a urine test to screen for use of opioids and illicit drugs (including amphetamine, barbiturates, cocaine, marijuana, etc.). The urine drug screen will be performed during the screening visit. A rapid urine drug screening that utilizes monoclonal antibodies to detect elevated levels of specific drugs in urine, will be used for this purpose. Results will be read five minutes after the test was started.

MR-PET visits

Participants eligible to continue into the study based on the screening visit and initial blood test will be asked to participate in a first MR-PET visit.

Prior to each scan session, subjects will complete screening checklists for MRI and PET. These checklists will ask the patients whether they have any contraindications for MR or PET scanning. Female participants of childbearing age will be asked to have ~3mL of their blood drawn in order to perform a serum pregnancy test on the day of the scan (blood will be sent to the core lab for super stat testing).

At the beginning of the scan session, an intravenous catheter will be placed in the participant's antecubital vein of the left or right arm, prior to going to the scanning area. Up to 15mL of blood will be drawn to assess the levels of various substances in the blood, such as the proinflammatory cytokines IL-6 and TNF-alpha. Blood will be collected in various vials (e.g., purple top K3EDTA).

Subjects will be instructed to remain still, with eyes open, for the total duration of the scans, except when prompted to express various ratings (e.g., pain intensity, unpleasantness, anxiety). The radioligand [^{11}C]PBR28 will used to determine whether patients with chronic pain exhibit evidence of microglial activation.

During the scan visit, subjects will be asked to complete the *BPI, BDI and PROMIS-29* assessments.

Brief Pain Inventory (BPI): The BPI is a 15-item questionnaire assessing pain location, and 0–10 ratings of pain intensity, relief, quality, pain-related quality of life, and function. It has been validated in cancer and noncancer pain conditions[56].

Beck Depression Inventory (BDI): The 21-item BDI has shown good sensitivity and specificity for major depression in chronic pain patients.

Patient Reported Outcomes Measurement Information System (PROMIS-29) questionnaire[57]*:* The PROMIS-29 is a 29-item self-report measure assessing physical, mental, and social health.

The total duration of each scanning visit will be approximately 4 hours (and up to 6 hours) (~45min for preparation, ~120 min for scanning procedures and ~60min for filling out questionnaires and observation, plus an additional ~90min to perform pregnancy test in women of childbearing age). In case of equipment failure (e.g., failure in radiosynthesis) delays of > 2 hours may be possible. In this case, we will ask the participant if he or she feels comfortable with staying longer than anticipated, or will prefer reschedule to another date.

Depending on the patients' level of discomfort and time constraints, we may occasionally shorten and simplify the scan visits. For instance, if the participant would feel too uncomfortable to lay down in the scanner for the full ~2:00 hours of scanning, we may administer the radioligand in the injection room and then scan the participant between 45 and 90 minutes post-injection.

The imaging visit, including all the procedures described above, will be repeated a second time after a 4-week trial of CBD.

Drug Administration Protocol

Following the behavioral visit, subjects will receive CBD. They will be instructed to take 2.5mg/kg twice daily (5mg/kg/day). Participants will follow a titration schedule, with 5 mg/kg/d in week 1, 10 mg/kg/d in week 2, 15 mg/kg/d in week 3, and 20 mg/kg/d in week 4. Subjects will increase to 20 mg/kg/d on the first day of the final week of the study (week 4) and take Epidiolex at this dose for the remainder of this final week. If participants report AEs (tiredness, dizziness, not tolerating the medication well), the physician will decrease the dose by 5 mg/kg. Participants will be treated for 4 weeks in total. Chronic CBD dosing up to 1500 mg/day has been reported to be tolerated well without AEs [58-61]; minor AEs were reported after CBD use in children with epilepsy being treated with multiple other medications in doses up to 50 mg/kg/day[62,63]. Accordingly, we believe an upper limit of 20 mg/kg/day orally is reasonable and safe.

Continuation of medication (e.g., NSAIDS) will be permitted on the condition that patients will be on a stable dose for at least 1 month before the baseline MR-PET scan.

The Research Pharmacy at Massachusetts General Hospital will prepare the CBD. The study drug will be labeled as Epidiolex 100 mg/mL. It will be labeled with the name of the sponsor, lot number, trial subject identification number, storage conditions and the statement "for clinical trial use only." Each container will be labeled with a unique number that will be recorded by study staff at the time of administration. As soon as possible after the 4-week CBD period, patients will be re-scanned and/or re-evaluated clinically to evaluate the hypothesis that CBD reduces glial activation and pain symptoms. Participants will be instructed to take CBD with food, rather than in a fasted state, and not to take CBD concurrently with alcohol.

Follow-up Visits

2-Week Visit. Patients will undergo a follow-up appointment at Week 2 with a study clinician, where health and adverse events will be assessed, and patients will complete questionnaires and will be reminded to increase medication dose. Participant may receive a ride to and from the study visit if requested.

4-Week Visit. Patients will undergo a second follow-up appointment immediately after the four-week treatment period with a study clinician, where we will assess back pain, general health, and adverse events. On the same day or as close as possible depending on scheduling, patients will be re-scanned, using identical protocols, to evaluate the hypothesis that CBD reduces glial activation. We will also repeat the questionnaires administered during the baseline visit to assess any changes in subjective pain. While this study is focused on mechanisms, and is not powered to detect a clinical effect, collecting pain scores will allow us to start evaluating the potential clinical significance of any reductions in neuroinflammation we will observe, and also provide important preliminary data for a subsequent clinical trial focused on efficacy. We will also take a small sample of blood for a follow-up liver function test. Participant may receive a ride to and from the study visit if requested.

6-Week Call. We will conduct a follow-up call 2 weeks after the discontinuation of the study medication. In this call, we will assess back pain, general health and adverse events.

DATA ACQUISITION AND ANALYSES

MR-PET scanning will be performed at 3 Tesla, using Siemens TIM Trio whole-body MRI with head PET camera, and/or Siemens Verio whole-body MRI, whole-body PET camera (Biograph mMR).

PET

During each visit, [^{11}C]PBR28 (up to 15 mCi, corresponding to ~3.7mSv) will be injected intravenously with a slow bolus over a 30-60s period[64]. This dose is compatible with a longitudinal study, because the total radiation of maximum ~7.4mSv received by the subjects participating in two scans will be significantly less than the maximum allowed annual whole-body dose set by the U.S. Food and Drug Administration at 5 REM, i.e., 50mSv). The catheter will be flushed post-injection with 0.9% saline solution. Dynamic data will be collected over ~120 minutes in list mode, and framed post-collection. Regional uptake of the tracer will be estimated as SUV, SUVR, Vt and/or DVR.

In addition, participants will be asked if they are willing to undergo spinal cord PET imaging, which will take ~20-30 minutes extra in the PET scanner at the end of the 90 minutes post-injection. This will not add significant risk, as it will not require extra injection.

MRI

MRI data will be acquired simultaneously to the PET data, and may include some or all of these standard sequences: BOLD fMRI (e.g., for functional connectivity analyses, to

estimate motion parameters), Diffusion-Spectrum or Diffusion-Tensor Imaging volumes (DSI) (to perform tractography analyses); Chemical shift imaging (to estimate brain metabolites); a high resolution structural volume (for anatomical reference, cortical reconstruction, volumetric segmentation, and attenuation correction); in addition to various T1 and T2 weighted structural volumes.

We may collect a 6-minute resting-state connectivity functional MRI scan. The additional MR sequences are only collected when all the core MRI data have been completed early, and the patient still needs to remain in the scanner to complete data acquisition. As mentioned before, this is an integrated PET/MR study, the MRI (including fMRI) data is collected throughout the PET data acquisition, and as such the participant needs to stay in the scanner for the 90 minutes to complete the brain scan. Therefore these additional MR sequences will not affect the duration of the scan. This additional data will be used for ancillary analyses.

Behavioral measures
Before, during and after the scans, subjects may be asked (either verbally, or using a button box connected to a computer) to express various behavioral ratings, including pain intensity, unpleasantness, and anxiety.

BIOSTATISTICAL ANALYSES

If time allows, we may collect resting-state fMRI scans. This will only be collected if core MRI data have been completed early, and the patient still needs to remain in the scanner to complete data acquisition. Therefore, the resting state fMRI will not affect the duration of the scan. All fMRI data will be stored on hard drive and storage clusters. Behavioral data will be recorded and stored in RedCap in preparation for statistical analyses.

In order to address our aim ('**Assess the effect of CBD on glial activation and pain**), we will perform paired t-tests in cLBP patients **before vs after** receiving CBD. We will also perform an ANOVA including age and sex as covariates of no interest. Additionally, secondary regression analyses will be performed in order to evaluate the association between imaging and behavioral data (e.g., questionnaire scores, pain duration, etc). For the exploratory assessment of the relationship between imaging and pain measures, we will use multiple regression analysis. We will use a thalamus-targeted region of interest analysis, in which mean SUVRs will be extracted from the voxels within the right and left thalamus labels of the Harvard-Oxford Subcortical Structural Atlas and will be correlated with differences in clinical pain scores from baseline to Week 4. To be consistent in our use of non-parametric methods, we will use a non-parametric regression method, Generalized Additive Models. We will compute residuals adjusting for the effect of genotype. The Ala147Thr polymorphism in the TSPO gene predicts binding affinity

for 11C-PBR28, with the Ala/Ala, Ala/Thr and Thr/Thr genotypes being associated with high, mixed and low affinity binding, respectively. For all analyses, we will adjust for the TSPO polymorphism.

Since the analysis plan requires a pre- and post-scan, we will not include patients with only one scan in the analysis for this pilot study. If the pilot study warrants a larger trial, we will use likelihood based mixed models to produce unbiased estimates of effects in the presence of missing data.

These analyses will be performed voxelwise for the brain data, using methods similar or identical to those we have previously used[36], as well for spinal cord data using methods developed by Dr. Cohen-Adad[65]. In addition to these voxelwise analyses, for the spinal cord we will perform region-of-interest (ROI) analyses focused on the spinal cord segments contained in the T11-L1 vertebral levels (as these contain the lower lumbar/upper sacral spinal cord segments).

All voxelwise analyses will be conducted using the FSL suite[66] for the brain, and using the Spinal Cord Toolbox[65] for the spinal cord. Group comparisons maps will be thresholded using threshold-free cluster enhancement[67], and a corrected threshold of p=0.05. All behavioral, demographic and ROI data will be analyzed using Statistica 10.0 (StatSoft Inc., USA), and an alpha level of 0.05.

Consideration of sex as a biological variable

In addition to the aforementioned analyses, the effect of sex will be evaluated using ANOVAs, because animal research suggests the presence of a possible sexual dimorphism in the role of glia in pain (as pain hypersensitivity may be microglial-dependent only in males[68]). The effect of menstrual cycle status will also be evaluated by comparing women in early follicular (day 2-7 after onset of menses) and midluteal (day 20-25 after onset of menses), based on self-report[69].

POWER ANALYSIS

The purpose of this pilot study is to collect data to adequately power a placebo-controlled trial. The sample size proposed in this study, n = 20, is similar to other cohorts in the PET literature. Most similar to our study is a report in Brain by Loggia et al that showed significant reductions in TSPO binding between 10 chronic back pain patients and 9 healthy controls (Loggia, 2015)**.** This is not a fully-powered trial. If we see a trend toward reduction in TSPO, this will warrant a fully-powered, placebo-controlled trial, for which an adequate sample size will be calculated. We will have dual criteria by which we will consider a trend-level effect to be worthy of consideration of applying for funding to support a larger placebo-controlled trial. If we either (1) observe a TSPO reduction in binding of 5-10% on average from post to pre-

CBD, or (2) observe a clear and consistent reduction in TSPO binding in those patients who also report pain reductions, we will consider this a trend worth pursuit.

RISKS AND DISCOMFORTS

All subjects will undergo a telephone or email screening to attempt to distinguish potential subjects from those not meeting eligibility criteria. Likely candidates will undergo a characterization and training session, which will include a clinical screening procedure. This procedure will involve answering questions about subjects' medical history recording of medical history review and answering questions about their medical situation including liver disease, kidney disease, blood disorders, heart disease, alcohol and opioid use, high blood pressure, asthma and other respiratory disorders.

PET/MR Procedure: The U.S. Food and Drug Administration (FDA) recently gave the first regulatory clearance of a hybrid PET/MRI scanner in the U.S. Additionally, FDA considers investigations of MRI software and hardware operating within FDA specific parameters as non-significant risk device studies. All studies will adhere to these FDA approved safety levels for the Siemens system. These safety parameters include static magnetic field, time varying magnetic fields (dB/dt), specific absorption rate (SAR), and acoustic noise levels. Subjects will be informed about minimal risks of routine high magnetic field and non-ionizing RF radiation involved in MR imaging.

Subjects will also be informed about the PET procedure and the minor risks associated with exposure to radiation. Subjects will also be informed about small space within the magnet and noises made by switching gradients. Subjects will be informed that if they feel uncomfortable with the study, they can choose to terminate the study at any time. They will be informed that their refusal to participate in the study or choosing to terminate it at some point will have no effect on care and treatment received by them at MGH now or in future. The subjects will be informed that their personal information will be protected as per the HIPAA guidelines.

Intravenous catheter: An intravenous catheter will be placed for this study. The subject will feel a slight pinprick, similar to a bee sting, and may feel some discomfort and have some bruising or bleeding at the site where the needle goes in. Depending on the length of time the catheter is in place, a bruise may last for a day or so. Rarely an infection may occur at this site. If infection does occur, it will be treated. About 24 hours after the beginning of the imaging procedures, we will give the subject a phone call to determine whether he or she is experiencing study related issues.

Radiation exposure: The radiation exposure in this study will be small and there is no evidence that it represents a major health risk. If subjects have participated in other research studies in the past 12 months that have involved radiation exposure, they will be

asked to inform the investigators or study staff (by writing initials on the consent form verifying that they have not been exposed to other radiation in the past 12 months). If it is determined that their prior radiation exposure exceeds our current guidelines, they may not be allowed to participate in this study.

We will use [^{11}C]PBR28 produced by the cyclotron/radiochemistry/radiopharmacy facility at the A. A. Martinos Center for Biomedical Imaging. The Martinos Center has studied several hundreds of people with this radioligand and have had no clinically detectable effects or side effects. Given the use of [^{11}C]PBR28 in a small clinical trial, we are in the process of requesting an IND from the FDA.

The IV injection will be administered by a licensed nuclear medicine technologist. Should there be an adverse event, Dr. Gilman will be responsible for communicating with the IRB within the stipulated time frame.

Imaging will be stopped should any untoward reaction be observed during the imaging session or if the participant so requests for whatever reason. Some subjects find it unpleasant or feel anxious when confined in the enclosed space of the scanner. If this happens, the study will be aborted. Patients will be required to use earplugs to decrease the noise perceived while in the scanner.

EPIDIOLEX (CBD): CBD is an FDA approved medication used to treat epilepsy. According to the FDA briefing document on Epidiolex, dated 04/19/2018, treatment-emergent AEs in controlled trials for Lennox-Gastaut and Dravet syndromes included decreased appetite, diarrhea, irritability, somnolence, fatigue, aggression, pneumonia, rash, and hepatic symptoms, and in a very small number of patients, an increase in suicidal thoughts. Of note, AE related to hepatic function were likely due to the interaction between CBD and anti-epileptic drugs; prescription of an anti-epileptic drug is an exclusionary criterion for this proposed study. Further, CBD may produce pharmacokinetic interaction effects when taken with opioids (Benowitz et al., 1980; Kotlinska-Lemieszek et al., 2015). Any subjects who at baseline had elevated AST/ALT levels but still met the eligibility criteria for the study will undergo a follow-up liver function tests at 2 weeks. In addition, we will perform a liver function test in subjects who at any time point during the study develop clinical signs or symptoms suggestive of hepatic dysfunction.

Given the use of EPIDIOLEX in a small clinical trial, we are in the process of requesting an IND (or an IND exemption) from the FDA.

Questionnaires: Minimal risks associated with completing questionnaires are subject fatigue and the possibility of minor psychological distress associated with answering sensitive questions regarding psychological functioning. Subjects will be instructed to complete the questionnaires to the best of their ability, but will have the option to leave

any question(s) blank. In the unlikely event that evidence of physical or psychological disorder is found, with the individual's permission, the information will be shared with his or her primary care physician who can direct care as needed.

Confidentiality: As detailed, the investigators are quite careful regarding the protection of confidentiality, and multiple procedures are in place to reduce the likelihood of a breach of confidentiality. However, there is a small risk that information about subjects could become known to people outside of this study, and this risk is identified in the informed consent form.

The key investigators will meet quarterly to discuss any potential adverse event and side effects. We will involve the MGH Human Research Committee and Radiation Safety Committee if any additional potential risks arise. Adverse events and unanticipated problems involving risks to subjects or others will be reported to the PHRC in accordance with PHRC adverse event and unanticipated problems reporting guidelines, as well as FDA when appropriate.

POTENTIAL BENEFITS:

It is unlikely that individual subjects will benefit from taking part in this study. While this study is powered to possibly observe a statistically significant reduction in pain due to CBD, it is unclear whether the effect will be clinically meaningful. However, findings from these studies will help advance our understanding of the pathophysiology of pain disorders. In particular, this project will assess the role of microglia in the establishment and/or maintenance of chronic pain, and how this may be affected by CBD.

REMUNERATION:

Subjects will be paid by check at the completion of the study for their participation.

We will pay up to $525. Payments will be as follows:

- $75 for the initial behavioral session (Visit 1)
- $50 for completing daily surveys
- $200 for each MR-PET scanning visit

If during the imaging visit(s) we cannot inject the subject with the radioligand (e.g., due to a failure in radiosynthesis, or to issues with the scanner) he/she will receive $50.

If the subject will need to stop the scan early for any reason, he/she will still receive $50 for his/her time. Additionally, parking fees will be covered as needed.

MONITORING AND QA

The proposed study will be monitored for safety, with monthly staff meetings reviewing adverse events and treatment outcomes and directly reporting any adverse events. The PI will also routinely monitor and assure the validity and integrity of collected data and adherence to the IRB-approved protocol. The trained staff members who carry out the procedures will also carefully monitor the study throughout its duration. The team will evaluate the progress of the study, verify that the rights and well-being of the subjects are protected, verify that the reported study data are accurate, complete and verifiable from source documents, and the conduct of the study is in compliance with the approved protocol and amendments. Outcome monitoring and adverse events will all be reported through appropriate channels of the Human Studies Committee as well to the FDA when appropriate.

If a patient develops clinical signs or symptoms suggestive of hepatic dysfunction (e.g., unexplained nausea, vomiting, right upper quadrant abdominal pain, fatigue, anorexia, or jaundice and/or dark urine), we will promptly measure serum transaminases and total bilirubin and interrupt or discontinue treatment with EPIDIOLEX, as appropriate. We will discontinue EPIDIOLEX in any patients with elevations of transaminase levels greater than 3 times the ULN and bilirubin levels greater than 2 times the ULN. Our physician monitoring group (Drs. Mao, Zhang, Schnitzer, and Evins) will consider stopping the study if back pain becomes significantly worse in 3 or more patients. If serum liver enzyme concentrations are significantly elevated (with elevations of transaminase levels greater than 3 times the ULN and bilirubin levels greater than 2 times the ULN) in 2 or more patients, which will be considered serious adverse events, the study will be stopped. In addition, if two or more patients experience any serious adverse event, the study will be stopped.

The Siemens MR-PET scanners have a built-in self-monitoring system that automatically shuts off if parameters exceed safe levels. For backup protection NMR technicians constantly monitor the subjects' physiological signs and the quality of the raw data.

Quality assurance of the scanner's performance is obtained by a daily quality assurance protocol. More extensive quality assurance protocols are performed monthly under the commercial service contract with Siemens Medical Systems. The daily quality assurance protocol consists of an image Signal-to-Noise measurement in a phantom and a stability run which checks the image-to-image variation in image intensity over 600 images using a standard echoplanar imaging sequence with a head-sized phantom. The images are analyzed by the technologist to provide data on SNR (as an absolute, unitless number)

and stability expressed as the peak-to-peak variation in the mean of a 15x15 pixel region of interest (ROI) in the center of the phantom expressed as a percentage of the mean of the ROI. Runs are performed at each of 3 TR values (300ms, 800ms, 1300ms). The time course of the means is also reviewed to check for periodicities (the TR values are chosen so as not to be multiples of one another). If the peak-to-peak variation is greater than 0.5% of the mean value, the Siemens Medical System service engineer is called. In addition to these daily quality assurance tests, the Siemens Medical System service engineer performs quality assurance tests once a month. These tests include a SNR test, a small sample stability test, a gradient stability test, a gradient eddy current test, a shim test, an image uniformity test, and an RF stability test.

BIBLIOGRAPHY

1 Merskey, H. & Bogduk, N. *Classification of Chronic Pain, Second Edition, Part III: Pain Terms, A Current List with Definitions and Notes on Usage*. (IASP Press, 1994).

2 Turk, D. C. & Okifuji, A. in *Bonica's management of pain (3rd ed.)* (ed S.M. Fishman, Ballantyne, J.C., Rathmell, J.P.) (Kluwer/Lippincott, Williams & Wilkins, 2010).

3 Brennan, F., Carr, D. B. & Cousins, M. Pain management: a fundamental human right. *Anesthesia and analgesia* **105**, 205-221, doi:10.1213/01.ane.0000268145.52345.55 (2007).

4 Harstall, C. How prevalent is chronic pain? *Pain: Clinical Updates, X, 1–4.* (2003).

5 McCool, W. F., Smith, T. & Aberg, C. Pain in women's health: a multi-faceted approach toward understanding. *J Midwifery Womens Health* **49**, 473-481 (2004).

6 Luo, X., Pietrobon, R., Sun, S. X., Liu, G. G. & Hey, L. Estimates and patterns of direct health care expenditures among individuals with back pain in the United States. *Spine (Phila Pa 1976)* **29**, 79-86 (2004).

7 National Institutes of Health. NIH guide: new directions in pain research: I. (1998).

8 Manchikanti, L., Damron, K. S., McManus, C. D. & Barnhill, R. C. Patterns of illicit drug use and opioid abuse in patients with chronic pain at initial evaluation: a prospective, observational study. *Pain Physician* **7**, 431-437 (2004).

9 Manchikanti, L. *et al.* Effectiveness of long-term opioid therapy for chronic non-cancer pain. *Pain Physician* **14**, E133-156 (2011).

10 The SUPPORT Principal Investigators. A controlled trial to improve care for seriously ill hospitalized patients. The study to understand prognoses and preferences for outcomes and risks of treatments (SUPPORT). . *Jama* **274**, 1591-1598 (1995).

11 Kuehn, B. M. Opioid prescriptions soar: increase in legitimate use as well as abuse. *Jama* **297**, 249-251 (2007).

12 Substance Abuse and Mental Health Services Administration. Substance abuse treatment admissions involving abuse of pain relievers: 1998 and 2008. (http://oas.samhsa.gov/2k10/230/230PainRelvr2k10.cfm). (2010).

13 Gosselin, R. D., Suter, M. R., Ji, R. R. & Decosterd, I. Glial cells and chronic pain. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* **16**, 519-531, doi:10.1177/1073858409360822 (2010).

14 Scholz, J. & Woolf, C. J. The neuropathic pain triad: neurons, immune cells and glia. *Nat Neurosci* **10**, 1361-1368 (2007).

15 Kreutzberg, G. W. Microglia: a sensor for pathological events in the CNS. *Trends Neurosci* **19**, 312-318 (1996).

16 Ji, R. R. & Suter, M. R. p38 MAPK, microglial signaling, and neuropathic pain. *Mol Pain* **3**, 33 (2007).

17 Colburn, R. W., Rickman, A. J. & DeLeo, J. A. The effect of site and type of nerve injury on spinal glial activation and neuropathic pain behavior. *Exp Neurol* **157**, 289-304 (1999).

18 Fu, K. Y., Light, A. R., Matsushima, G. K. & Maixner, W. Microglial reactions after subcutaneous formalin injection into the rat hind paw. *Brain Res* **825**, 59-67 (1999).

19 Lee, S., Zhao, Y. Q., Ribeiro-da-Silva, A. & Zhang, J. Distinctive response of CNS glial cells in oro-facial pain associated with injury, infection and inflammation. *Mol Pain* **6**, 79 (2010).

20 Wei, F., Guo, W., Zou, S., Ren, K. & Dubner, R. Supraspinal glial-neuronal interactions contribute to descending pain facilitation. *J Neurosci* **28**, 10482-10495 (2008).

21 Zhang, J. *et al.* Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. *Eur J Neurosci* **17**, 2750-2754 (2003).

22 Jin, S. X., Zhuang, Z. Y., Woolf, C. J. & Ji, R. R. p38 mitogen-activated protein kinase is activated after a spinal nerve ligation in spinal cord microglia and dorsal root ganglion neurons and contributes to the generation of neuropathic pain. *J Neurosci* **23**, 4017-4022 (2003).

23 Zhuang, Z. Y. *et al.* Role of the CX3CR1/p38 MAPK pathway in spinal microglia for the development of neuropathic pain following nerve injury-induced cleavage of fractalkine. *Brain Behav Immun* **21**, 642-651 (2007).
24 Gehrmann, J., Matsumoto, Y. & Kreutzberg, G. W. Microglia: intrinsic immuneffector cell of the brain. *Brain research. Brain research reviews* **20**, 269-287 (1995).
25 Mika, J. Modulation of microglia can attenuate neuropathic pain symptoms and enhance morphine effectiveness. *Pharmacol Rep* **60**, 297-307 (2008).
26 Roberts, J., Ossipov, M. H. & Porreca, F. Glial activation in the rostroventromedial medulla promotes descending facilitation to mediate inflammatory hypersensitivity. *Eur J Neurosci* **30**, 229-241 (2009).
27 Okada-Ogawa, A. *et al.* Astroglia in medullary dorsal horn (trigeminal spinal subnucleus caudalis) are involved in trigeminal neuropathic pain mechanisms. *J Neurosci* **29**, 11161-11171, doi:10.1523/JNEUROSCI.3365-09.2009 (2009).
28 Zhao, P., Waxman, S. G. & Hains, B. C. Modulation of thalamic nociceptive processing after spinal cord injury through remote activation of thalamic microglia by cysteine cysteine chemokine ligand 21. *J Neurosci* **27**, 8893-8902, doi:10.1523/JNEUROSCI.2209-07.2007 (2007).
29 Leblanc, B. W., Zerah, M. L., Kadasi, L. M., Chai, N. & Saab, C. Y. Minocycline injection in the ventral posterolateral thalamus reverses microglial reactivity and thermal hyperalgesia secondary to sciatic neuropathy. *Neurosci Lett* (2011).
30 Del Valle, L., Schwartzman, R. J. & Alexander, G. Spinal cord histopathological alterations in a patient with longstanding complex regional pain syndrome. *Brain Behav Immun* **23**, 85-91, doi:10.1016/j.bbi.2008.08.004 (2009).
31 Brisby, H., Olmarker, K., Rosengren, L., Cederlund, C. G. & Rydevik, B. Markers of nerve tissue injury in the cerebrospinal fluid in patients with lumbar disc herniation and sciatica. *Spine (Phila Pa 1976)* **24**, 742-746 (1999).
32 Papandreou, O. *et al.* Serum S100beta protein in children with acute recurrent headache: a potentially useful marker for migraine. *Headache* **45**, 1313-1316 (2005).
33 Banati, R. B. *et al.* Long-term trans-synaptic glial responses in the human thalamus after peripheral nerve injury. *Neuroreport* **12**, 3439-3442 (2001).
34 Banati, R. B., Myers, R. & Kreutzberg, G. W. PK ('peripheral benzodiazepine')--binding sites in the CNS indicate early and discrete brain lesions: microautoradiographic detection of [3H]PK11195 binding to activated microglia. *J Neurocytol* **26**, 77-82 (1997).
35 Banati, R. B. *et al.* The peripheral benzodiazepine binding site in the brain in multiple sclerosis: quantitative in vivo imaging of microglia as a measure of disease activity. *Brain : a journal of neurology* **123 (Pt 11)**, 2321-2337 (2000).
36 Loggia, M. L. *et al.* Evidence for brain glial activation in chronic pain patients. *Brain : a journal of neurology* **138**, 604-615, doi:10.1093/brain/awu377 (2015).
37 Bruni, N. *et al.* Cannabinoid Delivery Systems for Pain and Inflammation Treatment. *Molecules* **23**, doi:10.3390/molecules23102478 (2018).
38 Pisanti, S. *et al.* Cannabidiol: State of the art and new challenges for therapeutic applications. *Pharmacol Ther* **175**, 133-150, doi:10.1016/j.pharmthera.2017.02.041 (2017).
39 Sarne, Y. & Mechoulam, R. Cannabinoids: between neuroprotection and neurotoxicity. *Curr Drug Targets CNS Neurol Disord* **4**, 677-684 (2005).
40 Mechoulam, R. & Parker, L. A. The endocannabinoid system and the brain. *Annu Rev Psychol* **64**, 21-47, doi:10.1146/annurev-psych-113011-143739 (2013).
41 Ehrhart, J. *et al.* Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. *J Neuroinflammation* **2**, 29, doi:10.1186/1742-2094-2-29 (2005).
42 Zigmond, A. S. & Snaith, R. P. The hospital anxiety and depression scale. *Acta Psychiatr Scand* **67**, 361-370 (1983).
43 Sullivan, M. J., Bishop, S. R. & Pivik, J. The Pain Catastrophizing Scale: Development and Validation. *Psychol Assess* **7**, 524-532 (1995).

44 Freynhagen, R., Baron, R., Gockel, U. & Tolle, T. R. painDETECT: a new screening questionnaire to identify neuropathic components in patients with back pain. *Current medical research and opinion* **22**, 1911-1920, doi:10.1185/030079906X132488 (2006).
45 Cutrona, C. E. & Russell, D. W. The provisions of social relationships and adaptation to stress. *Advances in personal relationships* **1**, 37-67 (1987).
46 Snaith, R. P. *et al.* A scale for the assessment of hedonic tone the Snaith-Hamilton Pleasure Scale. *The British journal of psychiatry : the journal of mental science* **167**, 99-103 (1995).
47 Imaizumi, M. *et al.* Brain and whole-body imaging in nonhuman primates of [11C]PBR28, a promising PET radioligand for peripheral benzodiazepine receptors. *Neuroimage* **39**, 1289-1298 (2008).
48 Brown, A. K. *et al.* Radiation dosimetry and biodistribution in monkey and man of 11C-PBR28: a PET radioligand to image inflammation. *J Nucl Med* **48**, 2072-2079, doi:10.2967/jnumed.107.044842 (2007).
49 Kreisl, W. C. *et al.* Comparison of [(11)C]-(R)-PK 11195 and [(11)C]PBR28, two radioligands for translocator protein (18 kDa) in human and monkey: Implications for positron emission tomographic imaging of this inflammation biomarker. *Neuroimage* **49**, 2924-2932 (2010).
50 Owen, D. R. *et al.* An 18kDa Translocator Protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. *Journal of Cerebral Blood Flow and Metabolism* (in press).
51 Lee, H. K., Morin, P. & Xia, W. Peripheral blood mononuclear cell-converted induced pluripotent stem cells (iPSCs) from an early onset Alzheimer's patient. *Stem Cell Res* **16**, 213-215, doi:10.1016/j.scr.2015.12.050 (2016).
52 Agu, C. A. *et al.* Successful Generation of Human Induced Pluripotent Stem Cell Lines from Blood Samples Held at Room Temperature for up to 48 hr. *Stem Cell Reports* **5**, 660-671, doi:10.1016/j.stemcr.2015.08.012 (2015).
53 Lee, H. K., Morin, P., Wells, J., Hanlon, E. B. & Xia, W. Induced pluripotent stem cells (iPSCs) derived from frontotemporal dementia patient's peripheral blood mononuclear cells. *Stem Cell Res* **15**, 325-327, doi:10.1016/j.scr.2015.07.004 (2015).
54 Wainger, B. J. *et al.* Modeling pain in vitro using nociceptor neurons reprogrammed from fibroblasts. *Nat. Neurosci.* **18**, 17-24, doi:10.1038/nn.3886 (2015).
55 Wainger, B. J. *et al.* Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. *Cell reports* **7**, 1-11, doi:10.1016/j.celrep.2014.03.019 (2014).
56 Tan, G., Jensen, M. P., Thornby, J. I. & Shanti, B. F. Validation of the Brief Pain Inventory for chronic nonmalignant pain. *J Pain* **5**, 133-137, doi:10.1016/j.jpain.2003.12.005 (2004).
57 Hinchcliff, M. *et al.* Validity of two new patient-reported outcome measures in systemic sclerosis: Patient-Reported Outcomes Measurement Information System 29-item Health Profile and Functional Assessment of Chronic Illness Therapy-Dyspnea short form. *Arthritis care & research* **63**, 1620-1628, doi:10.1002/acr.20591 (2011).
58 Chiang, C. N. & Rapaka, R. S. Pharmacokinetics and disposition of cannabinoids. *NIDA research monograph* **79**, 173-188 (1987).
59 Huestis, M. A. Pharmacokinetics and metabolism of the plant cannabinoids, delta9-tetrahydrocannabinol, cannabidiol and cannabinol. *Handbook of experimental pharmacology*, 657-690 (2005).
60 Deiana, S. *et al.* Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Delta(9)-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour. *Psychopharmacology* **219**, 859-873, doi:10.1007/s00213-011-2415-0 (2012).
61 Ujvary, I. & Hanus, L. Human metabolites of cannabidiol: A review on their formation, biological activity, and relevance in therapy. *Cannabis and cannabinoid research* **1**, 90-101, doi:10.1089/can.2015.0012 (2016).
62 Devinsky, O. *et al.* Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. *The New England journal of medicine* **376**, 2011-2020, doi:10.1056/NEJMoa1611618 (2017).

63 Devinsky, O. *et al.* Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. *The Lancet. Neurology* **15**, 270-278, doi:10.1016/s1474-4422(15)00379-8 (2016).
64 Debruyne, J. C. *et al.* PET visualization of microglia in multiple sclerosis patients using [11C]PK11195. *Eur J Neurol* **10**, 257-264 (2003).
65 Cohen-Adad, J. *et al.* in *Proceedings of the 20th Annual Meeting of OHBM* 3633 (Hamburg, Germany, 2014).
66 Nichols, T. E. & Holmes, A. P. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum. Brain Mapp.* **15**, 1-25 (2002).
67 Smith, S. M. & Nichols, T. E. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage* **44**, 83-98, doi:10.1016/j.neuroimage.2008.03.061 (2009).
68 Sorge, R. E. *et al.* Different immune cells mediate mechanical pain hypersensitivity in male and female mice. *Nat. Neurosci.* **18**, 1081-1083, doi:10.1038/nn.4053 (2015).
69 Chung, K. C. *et al.* The Influence of Menstrual Cycle and Androstadienone on Female Stress Reactions: An fMRI Study. *Front Hum Neurosci* **10**, 44, doi:10.3389/fnhum.2016.00044 (2016).