Evaluating the Role of Neuroinflammation in Low Back Pain

NCT03106740

Version Date: May 12, 2022

Mass General Brigham

Institutional Review Board
Intervention/Interaction Detailed Protocol

Principal Investigator:	Marco Loggia, PhD
Project Title:	Imaging Glia-mediated NeuroInflammation and Treatment Efficacy (the IGNITE study)
Version Date:	5/12/2022

1. 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, our group 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.

In the present study, we propose to test a model built upon observations from our own human data as well as from the animal literature, which describes the temporal dynamics, spatial extent, somatotopic specificity, and clinical significance of glial activation in humans with low back pain. According to this model, glial activation: (1) originates during the acute or subacute stage in the central nervous system sites most proximal to the back (i.e., spinal cord); (2) propagates trans-synaptically[26,27] up to the brain, with increasing pain duration and (3) presents a somatotopic organization in the brain (in the primary somatosensory and motor cortices; S1/M1),

which reflects the bodily distribution of patients' pain symptoms. Finally, the model predicts that (4) pharmacological glial inhibition reduces pain and that (5) the presence of glial activation in the subacute stage predicts transition to chronic pain.

In order to test this model, we will study patients with subacute (pain duration 1-4 months) and chronic (pain duration > 6 months) low back pain longitudinally. Most participants will be studied before and after a treatment with minocycline or placebo. In animal models minocycline is commonly used as a glial inhibitor, including in preclinical models of pain[25]. Minocycline is readily absorbed by the gastrointestinal tract and easily crosses the blood-brain barrier[37], reaching cerebrospinal fluid levels of around 25%- 30% of serum concentration[38]. In humans with subacute lumbar radiculopathy, minocycline was shown to induce a small but statistically significant reduction in pain[39], however it is unclear whether it reduces glial activation in humans.

2. Specific Aims and Objectives

Aim 1. Assess spatial extent and and somatotopic organization of glial activation in chronic LBP

Hypothesis 1.1. The cLBP group will demonstrate higher PET signal in the *brain* (thalamus, S1/M1 representation of the lumbar spine), thus corroborating our previous observations[36] in a larger sample, as well as in the lower lumbar/upper sacral spinal cord segments (situated at the T11-L1 vertebral levels).

Hypothesis 1.2. Patients with radicular cLBP (i.e., pain in the back, shooting down the leg below the knee), but not those with axial cLBP (i.e., pain in the back only), will demonstrate an additional PET signal increase at the level of the S1/M1 representation of the leg, in the paracentral lobule. This observation will provide evidence for *somatotopic* organization of glial activation in the brain of cLBP patients.

Aim 2. Assess temporal evolution of glial activation in subacute LBP

Hypothesis 2.1. In the sLBP group, the *spinal* PET signal (but, unlike in the cLBP group, not the brain signal) will be higher than in healthy controls. This observation will provide evidence of *spinal* glial activation in sLBP, and indicate that chronicity of pain is needed for glial activation to occur in the brain.

Hypothesis 2.2. The baseline spinal PET signal will be heightened in sLBP patients who eventually transition to cLBP, but not in sLBP patients who subsequently heal. This observation will suggest that spinal glial activation is a predisposing factor for pain chronification.

Aim 3. Assess the effect of minocycline on glial activation and pain, and the role of minocycline as predictor of response to minocycline

Hypothesis 3.1. On average, a 2-week treatment with the glial inhibitor minocycline, compared to the placebo group, will reduce pain and [^{11}C]PBR28 PET signal.

Hypothesis 3.2. Higher pre-treatment glial activation will predict larger behavioral and imaging responses to minocycline.

3. General Description of Study Design

In brief, the structure of the study will be as follows (see **Figure 1**):

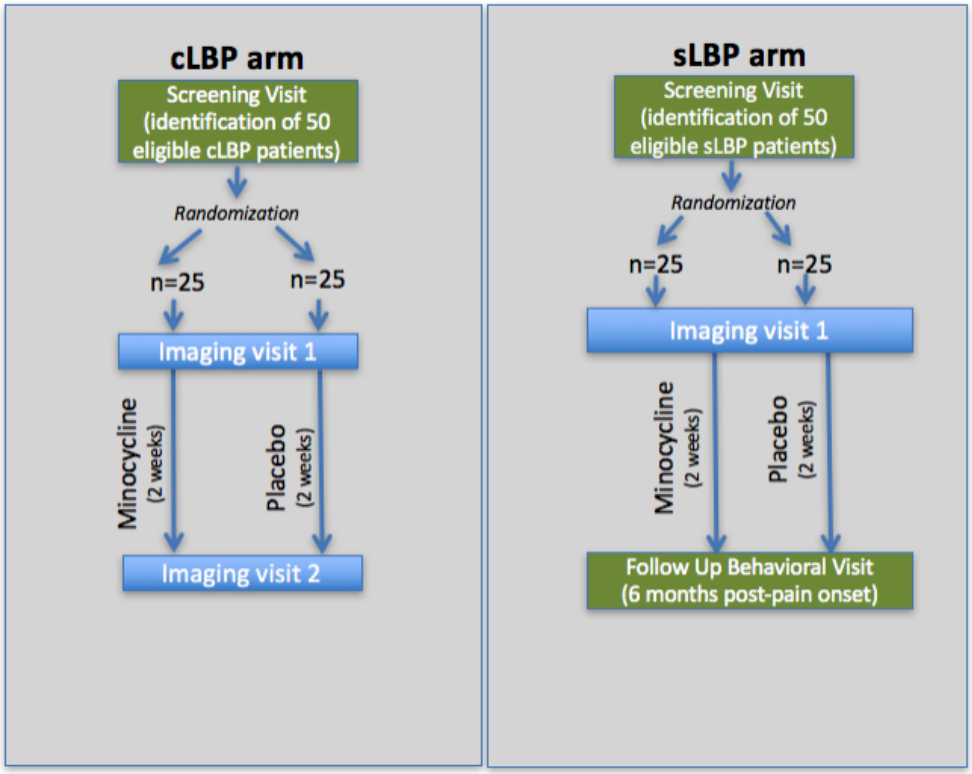

Figure 1. Study schema

-**CLBP arm**: After a behavioral visit aimed at determining eligibility, 50 cLBP patients will be randomized to receive either Minocycline 100mg (N=25) or Placebo (N=25). Following randomization, subjects will then participate in their first imaging visit, during which they will undergo a simultaneous MR-PET scan. At this visit, subjects who are assigned to the clinical trial groups will also receive the treatment corresponding to the group to which they are assigned. 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 2-week drug trial period, all subjects will be scanned again.

-**SLBP arm**: After a behavioral visit aimed at determining eligibility, 50 sLBP patients will be randomized to receive either Minocycline 100mg (N=25) or Placebo (N=25). Following randomization, all subjects will then participate in an imaging visit (at 1 to 4 months after reported pain onset), during which they will undergo a simultaneous MR-PET scan. At this visit, subjects who are assigned to the clinical trial groups will also receive the treatment corresponding to the group to which they are assigned. During the drug trial period, subjects will also be sent a daily survey to assess the treatment effect of the medication on their pain.

All sLBP patients will be sent a survey once per week to assess the status of their pain and track any interventions they receive. These patients will be re-evaluated clinically at 6 months post-pain onset.

4. Subject Selection

We plan to identify 50 patients with subacute low back pain (sLBP; i.e., with a pain duration between 1 to 4 months at time of behavioral screening), as well as 50 patients with chronic low back pain (cLBP; i.e., with a pain duration longer than 6 months), who will complete the study. In order to achieve the final sample size of 100 study completers, we will consent up to a total of 175 participants, in order to account for screen fails and attrition.

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 for sLBP:

- age ≥ 18 and ≤ 75;
- the ability to give written, informed consent;
- fluency in English;
- Subacute low back pain, ongoing for 1-4 months prior to enrollment with 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;
- Medical records confirming diagnosis of low back pain; however, subjects can be exempted from providing medical records if sufficient evidence for low back pain is found upon clinical evaluation.

Inclusion Criteria for cLBP:

- age ≥ 18 and ≤ 75;
- the ability to give written, informed consent;
- fluency in English;
- Chronic low back pain, ongoing for at least 6 months prior to enrollment, with 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;
- Medical records confirming diagnosis of low back pain.

Exclusion Criteria for sLBP:

- any interventional pain procedures during the 2-week drug trial period or between the first scan visit and completion of the drug trial period;
- 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);
- History of disease that may be exacerbated by minocycline administration i.e.

Clostridium Difficile colitis, lupus, or vasculitis;
- any history of neurological illness or major medical illness affecting the central nervous system;
- 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 5 years;
- peripheral neuropathic pain of greater severity than that of the back pain;
- pregnancy or breast feeding;
- history of head trauma requiring hospitalization;
- history of impaired elimination or abnormal kidney function testing (e.g., renal insufficiency with GFR < 60);
- history of diagnosed liver disease or abnormal liver function testing within the past 6 weeks (e.g. total protein, albumin/globulin ratio, bilirubin, or alkaline phosphatase levels falling outside of normal ranges);
- routine use of benzodiazepines or any use in the past 2 weeks except clonazepam (Klonopin), lorazepam (Ativan), and alprazolam (Xanax);
- current bacterial or viral infection;
- Contraindications to minocycline administration including:
    - Allergy to minocycline or other tetracycline medications;
    - Taking medications known to interact with minocycline including: anticoagulant therapies; penicillin; antacid medications containing: aluminum, calcium, magnesium, or iron; methoxyflurane; isotretinoin; ergot alkaloids or their derivatives; or any other drugs determined by the study physician to be unsafe, taken in concomitance with minocycline;
    - Any other contraindications to minocycline administration noted by the study physician;
- History of COVID-19 infection within the past 3 months WITH neurological symptoms that required hospitalization.

Exclusion Criteria for cLBP are the same as for sLBP, with the addition of:
- outpatient surgery within 2 months and inpatient surgery within 6 months from the time of scanning;
- epidural steroid injections 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;
- current or past history within the last 5 years of major medical illness not affecting the central nervous system, other than chronic pain;
- peripheral nerve injury;
- major cardiac event within the past 10 years;
- any use of recreational drugs in the past 6 months;
    - marijuana use, medical or recreational, is an exclusion only if average use exceeds 1x/week;
- an abnormal physical exam (e.g., peripheral edema);
- routine use of opioids ≥ 60 mg morphine equivalence;
- history of substance abuse;

- use of immunosuppressive medications, such as prednisone, TNF medications within 2 weeks of the visit.

Additional Exclusion Criteria for sLBP only:

- a separate episode of back pain within the same year, lasting at least 1 month and requiring medical attention;
- epidural steroid injections within 6 weeks prior to scanning procedure or during the 2-week drug trial period;
- Surgical intervention or introduction/change in opioid regimen during the 2-week drug trial period.

Exclusion Criteria for placement of arterial line (sLBP and cLBP):

- contraindications to placement of arterial line, such as abnormal result on modified Allen's test on both hands; Raynaud syndrome; bleeding disorder; use of anticoagulants such as Coumadin, Plavix or Lovenox.

5. 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. To recruit subjects, we will also use multiple research databases such as the Partners' RSVP for Health system, Partners Clinical Trials, ResearchMatch, EPIC, and RPDR. We will run queries on EPIC and RPDR to find subjects with low back pain. Subjects identified through these mechanisms will receive a recruitment letter in the mail from the Principal Investigator. In addition, methods that advertise the study to the greater community will be used such as Craig's List, 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. Finally, we will be collaborating with a research team at the Emergency Department in the Brigham and Women's Hospital, the Phyllis Jen Center for Primary Care at Brigham and Women's Hospital, the Walk-In Clinic at MGH, etc. who will help us identify potential participants for the study. The personnel in those clinics will ask the patients if they are interested in hearing about our study, and obtain their permission for study staff to approach them in the waiting rooms to discuss it.

All subacute low back pain subjects will undergo a telephone screening to attempt to distinguish potential subjects from those not meeting eligibility criteria. For acute low back pain patients, the BWH ED staff will explicitly ask for their permission to receive an email communication containing the REDCap Pain Catastrophizing Scale survey from our team. We will use "send secure" to deliver these emails, if requested by the potential participants. The objective of this survey will be to identify potential candidates for our study based on their perceptions of pain. 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 or a Nurse Practitioner will obtain informed consent, as in all other protocols involving [^{11}C]PBR28 PET/MR scanning (e.g., 2011P002311).

6. STUDY PROCEDURES

Screening visit: Subjects eligible to participate will be recruited to participate in a 2-hour characterization and training 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 data on demographics and socioeconomic status. Computer-based tasks will be completed on a laptop. All assessments, including those involving experimental noxious stimuli, 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), who has ample experience in, and familiarity with, the use of experimental pain stimuli in research studies.

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[40]. 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.

The Pain Catastrophizing Scale (PCS): It is a 13-item self report scale which measures pain-related Rumination, Magnification and Helplessness[41].

PainDETECT[42]: 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.[43]

Adverse Childhood Experiences Scale (ACE): The ACE scale asks about 10 common adverse events/experiences that affect development such as childhood maltreatment, parental divorce, and parental incarceration. Increased ACE scores have been associated with negative health outcomes such as shortened life span, mental illness, and chronic illness.[44]

Brief Trauma Questionnaire (BTQ)[45,46] : The BTQ will be used to determine whether an individual meets Criterion A1 for traumatic exposure according to the DSM-V PTSD diagnosis. It is a brief self-report questionnaire designed to assess 10 traumatic events including physical assault, car accidents, natural disasters, and unwanted sexual contact. It is derived from the *Brief Trauma Interview.*

Snaith-Hamilton Pleasure Scale (SHAPS)[47]*:* 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.

Thermal stimuli: We will also test for participants' sensitivity to cool/warm and painful stimuli. Brief (1-15s) thermal stimuli will be delivered from a neutral baseline temperature (e.g., 32-38°C) to different part of the body (back, leg, and/or arm) using a computer-controlled system (Medoc Pathway). Subjects will be asked to rate the intensity of each stimulus using various scales (e.g., a scale of 0 to 10). These procedures will improve the characterization of our patient population, as they will allow us to assess whether the degree of 'central sensitization' will be a predictor of symptom characteristics. Similar procedures are used in many other studies of chronic pain patients, including in studies approved by the Partners IRB (e.g., # 2010P000978, 2015P001150 and 2015P002373, PIed by our colleague Robert Edwards).

Electrical Stimulation: Electrical stimulation will be applied to the back and legs, using electrodes connected to a muscle/nerve stimulator. The frequencies will elicit different levels of tolerable stimulation at both non-painful and painful levels, following the procedures similar to those used in our center's protocols (e.g., 2011P001364). We will give several stimuli at precisely controlled current intensities, within a safe and tolerable range, to test for the participants' sensitivity to pain and to identify appropriate stimulus intensity to be used to produce a moderately painful percept in the imaging visit. During the behavioral visit, we will present up to 25 stimuli, each lasting a maximum of 3s, plus 1-2 stimuli lasting a maximum of 10s, per body site.

History and physical examination: an MD or NP will also collect medical history and perform a formal physical examination, including a modified Allen's test to evaluate safety of the arterial line (a-line) placement and the recording of vital signs (heart rate, blood pressure, and body temperature).

Blood and saliva tests: a trained member of the study staff will draw venous blood (up to 10 ml) from all subjects considered for potential participation in a [^{11}C]PBR28 scan in order to have them genotyped for the **Ala147Thr TSPO polymorphism** in the *TSPO* gene (rs6971) **(unless this genotype information is already available)**. In addition, up to 50 participants may be asked to provide a saliva sample. This dual collection of samples will allow us to validate the genotyping results using DNA extracted from saliva, and may in the future render blood draws unnecessary for the purpose of genotyping.

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[49], it also presents a potential limitation, in that about 10% of human subjects show no binding to PBR28[50] (whereas [^{11}C](R)-PK11195 has never been associated with non-binding[51]). A recent study has demonstrated that the rs6971 polymorphism predicts PBR28 binding affinity in human platelets[52]. Since **the low-affinity binder phenotype is consistent across all tissues within the same subject [51], 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** [^{11}C]PBR28 PET scan(s). Under special circumstances, some patients may be scanned before the genotyping results become available to us, as we currently do for one of our protocols (2014P001709, 2016P001009; PI: Loggia). We would do so if having to wait for the genotype information to become available could compromise participation or completion of the study (for instance, if a subacute patient at the time of screening were about to fall out of the 1 to 4 -month window from pain onset, or if a patient had a surgical or other procedure scheduled in the immediate future that would conflict with the 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.

Of the small number of participants that we anticipate needing to scan without prior genotyping, only about 10% will be low affinity binders. In addition, having some low affinity binder participants may allow us to perform sub analyses that will determine whether excluding these participants is the most appropriate strategy, rather than modeling genotype as a covariate in our analyses. Should a low affinity binder be scanned under such circumstances, their data will still provide useful information regarding the distribution of the PET signal across genotypes.

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[53-55] to assess in-vitro alterations in patient-derived neural or glial cells[56,57].

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), as well as to monitor urine riboflavin levels to aid in the assessment of trial compliance. The urine drug screen will be performed during the screening visit and on the day of the scan. 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. Riboflavin will be

measured using a UV light to detect for the baseline and post-treatment changes in riboflavin excreted through the urine. This will allow us to measure compliance with the prescribed treatment regimen.

Following the screening visit, all participants will receive daily web-based surveys, to assess their pain prior to treatment. These surveys will be administered for 7 days prior to the commencement of the assigned study treatment. If the treatment needs to be rescheduled, then the surveys will be restarted 7 days prior to the new start date.

Follow up behavioral visits*:*

For all sLBPs, some of the assessments listed as part of the screening and imaging visits will be performed at the follow-up behavioral visit (see 'Behavioral visit 2' in Figure 1) along with blood tests to measure changes in the levels of various substances in the blood, such as the proinflammatory cytokines IL-6 and TNF-alpha. Up to 15mL of blood will be drawn at the follow up visit.

In order to comply with public health efforts to address COVID- 19, virtual visits may be conducted as necessary. Virtual visits will be conducted via MGB approved platforms (i.e. video calls over Zoom and phone conferences via Doximity Dialer) and will mirror in-person visits with the identical personnel present as explained above. All questionnaires typically collected during the in-person behavioral visit may be collected during the remote behavioral visit, as they are largely already completed on secure online platforms (i.e. StudyTrax). All behavioral visit study procedures may be performed during the remote behavioral visit, with the exception of the urine drug test, physical exam, and extensive back pain exam which will be performed at the first in-person visit (i.e. first imaging visit). When electronic written consent is obtained via REDCap, the REDCap e-consent template being utilized is equivalent to written consent and is FDA compliant.

Blood may be drawn for SARS-CoV-2 antibody serology testing, and the presence of antibodies will be used to explore the possible effects of prior exposure to the coronavirus on neuroinflammation, in exploratory analyses. Up to 10 ml of blood will be drawn for this purpose. This testing will be performed through a third party vendor or through the MGH core lab.

MR-PET visit(s)

Participants eligible to continue into the study based on the screening visit and initial blood/saliva 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). In addition, a urine drug test will be repeated on the day of each MR-PET visit. Subjects who are participating in the treatment arms of the study will also have their urine

tested with a UV light to detect for the presence of riboflavin. This will allow us to know whether subjects complied with their prescribed drug regimen.

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. Blood will be drawn to evaluate the circulating level of on interleukins, TNF-alpha, other inflammatory markers, for protein binding analysis, for SARS-CoV-2 antibody serology testing, and for other serum and cell processing (e.g. PBMC, PaxGene, TruCulture). During the first scanning visit, up to 55 mL of blood may be drawn for this purpose. During the second scanning visit, up to 30 mL of blood may be drawn for this purpose. If applicable, female participants will also be asked how many days after onset of menses they are on the day of the scan, and the information will be used for the blood analyses.

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), or while engaged in any experimental tasks. The radioligand [^{11}C]PBR28 will used to determine whether patients with chronic pain exhibit evidence of microglial activation.

Following procedures identical to those adopted in other PET studies (including using [^{11}C]PBR28) from our center (2015P001594, 2013P001297, 2011P001546, 2011P002311, 2016P001009, etc), an arterial line will be placed in a radial artery with local anesthesia (20 or 18 gauge cannula, 2-5 ml of lidocaine 1% intradermal and subcutaneous) using sterile techniques, if the participant has consented to this procedure. The placement of an arterial line will be presented as optional to the participants, and we will ask for the participants' consent each time. The arterial line will be placed in the arm contralateral to the intravenous line that is used for the [^{11}C]PBR28 radiotracer injection. The arterial line will enable blood sampling (1mL to 12mL) at various times during the imaging study for at most 160 mL of blood. The collected arterial blood will be used to compute metabolite-corrected arterial input function for kinetic modeling analyses (see Data Acquisition and Analyses). The catheter will be placed by an individual with anesthesia training(i.e., board-certified anesthesiologist, fully licensed anesthesia resident, or a certified registered nurse anesthetist), monitored throughout and accessed by an experienced research nurse. The catheter will be discontinued at the end of the study by a physician, a nurse practitioner, or a certified registered nurse anesthetist.

Of note, over the course of the three visits, up to 385 mL over three possible visits. This includes 160 mL from the arterial line for each scan and from the IV catheter up to 45 mL at the first scan and up to 20 mL at the second scan.

During the scan visit, subjects may be asked to complete one more time some or all of the assessments completed during the screening visit. In addition, subjects may be asked to complete a few additional assessments including *BPI, PROMIS-29, the Trail Making Test, Part B* and the *Rey*-Osterrieth *Complex Figure*.

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[58].

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[59]*:* The PROMIS-29 is a 29-item self-report measure assessing physical, mental, and social health.

Post-Lockdown Impact Survey: This survey will be used to assess patient wellbeing between the beginning of the pandemic and the date of their scan.

Trail Making Test, Part B[60]*:* The *Trail Making Test, Part B* is a common neuropsychological assessment used to gauge a participant's cognitive flexibility and working memory. Participants are asked to trace a series of numbers and letters around a paper in the proper sequence (i.e. 1-A-2-B). Participants are timed while they trace from the start of the sequence until they reach the end of the sequence. If a participant makes an error in the sequence, they will be asked to return to the last correct point, and start again from there while the time continues to be recorded.

Rey-Osterrieth Complex Figure[61,62]*:* The Rey-Osterrieth Complex Figure is another common neuropsychological assessment used to gauge a participant's visual and executive functioning. Participants are shown a picture of an abstract figure. The figure is then removed and participants are asked to draw the figure from their memory. Participants are again asked to draw the figure following a 2 minute delay. Participants are scored based on the accuracy of their representation as compared to the original figure and their approach to the drawing.

During the scan, participants may be asked to complete the *Monetary Incentive Delay (MID) task*. The MID task features balanced incentive delivery and analytic strategies designed to identify activity specific to anticipation or consumption of incentives. The structure of the MID paradigm will be as follows: Individual trials begin with presentation of one of three visual cues (0.5 s) signaling potential trial outcomes (reward: +$; loss: -$; no-incentive: 0$). Following a jittered ISI (approximately 3s), a red square target stimulus will be presented; participants are instructed to respond to the target with a button press. Following a second ISI (approximately 3s), visual feedback (1.25 s) will be provided. In the reward condition, successful trials are associated with monetary gains ($1.96 to $2.34) whereas unsuccessful trials lead to no change. In the loss condition, successful trials are associated with no change whereas unsuccessful trials are associated with monetary penalties (-$1.81 to -$2.19). No feedback about cumulative earnings will be provided. Intertrial intervals (ITIs) will be approximately 3s. The task will include up to five blocks of up to 24 trials (8 reward, 8 loss, 8 no-incentive). Thus, up to 40 trials/ condition will be available for the analyses of anticipatory activation, whereas up to 20 trials/condition will be used for post-feedback responses. Participants will be told that faster reaction times (RT) will increase the likelihood of obtaining rewards and avoiding losses. RT data in three independent control samples confirmed that the task induces motivated responding: mean RT was fastest on reward trials, intermediate on loss trials, and slowest on no-incentive trials (all ps<.001; [63] [64]).

In addition, during the scan subjects may receive electrical stimuli similar to those received in the behavioral visit. This stimulation paradigm, will allow us to assess the relationship between brain activation in response to acute pain and neuroinflammation. The subjects who will receive

electrical stimuli will be chosen based on several considerations, including how well the subject has tolerated these stimuli during the behavioral visit.

Vital signs (heart rate, blood pressure, and body temperature) will also be recorded for all subjects before and after [^{11}C]PBR28 injection to monitor for any adverse events related to radioligand administration. For subjects in the cLBP groups the pre-administration vital signs will also be compared between imaging visit 1 and imaging visit 2 to assess for significant deviations that may be related to adverse events caused by the study treatment.

At the end of the scan, for those participants who received an a-line, an experienced nurse practitioner or MD will remove the catheter. These subjects will be kept under observation for 30 minutes.

The total duration of each scanning visit will be up to 6 hours (~45min for preparation, ~30min for a-line placement, if applicable, ~120 min for scanning procedures and 30 minutes (up to 60 minutes based on clinical opinion of NP) for filling out questionnaires and observation, plus an additional ~1.5hr 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 cannot remain for a full 6 hour scan visit, it will be acceptable to forego the placement of the arterial line. Eliminating this procedure will save the time needed for the placement and the 30 minutes of observation needed after the removal of the a-line (from these scans we will derive metrics that do not depend on arterial sampling, such as SUVR). Additionally, 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.

We will follow up with all subjects by phone 24-48 hours following the imaging visit to monitor for adverse events that may be related to radioligand administration and/or the initiation of study treatment.

The imaging visit, including all the procedures described above, will be repeated a second time after either a 2-week trial of minocycline or placebo (cLBP).

Randomization

Following the behavioral visit, sLBP subjects will be randomized to either the minocycline or placebo group. The sLBP and cLBP **s**ubjects, will be randomized in a 1:1 ratio to receive either: placebo (lactose powder), or minocycline 100mg once daily for 2 weeks. Randomization will be performed by assigning each subject a randomization number via a computerized random number generator. However, the Clinical Trials Pharmacy will maintain the specific subjects'

treatment assignments (minocycline or placebo) for later identification. Patients and study staff will be blinded to minocycline or placebo assignment.

Intervention arms

We will follow the same dosage and administration protocol used by Vanelderen et al to demonstrate a statistically significant reduction in pain in patients with subacute lumbar radiculopathy (N=17)[39]. Specifically, patients will be asked to take the study medication in the morning with a glass of water 1h before or 1h after breakfast, to prevent interference of food with gastrointestinal absorption of the drug[37]. 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. Because minocycline is known to reduce the effectiveness of oral contraceptives, all subjects taking oral contraceptives will be counseled to use alternative forms of birth control while taking the study medication.

The Jackson Compounding Center at Massachusetts General Hospital will prepare the minocycline and placebo, encapsulated in identical opaque capsules. Minocycline and placebo will be stored at controlled room temperature between 59-86 °F in a container that is kept tightly closed and stored out of direct sunlight. Both the minocycline and placebo will have 25 mg of riboflavin (vitamin B2) added to them for the purposes of tracking treatment compliance. Riboflavin acts as a tracer and will allow us to do urine testing using a UV light to ensure that subjects are taking the medication as instructed. 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 2-week minocycline or placebo period, patients will be re-scanned and/or re-evaluated clinically to evaluate the hypothesis that minocycline reduces glial activation and pain symptoms.

During the drug trial period, subjects will also be sent a daily survey via email (administered through StudyTrax) to assess the treatment effect of the medication on their pain. The surveys will also include contact information for the study physician in case they have any concerns about the medication. If subjects do not have email access, they will be able to complete the surveys over the phone.

Following the completion of the drug trial period, participants will be asked to complete a brief questionnaire assessing their experience of the study medication.

Following the drug trial period, sLBP subjects will be sent a weekly survey via email (administered through StudyTrax) to assess the progression of their pain. The surveys will also inquire about pain management interventions used in the past week so that these can be tracked throughout study enrollment and the effect on pain outcomes can be assessed. They will complete these weekly surveys until their 6-month follow-up visit. Again, if subjects do not have email access, they will be given the opportunity to complete the surveys over the phone.

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

Payments will be as follows:

- $75 for the initial behavioral session (Visit 1)
- $2 per daily baseline survey
- $2 per daily survey for all treatment arms plus a $25 bonus for completing at least 12 of the surveys
- $5 per weekly survey for sLBP patients $25 bonus for completing at least 10 of the surveys
- $200 for each MR-PET scanning visit
- $100 for each follow-up behavioral session
- $50 for each arterial line placement
- $25 for each blood test to exclude pregnancy (if the subject is female of childbearing age)

Participants will be able to earn up to an additional $17-$22 during the Monetary Incentive Delay task.

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) and we HAVE NOT yet placed the arterial line, he/she will receive $50. If we cannot inject the subject with the radioligand, and we HAVE already placed the arterial line, he/she will receive $100.

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.

If a cost is incurred by the subject for obtaining medical records to confirm the diagnosis of low back pain, we will reimburse the subject for this expense.

If subjects have difficulties traveling to our facility, we may offer travel reimbursement for their cab or Uber fare up to $100.

If subjects are eligible to scanned but have difficulties with travel (i.e. out-of-state), we may offer them 1 night's stay at a hotel not exceeding $200.

Individuals will be compensated $25 for participation in the "Post-Lockdown Impact Survey" if they were scanned prior to 7/1/2021. Participants scanned after this date will complete this optional survey as part of the questionnaire set included in the MRI-PET visit for no additional compensation.

7. 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 minocycline, 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.

As such, we envision that in the future the information obtained from the proposed research will enhance the diagnosis and management of a variety of chronic pain conditions.

8. 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.

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.

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.

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. Also, an intra-arterial catheter will be placed by an individual with anesthesia training(i.e., board-certified anesthesiologist, fully licensed anesthesia resident, or a certified registered nurse anesthetist), on the arm opposite to the radio-ligand injection line, for blood draws during the PET study. Local infection, swelling, and redness could occur at the sites of line placement, as well as temporary loss of pulse at the wrist. This area may have a bruise or feel uncomfortable for 2-3 days after the catheter is removed. The risks associated with having blood drawn include: bruising, local discomfort, or infection at the site of the needle puncture.

Rarely an infection may occur at this site, and if an infection does occur, it will be treated. Inserting an arterial line (A-line) can hurt more than having a regular IV or having blood drawn with a needle. We will place the A-line under local anesthesia (i.e., lidocaine), which may cause an allergic reaction. Even if we numb the wrist area first, the insertion may still hurt. Once the A-line is in place, it usually does not hurt.

The subject may experience pain, bleeding, swelling or redness at the wrist, short loss of pulse at the wrist if blood flow in the artery is briefly stopped, damage to the artery wall or nearby nerves, or catheter breaking or falling out. There have been reports of decreased blood flow to the hand, which resulted in the need for surgery. This is very rare and has not been reported when catheters have been in place for only a few hours for research. A subject who will not pass the modified Allen's test during the screening visit will not be considered eligible for the imaging visit, including A-line placement. Additionally, the insertion or removal of the A-line might cause temporary dizziness, nausea or fainting. After the anesthesiologist, anesthesia resident, certified nurse anesthetist or RN removes the catheter and has held pressure for several minutes, we'll ask the subject to stay for 30 minutes (up to 60 minutes based on clinical opinion of NP) so we can monitor him/her in order to assess the occurrence of any adverse event. Dr. Loggia will file a report on Insight within the timeframe stipulated by the IRB (5 working days/7 days) should any adverse event occur. The subject may have a bruise or feel tenderness for 2-3 days around the area where the catheter was placed. We will instruct the subject to avoid lifting anything heavier than a small bag of flour for a day. We will instruct the subject to call us if bleeding occurs after the subject leaves (rare), and/or if the wrist area is painful or red or swollen. 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.

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. Loggia 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.

Minocycline is an FDA approved antibiotic used to treat infections. Common side effects include dizziness (9 in 100 people), drowsiness (2 in 100 people), headache (23 in 100 people). Less common side effects (1 in 1,000 to 1 in 10,000 people) include sun sensitivity (photosensitivity). Uncommon side effects (less than 1 in 10,000 people) include the development of drug resistant infections. Additionally, side effects with unreported frequency include Clostridium difficile colitis (severe diarrhea caused by infection), gastrointestinal disturbance, diarrhea, indigestion, loss of appetite, nausea, vomiting, lightheadedness, sore mouth, throat, or tongue, tooth discoloration. Some of the side effects, especially diarrhea, may continue even after minocycline is no longer being taken. Subjects will be informed of all these potential adverse effects, and will be asked to immediately contact the Dr. Zhang (the main study MD investigator) if they experience any of them.

Riboflavin is a commonly consumed supplement, and is often use as a tracer to monitor compliance in clinical studies[75-80]. There is no evidence for riboflavin toxicity produced by doses comparable to those used in the present study. After administering 400mg of riboflavin daily (16 times the daily dose used in this study) for three months (i.e., a treatment period 6 times longer than in this study), no short term side effects were reported [81].

Minimal risks associated with completing questionnaires are subject fatigue and the possibility of minor psychological distress associated with answering sensitive questions regarding psychological functioning. Minimal discomfort may occur due to the time and effort related to completing the Monetary Incentive Delay and Probabilistic Reward Tasks.

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.

In this study, we aim to administer only tolerable thermal or electrical pain stimuli. However, if a participant feels that the stimuli has become intolerable, the participant can stop the stimulation at any time by letting the experimenters know. There is a very small chance that the heating device might produce a minor burn, but the risks associated with relatively small amount of heat applied to the skin (maximum of 50°C, for very short periods of time) are very low. Our group[82-88] and many others around the world have used similar or identical heat pain testing procedures in a number of previous studies with healthy adults and patients with chronic pain. No serious side effects were ever reported.

9. Statistical Analysis

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[67]. 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. At various times during the PET acquisition we will perform arterial blood sampling as described above, if the participant has consented to receive an arterial line. Regional uptake of the tracer will be estimated as SUV, SUVR, Vt and/or DVR.

MRI
MRI data will be acquired simultaneously to the PET data, and may include some or all of these standard sequences: BOLD fMRI (for functional connectivity analyses, to evaluate brain responses to the reward task, and/or to estimate motion parameters), Diffusion-Spectrum or Diffusion-Tensor Imaging volumes (DSI) (to perform tractography analyses), Arterial Spin Labeling (to estimate resting state regional cerebral blood flood); 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.

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.

All fMRI data will be stored on hard drive and storage clusters. Behavioral data will be recorded and stored in StudyTrax in preparation for statistical analyses. StudyTrax is approved by Partners Research Information Science and Computing and is compliant with 21 CFR Part 11.

In order to address the first aim ('**Assess spatial extent and and somatotopic organization of glial activation in chronic LBP**'), we will compare [^{11}C]PBR28 PET brain and spinal cord maps between radicular cLBP patients, axial cLBP patients and healthy volunteers. We will perform an unpaired comparison of the imaging data between all 3 groups, including age, sex and TSPO genotype (which predicts binding affinity[52]) as covariates of no interest. Additional, secondary regression analyses will be performed in order to evaluate the association between imaging and behavioral data (e.g., questionnaire scores, pain duration, etc). The effect of painkillers (e.g., NSAIDs) on the PET signal will be evaluated by performing subanalyses on

patients not taking any medication, or by adding use of medication as a covariate of no interest, depending on the profile of drug usage observed in the samples we will have recruited.

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[68]. 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). The ROI data, as well as demographic and behavioral data (age, sex, polymorphism, axial vs radicular symptoms, and the questionnaires) will be compared across groups with a one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc pairwise tests. In addition, we will run regression analyses to evaluate the association between the [^{11}C]PBR28 data, and sensory/affective pain ratings as well as negative affect scores and blood data (e.g., levels of IL-6 and TNF-alpha).

Using ANOVAs we will compare ratings of thermal stimuli across groups to determine whether they differ in terms of pain sensitivity. We will also evaluate the association between imaging metrics (e.g., PET signal) and pain sensitivity to test the ancillary hypothesis that higher pain sensitivity is correlated with higher neuroinflammation.

For the second aim ('**Assess temporal evolution of glial activation in subacute LBP**), we will perform unpaired comparison between sLBP patients and cLBP patients, for both spinal cord and brain. In addition, we will separate the sLBP subjects in 2 groups, based on their follow-up clinical evaluation at 6 months post pain-onset: those who have healed, and those who have transitioned to cLBP. We will then compare the imaging as well as behavioral data from the baseline scans across these two subgroups, to assess whether imaging data can be used as predictor of pain chronification. Comparisons of voxelwise, ROI and demographic/ behavioral data will be performed as described above.

For the last aim ('**Assess the effect of minocycline on glial activation and pain, and the role of minocycline as predictor of response to minocycline**'), we will perform paired analyses in cLBP receiving minocycline or placebo (pre vs post treatment) and test for a treatment*time interaction. In addition, we will use regression analyses to assess the hypothesis that baseline PET signal predicts changes in pain after minocycline treatment.

In addition to these main analyses done with the PET data, additional, exploratory analyses will be performed on the MRI data (BOLD resting state, diffusion, spectroscopy, etc) collected simultaneously to the PET data.

All voxelwise analyses will be conducted using the FSL suite[69] for the brain, and using the Spinal Cord Toolbox[68] for the spinal cord. Group comparisons maps will be thresholded using threshold-free cluster enhancement[70], 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 for all aims, because animal research suggests the presence of a possible sexual dimorphism in a) the role of glia in pain (as pain hypersensitivity may be microglial-dependent only in males[71]) and in b) the effects of minocycline (as this drug may decrease the expression of glial activation markers and increase acute morphine analgesia only in males)[72]. 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[73].

Functional MRI data from the Monetary Incentive Delay (MID) task as well as resting state scans, MRS, PCASL, etc will be analyzed with established analytic procedures (regression analysis, General Linear Model) using commonly used imaging software (FSL, FreeSurfer, LCModel, etc).

Effect of coronavirus on neuroinflammation. Finally, in addition to the aforementioned analyses, the effect of prior exposure to coronavirus (as detected by the presence of antibodies to SARS-CoV-2) will be evaluated in both ROI and voxelwise analyses, in exploratory analyses. While we will be explicitly excluding for participants who had been diagnosed with COVID-19 in the previous 3 months with neurological symptoms that required hospitalization (to avoid that the acute/subacute effects of the disorders might confound our main analyses on the role of neuroinflammation in chronic back pain patients), identifying participants who are positive to the antibodies might allow us to test the exploratory hypothesis that prior exposure to the coronavirus can lead to neuroinflammation even without having experienced overt acute COVID-19 symptoms.

In order to power the study, we computed the sample size required for a mixed between subject (Treatment Group: Minocycline/Placebo) and within subject (Time: Pretest/Posttest) repeated measures ANOVA design using the pain ratings on a 0-100 numerical ratings scale as the dependent variable. Alpha was set at 0.05 (2 tailed test). Based on data from a previously studied cohort of cLBP[36], we assumed a mean rating of 50 and a standard deviation of 20 before treatment. In addition, we assumed a test-retest correlation of r=0.65, based on a large (n=200) test-retest study[74]. In terms of effect size, we assumed a reduction of 15 points in the minocycline group (in the Vanelderen trial a reduction of ~1.5 was observed on a 0-10 scale)[39], and a negligible (1 point) reduction in the placebo group. For these specifications and 80% power to detect the interaction of Treatment Group X Time, the total sample size required for the study was computed to be 48 (i.e., 24 per group).

In addition, we powered our study to be able to detect differences between patients and controls. This analysis was performed using the fMRIpower software package (fmripower.org). This method, which can be used for any brain imaging data including PET, estimates power for detecting significant effects within specific regions of interest, with the assumption that the planned studies will have the same number of runs per subject, runs of the same length, and data analysis with a comparable model. The effect sizes have been expressed in standard deviation (SD) units, which is analogous to the Cohen's D measure. These power calculations are based on our previous brain [^{11}C]PBR28 study in chronic low back pain patients[4], and employed the same thalamic region of interest (ROI) used from that study (the thalamic labels from the Harvard Oxford atlas), with a p-value threshold of 0.05 for a 1-sided hypothesis test. With 23 patient-

control pairs, we will have > 90% power to detect an effect of size of 0.64 SD in the left thalamus, and of 0.62 SD in the right thalamus. Thus, with the proposed sample size we will be able to also detect a group difference in [^{11}C]PBR28 signal.

In addition, we will explore whether environmental exposure may be a potential confound to our results. To do so, we will evaluate – in ancillary/exploratory analyses – whether neuroinflammation might be influenced by environmental and other factors, such as pollution and proximity to roads, that are thought to induce neuroimmune activation. We will estimate environmental exposure by linking residential address at time of scan with pollution levels (e.g., $PM_{2.5}$, ozone levels, etc.) extracted from publicly available (i.e., meteorological) records, and use this information to run regression analyses against brain and clinical/behavioral data.

We will also perform kinetic modeling analyses of the PET data, morphometric analyses of MRI data, and/or implement machine learning algorithms to create objective classifiers that can reliably distinguish between healthy volunteers and patients with chronic pain. Using our MRI data, we would like to evaluate potential anatomo-functional alterations, and their association with neuroinflammation, in patients with chronic pain.

10. Monitoring and Quality Assurance

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.

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.

11. Privacy and Confidentiality

- ☒ Study procedures will be conducted in a private setting
- ☒ Only data and/or specimens necessary for the conduct of the study will be collected
- ☒ Data collected (paper and/or electronic) will be maintained in a secure location with appropriate protections such as password protection, encryption, physical security measures (locked files/areas)
- ☒ Specimens collected will be maintained in a secure location with appropriate protections (e.g. locked storage spaces, laboratory areas)
- ☒ Data and specimens will only be shared with individuals who are members of the IRB-approved research team or approved for sharing as described in this IRB protocol
- ☒ Data and/or specimens requiring transportation from one location or electronic space to another will be transported only in a secure manner (e.g. encrypted files, password protection, using chain-of-custody procedures, etc.)
- ☒ All electronic communication with participants will comply with Mass General Brigham secure communication policies
- ☒ Identifiers will be coded or removed as soon as feasible and access to files linking identifiers with coded data or specimens will be limited to the minimal necessary members of the research team required to conduct the research
- ☒ All staff are trained on and will follow the Mass General Brigham policies and procedures for maintaining appropriate confidentiality of research data and specimens
- ☒ The PI will ensure that all staff implement and follow any Research Information Service Office (RISO) requirements for this research
- ☐ Additional privacy and/or confidentiality protections

12. References

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 Res Brain Res Rev* **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* **123 (Pt 11)**, 2321-2337 (2000).
36 Loggia, M. L. *et al.* Evidence for brain glial activation in chronic pain patients. *Brain* **138**, 604-615, doi:10.1093/brain/awu377 (2015).
37 Leyden, J. J. Absorption of minocycline hydrochloride and tetracycline hydrochloride. Effect of food, milk, and iron. *J. Am. Acad. Dermatol.* **12**, 308-312 (1985).
38 Brogden, R. N., Speight, T. M. & Avery, G. S. Minocycline: A review of its antibacterial and pharmacokinetic properties and therapeutic use. *Drugs* **9**, 251-291 (1975).

39 Vanelderen, P. *et al.* Effect of minocycline on lumbar radicular neuropathic pain: a randomized, placebo-controlled, double-blind clinical trial with amitriptyline as a comparator. *Anesthesiology* **122**, 399-406, doi:10.1097/ALN.0000000000000508 (2015).
40 Zigmond, A. S. & Snaith, R. P. The hospital anxiety and depression scale. *Acta Psychiatr. Scand.* **67**, 361-370 (1983).
41 Sullivan, M. J., Bishop, S. R. & Pivik, J. The Pain Catastrophizing Scale: Development and Validation. *Psychol Assess* **7**, 524-532 (1995).
42 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).
43 Cutrona, C. E. & Russell, D. W. The provisions of social relationships and adaptation to stress. *Advances in personal relationships* **1**, 37-67 (1987).
44 Felitti, V. J. *et al.* Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: The Adverse Childhood Experiences (ACE) Study. *American journal of preventive medicine* **14**, 245-258 (1998).
45 Schnurr, P. P., Lunney, C. A., Sengupta, A. & Spiro III, A. A longitudinal study of retirement in older male veterans. *Journal of Consulting and Clinical Psychology* **73**, 561 (2005).
46 Schnurr, P. P., Spiro III, A., Vielhauer, M. J., Findler, M. N. & Hamblen, J. L. Trauma in the lives of older men: Findings from the Normative Aging Study. *Journal of Clinical Geropsychology* **8**, 175-187 (2002).
47 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).
48 Scholz, J. *et al.* A novel tool for the assessment of pain: validation in low back pain. *PLoS medicine* **6**, e1000047, doi:10.1371/journal.pmed.1000047 (2009).
49 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).
50 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 (2007).
51 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, doi:10.1016/j.neuroimage.2009.11.056 (2010).
52 Owen, D. R. *et al.* An 18kDa Translocator Protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. *J. Cereb. Blood Flow Metab.* (in press).
53 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).
54 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).
55 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).
56 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).
57 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).
58 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).

59 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).
60 Reitan, R. M. Validity of the Trail Making Test as an indicator of organic brain damage. *Perceptual and motor skills* **8**, 271-276 (1958).
61 Osterrieth, P. A. Le test de copie d'une figure complexe; contribution à l'étude de la perception et de la mémoire. *Archives de Psychologie* (1944).
62 Rey, A. L'examen psychologique dans les cas d'encéphalopathie traumatique. (Les problems.). [The psychological examination in cases of traumatic encepholopathy. Problems.]. *Archives de Psychologie* **28**, 215-285 (1941).
63 Dillon, D. G. *et al.* Dissociation of neural regions associated with anticipatory versus consummatory phases of incentive processing. *Psychophysiology* **45**, 36-49, doi:10.1111/j.1469-8986.2007.00594.x (2008).
64 Pizzagalli, D. A. *et al.* Reduced caudate and nucleus accumbens response to rewards in unmedicated individuals with major depressive disorder. *The American journal of psychiatry* **166**, 702-710, doi:10.1176/appi.ajp.2008.08081201 (2009).
65 Baliki, M. N. *et al.* Corticostriatal functional connectivity predicts transition to chronic back pain. *Nat. Neurosci.* **15**, 1117-1119, doi:10.1038/nn.3153 (2012).
66 Slater, M. A. *et al.* Preventing progression to chronicity in first onset, subacute low back pain: an exploratory study. *Arch. Phys. Med. Rehabil.* **90**, 545-552, doi:10.1016/j.apmr.2008.10.032 (2009).
67 Debruyne, J. C. *et al.* PET visualization of microglia in multiple sclerosis patients using [11C]PK11195. *Eur. J. Neurol.* **10**, 257-264 (2003).
68 Cohen-Adad, J. *et al.* in *Proceedings of the 20th Annual Meeting of OHBM* 3633 (Hamburg, Germany, 2014).
69 Nichols, T. E. & Holmes, A. P. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum. Brain Mapp.* **15**, 1-25 (2002).
70 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).
71 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).
72 Posillico, C. K., Terasaki, L. S., Bilbo, S. D. & Schwarz, J. M. Examination of sex and minocycline treatment on acute morphine-induced analgesia and inflammatory gene expression along the pain pathway in Sprague-Dawley rats. *Biology of sex differences* **6**, 33, doi:10.1186/s13293-015-0049-3 (2015).
73 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).
74 Jensen, M. P. & McFarland, C. A. Increasing the reliability and validity of pain intensity measurement in chronic pain patients. *Pain* **55**, 195-203 (1993).
75 Herron, A. J. *et al.* Assessment of riboflavin as a tracer substance: comparison of a qualitative to a quantitative method of riboflavin measurement. *Drug Alcohol Depend.* **128**, 77-82, doi:10.1016/j.drugalcdep.2012.08.007 (2013).
76 Ramanujam, V. M., Anderson, K. E., Grady, J. J., Nayeem, F. & Lu, L. J. Riboflavin as an oral tracer for monitoring compliance in clinical research. *Open Biomark J* **2011**, 1-7 (2011).
77 Switzer, B. R. *et al.* Development of a urinary riboflavin adherence marker for a wheat bran fiber community intervention trial. *Cancer Epidemiol. Biomarkers Prev.* **6**, 439-442 (1997).

78 Del Boca, F. K., Kranzler, H. R., Brown, J. & Korner, P. F. Assessment of medication compliance in alcoholics through UV light detection of a riboflavin tracer. *Alcohol. Clin. Exp. Res.* **20**, 1412-1417 (1996).
79 Babiker, I. E., Cooke, P. R. & Gillett, M. G. How useful is riboflavin as a tracer of medication compliance? *J. Behav. Med.* **12**, 25-38 (1989).
80 Dubbert, P. M. *et al.* Riboflavin as a tracer of medication compliance. *J. Behav. Med.* **8**, 287-299 (1985).
81 Boehnke, C. *et al.* High-dose riboflavin treatment is efficacious in migraine prophylaxis: an open study in a tertiary care centre. *Eur. J. Neurol.* **11**, 475-477, doi:10.1111/j.1468-1331.2004.00813.x (2004).
82 Loggia, M. L. *et al.* The catechol-O-methyltransferase (COMT) val158met polymorphism affects brain responses to repeated painful stimuli. *PLoS One* **6**, e27764, doi:10.1371/journal.pone.0027764 (2011).
83 Wasan, A. D. *et al.* Neural correlates of chronic low back pain measured by arterial spin labeling. *Anesthesiology* **115**, 364-374, doi:10.1097/ALN.0b013e318220e880 (2011).
84 Loggia, M. L., Juneau, M. & Bushnell, M. C. Autonomic responses to heat pain: Heart rate, skin conductance, and their relation to verbal ratings and stimulus intensity. *Pain* **152**, 592-598, doi:10.1016/j.pain.2010.11.032 (2011).
85 Kong, J. *et al.* Exploring the brain in pain: activations, deactivations and their relation. *Pain* **148**, 257-267, doi:10.1016/j.pain.2009.11.008 (2010).
86 Loggia, M. L. *et al.* Carriers of recessive WNK1/HSN2 mutations for hereditary sensory and autonomic neuropathy type 2 (HSAN2) are more sensitive to thermal stimuli. *J Neurosci* **29**, 2162-2166, doi:10.1523/JNEUROSCI.4633-08.2009 (2009).
87 Loggia, M. L., Mogil, J. S. & Bushnell, M. C. Experimentally induced mood changes preferentially affect pain unpleasantness. *J Pain* **9**, 784-791, doi:10.1016/j.jpain.2008.03.014 (2008).
88 Loggia, M. L., Mogil, J. S. & Bushnell, M. C. Empathy hurts: compassion for another increases both sensory and affective components of pain perception. *Pain* **136**, 168-176, doi:10.1016/j.pain.2007.07.017 (2008).

APPENDIX A

Data Monitoring Committee / Data and Safety Monitoring Board Appendix

- *To be completed for studies monitored by Data Monitoring Committee (DMC) or Data and Safety Monitoring Board (DSMB) if a full DMC/DSMB charter is not available at the time of initial IRB review.*
- *DMC/DSMB Charter and/or Roster can be submitted to the IRB later via Amendment, though these are not required.*

A Data Monitoring Committee (DMC) or Data and Safety Monitoring Board (DSMB) will be convened for safety monitoring of this research study. The following characteristics describe the DMC/DSMB convened for this study (Check all that apply):

☐ The DMC/DSMB is independent from the study team and study sponsor.

☐ A process has been implemented to ensure absence of conflicts of interest by DMC/DSMB members.

☐ The DMC/DSMB has the authority to intervene on study progress in the event of safety concerns, e.g., to suspend or terminate a study if new safety concerns have been identified or need to be investigated.

☐ Describe number and types of (i.e., qualifications of) members:
Click or tap here to enter text.

☐ Describe planned frequency of meetings:
Click or tap here to enter text.

☐ DMC/DSMB reports with no findings (i.e., "continue without modifications") will be submitted to the IRB at the time of Continuing Review.

☐ DMC/DSMB reports with findings/modifications required will be submitted promptly (within 5 business days/7 calendar days of becoming aware) to the IRB as an Other Event.