Title of Study: The Effect of Minocycline on Opioid-Induced Hyperalgesia in Opioid-Maintained Patients

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3. Title of Project: The Effect of Minocycline on Opioid-Induced Hyperalgesia in Opioid-Maintained Patients

4. Purpose: Opioids are the most commonly utilized pharmacological treatment for moderate to severe pain. However, their clinical value is hindered by the development of opioid-induced hyperalgesia (OIH). OIH manifests as heightened pain sensitivity, and is an increasingly challenging drawback to the efficacy of opioid treatment. Although the mechanism of action modulating OIH is not completely understood, previous animal studies suggest that this phenomenon is a result of proinflammatory responses. Thus, administering an adjunct anti-inflammatory agent may attenuate OIH. Minocycline is one such agent; it is a tetracycline derivative antibiotic that inhibits microglia activation, nitric oxide (NO) production, and the release of pro-inflammatory cytokines and chemokines. In fact, recent evidence suggests that minocycline may attenuate the neuroinflammatory effects of opioids while enhancing their antinociceptive effects. Therefore, we will determine if minocycline will mitigate OIH in opioid-maintained patients.

Hypothesis**:** We hypothesize that adjunct minocycline (200mg) will attenuate hyperalgesia often observed in patients undergoing opioid maintenance treatment.

5-6. Background and Significance

Introduction

Opioids are the most prominent pharmacological treatment for moderate to severe pain that results from health issues such as cancer, chronic injuries, and surgical procedures. However, this drug class has an infamously high abuse liability, which casts a dark cloud over their clinical utility. Prescription of opioids over the past two decades has increased dramatically, surpassing 257 million in 2009 (Food and Drug Administration (FDA), 2010). Subsequent to this increase in prescription comes a drastic increase in the number opioid dependent persons. Consequently, there has been a striking increase in the number of deaths due to prescription opioids, from approximately 4,000 in 1999, to over 16,000 in 2010 (Centers of Disease Control and Prevention, 2012). Furthermore, opioid dependence has an estimated economic cost of $72.5 billion in health care services (Coalition Against Insurance Fraud, 2007), and those who abuse this drug class generate an average annual health care cost that is 8.7 times higher than non-

abusers (White et al., 2005). With that said, opioid abuse, addiction, and overdose have become part of a major societal epidemic.

Hyperalgesia as a clinical problem

In addition to abuse, opioids have a range of unfavorable side effects that include respiratory depression, as well as cardiovascular and gastrointestinal effects. Furthermore, opioids notoriously elicit a paradoxical state of increased pain sensitivity, known as hyperalgesia. Hyperalgesia is defined as an increased sensitivity to painful stimuli, as well as allodynia, which is pain evoked by a stimulus that is not under normal circumstances considered painful (Heger, Mair, Otter, Helwig, & Suttorp, 1999). Such a phenomenon is typically evident following administration of titrated doses of opioids, which is almost always necessary after the inevitable development of tolerance (Pasero & McCaffery, 2012). While hyperalgesia can be reliably elicited in preclinical models (Woolf, 1981; Mao et al., 1994; Ossipov, Lai, Vanderah, & Porreca, 2003; Waxman et al., 2009; Juni et al. 2010; Waxman et al., 2010), in humans this paradoxical state is less predictable and a quite serious complication of clinical opioid treatment (Compton, 2008; De Conno et al., 1991; Ossipov et al., 2004; Sjøgren et al., 1994; Sjøgren et al., 1998).

Although a majority of research on OIH is comprised of animal studies, the obvious application of such research is the use of opioids in clinical settings involving treatment of pain, where this phenomenon inserts a complication. The success of pain treatment in humans is hindered by the appearance of hyperalgesia, where opioid titration and rotation become a potentially dangerous guessing game. Further complicating the issue is the fact that clinicians cannot effectively treat OIH, as there is no consensus regarding the mechanism by which hyperalgesia manifests. Since opioids are the cornerstone for treatment of pain, understanding the mechanisms underlying OIH is imperative. As we advance our understanding of OIH, clinicians will be better able to effectively address patient needs, improve patient care, and prevent further medical complications arising from opioid use.

Human studies of OIH

Clinical investigations of OIH tend to focus on defining the contextual characteristics in which OIH appears. Investigations have been sporadically conducted in humans using observational case studies, as well as cross-sectional methodology. A majority of these studies focus on a few particular cohorts; for example, patients suffering from chronic illnesses such as cancer (Sjøgren et al., 1994; Mercadante & Acuri, 2005), those with acute pain following various surgical procedures (Lee, Lee, & Kim, 2013; Chia et al., 1999), populations of opioid addicts (both former and current) (Doverty et al., 2001a; Compton et al., 2010), opioid-naïve pain patients beginning opioid therapy for chronic pain (Chu et al., 2006), and healthy volunteers undergoing human experimental pain testing (Compton et al., 2003). Results of these studies indicate that opioid rotation is sometimes sufficient to eliminate hyperalgesia in the aforementioned populations. Others suggest that complete discontinuation is the most efficacious way to relieve this opioid-induced increased pain sensitivity. However, while it might be an ideal solution, opioid discontinuation is not always realistic or appropriate. As such, a solution that attenuates OIH such that the patient may continue benefitting from chronic opioids is needed. For example,

recent evidence suggests the addition of adjunct pregabalin to effectively diminish OIH in patients receiving chronic opioids (Compton et al., 2010; Lee et al., 2013).

Clinical models of hyperalgesia

Doverty et al. (2001a) attributed inconsistencies in the literature to the array of pain modalities and tests used to assess opioid-induced hyperalgesia. Indeed, a complicating factor exists in the wide variance of assessments and populations used to measure this phenomenon, ranging from cold pain, to chemical pain, to pressure pain models in patients with acute surgical pain to those maintained on methadone. Illustrated in both animal and human studies, manipulations that produce profound OIH on one pain assay in one individual may show no or varying hyperalgesic liabilities on other assays in another individual, suggesting that OIH is drug and modality specific, and likely influenced by varying genetic backgrounds (Angst & Clark, 2006; Mogil et al., 1999a; Mogil et al., 1999b; Doverty et al., 2001a; Chu, Clark, & Angst, 2006; Krishnan et al., 2012).

The most common assessments used to measure pain are the cold pressor test, electrical stimulation, and heat-capsaicin. In the most reliable test for assessing opioid effects (Krishnan et al., 2012), the cold pressor test requires that study participants submerge their hands and forearms into ice water, and are instructed to report initial detection of pain as well as the point at which they can no longer tolerate said pain. This test is quite robust and reliable when measuring pain related to opioid administration (Modir & Wallace, 2010; Olesen et al., 2012). Another common measure for analyzing opioid effects is electrical stimulation. In this assessment, cutaneous electrodes are attached to the participants' ear lobe, whereby increasing pulses of electricity are delivered. Participants verbally indicate the first perception of the stimulus as painful, and resultant increases in pain up until they can no longer tolerate it (Doverty et al., 2001a; Olesen et al., 2012). However, this measure yields unfavorable results in regard to opioid-induced hyperalgesia in both methadone and buprenorphine maintenance patients (Doverty et al., 2001a; Luginbühl et al., 2003; Compton et al., 2012). Finally, the capsaicin model produces primary and secondary hyperalgesia after a subcutaneous injection of capsaicin is applied. This model is widely used in opioid-induced pain research, but is not ideal for repeated use.

Mechanisms of Hyperalgesia

Several prominent hypotheses of opioid-induced hyperalgesia have emerged over the past two decades. While there is no consensus on a definitive mechanism underlying OIH, a theory that is steadily gaining popularity postulates that hyperalgesia is a result of glial cell-regulated immune responses via action at the toll-like receptor-4 subtype (TLR4) (Hutchinson et al., 2009; Lewis et al., 2010; Watkins, Wiertelak, Goehler, Mooney-Heiberger, Martinez, et al., 1994; Watkins, Hutchinson, Rice, & Maier, 2009). Opioids have been shown to activate TLR4s in addition to their well-known action at opioid receptors. Of particular interest is the fact that two of the three intracellular pathways activated by TLR4s are responsible for proinflammatory glial responses. Furthermore, there is some evidence to suggest that the aversive side effects of opioids are modulated by this receptor population (Watkins et al., 2009). As such, glial-regulated TLR4 pathways present a logical potential culprit for the mechanism underlying OIH (Hutchinson et al., 2009).

Indeed, the molecular mechanisms underlying OIH are not well understood (Bian et al., 2012). Aforementioned cumulating evidence suggests that glial cells, particularly microglia, play an essential role in development of OIH. About 70% of the cells in the CNS are glial cells, and microglia represent 5 to 10% of these glial cells. Microglia are intrinsic immune cells of the brain and express many neurotransmitter receptors (Pocock & Kettenmann, 2007). Stress, alcohol, and drugs of abuse such as cocaine, methamphetamine, and opioids activate microglia (Coller & Hutchinson, 2012), which results in the release of cytokines (TNF-α,IL-1β, and IL-6 and others), chemokines, lipid mediators of inflammation, matrix metalloproteases (MMPs) and nitric oxide (Pocock and Kettenmann, 2007). It has been suggested that activation of microglial cells in spinal and cortical areas contributes to development of OIH. Pharmacological treatments that inhibit microglial activation have been shown to block development of morphine tolerance in rodents (Mika et al., 2009), and increase analgesic potency (Hutchinson et al., 2008). Such findings suggest that pharmacological treatments targeting microglia activation may have utility in preventing OIH. One such medication is minocycline.

Minocycline: an antibiotic with multiple CNS effects

Minocycline is a second-generation tetracycline analog antibiotic commonly used for the treatment of acne. Minocycline is a highly lipophilic molecule that easily penetrates the blood-brain barrier (Kim & Suh, 2009). In addition to its antimicrobial effects, minocycline has significant neuroprotective effects and is currently under investigation for the treatment of Alzheimer's disease, Parkinson's disease, Huntington's disease, stroke, schizophrenia, depression and more recently for methamphetamine dependence (Chaudhry et al., 2012; Kim and Suh, 2009; Pae et al., 2008).

Both preclinical and clinical studies have demonstrated the anti-inflammatory and neuroprotective properties of minocycline (Kim & Suh, 2009). Inhibition of microglia activation is likely the main mechanism for these effects. The mechanism of minocycline's anti-inflammatory and neuroprotective effects may be at least partly due to its capacity to inhibit P38 mitogen-activated protein kinases (MAPK). The p38 MAPKs modulate multiple cell functions including proliferation, gene expression, mitosis, cell survival and apoptosis. Of particular interest is the fact that they are critically involved in the activation of microglia (Coller & Hutchinson, 2012). In neurons, they play an important role in neuronal death. Inhibition of the p38 MAPK may contribute both the anti-inflammatory and neuroprotective effects of minocycline.

Significance: As evidenced, OIH is a complicated phenomenon that remains nebulous. As there is no clear consensus on a theoretical model or mechanism, treating OIH in clinical settings remains quite challenging. It is possible that the increased pain sensitivity resulting from chronic opioid treatment is directly related to upward dose titration, tolerance, and addiction. With opioid use, abuse, addiction, and related deaths steadily rising, variables inciting this epidemic must be addressed. While there are active efforts to find more efficacious treatment alternatives for chronic pain (evidenced by the recent FDA approval of Phase 2 clinical trials for two glial-targeting drugs for neuropathic pain) (Watkins et al., 2009), research is needed that will aid the utility of currently approved and available pharmacotherapies. If a way to treat OIH can be

elucidated, this will allow clinicians to continue prescribing opioids without concern over paradoxically increasing the very symptom they set out to treat: pain.

7. Subjects

Veterans and non-veterans who meet the following criteria will be eligible for participation:

Inclusion criteria:

- Males and females, between the ages of 18 and 60
- Diagnosed with opioid dependence and currently enrolled in methadone or buprenorphine maintenance treatment
- Compliant in opioid maintenance treatment and on a stable dose for two weeks or greater
- No current dependence or abuse of any other drugs (other than tobacco or marijuana)
- No current medical problems deemed contraindicated for participation by physician investigator
- For women, not pregnant as determined by pregnancy screening; not breast feeding; using acceptable birth control methods; not experiencing moderate to severe premenstrual symptoms (may interfere with pain assessment); regular menstrual cycles

Exclusion criteria:

- Current major untreated, unstable psychiatric illnesses including mood, psychotic, or anxiety disorders
- History of major medical illnesses, including liver diseases, heart disease, or other medical conditions that the physician investigator deems contraindicated for inclusion in the study
- Current use of over-the-counter or prescription psychoactive drugs known to affect pain threshold or pain tolerance (including NSAIDS, serotonin-norepinephrine reuptake inhibitors (SNRIs), (e.g.,venlafaxine, duloxetine), tricyclic antidepressants (e.g., nortriptyline, amitriptyline), anticonvulsant medications (e.g., topiramate, tegretol), benzodiazepines (e.g., alprazolam, diazepam), and other opioid drugs)
- Liver function tests (ALT or AST) greater than 3x normal
- Allergy to minocycline or other tetracyclines

8. Privacy

All information that is obtained from subjects will be used for the specifically stated purposes that are described in this Project Description and have been approved by the HSS. The personal identifiers that are necessary for this research and that will be obtained are the following: Name, Medical Record Number, Age, Gender, Medical and Psychiatric History, and Laboratory Examination. The procedures for data collection and recruitment of subjects, described elsewhere in the project description, are the least intrusive consistent with obtaining the information necessary to complete this project. Medical records will be reviewed to extract research information by Mehmet Sofuoglu M.D., Ph.D., and Ellen Mitchell R.N. All members of the research team will review, use, and record the minimum amount of information necessary to accomplish the goals outlined in the protocol. All members of the research team have been

trained on VHA privacy regulations and policies and training are current. The settings in which informed consent discussions, other subject interviews or research procedures occur will provide that same privacy protections that would exist if these discussions, interviews, or procedures were carried out for required clinical care (private rooms, drawn curtains, etc.). The information will be used for this project, or disclosed to others, only as permitted by the Privacy Act, the HIPAA Privacy Rule, and VA policy.

9. Selection

Sixty completers will be recruited through the VA methadone and buprenorphine clinics, as well as through the APT Foundation Methadone and Buprenorphine Maintenance Programs. After the initial phone screening, potential subjects will undergo a comprehensive evaluation which will include medical, psychiatric, and drug use histories as well as physical, psychiatric, and laboratory examinations. Laboratory examination will include CBC, liver and thyroid function tests, serum electrolytes, BUN, creatinine, PT, PTT, urine analysis (including urine pregnancy for women) and urine toxicology screening.
Participants will be terminated from the study following opioid relapse, or use of any other psychotropic drugs/medications. If participants are noncompliant (no-show, positive urine screening, noncompliance with medication protocol/missing more than one dose of minocycline/placebo), participation will be terminated.

10. Research Plan

A. Overview: This double-blind, randomized clinical trial will randomize male and female veterans and non-veterans currently undergoing opioid maintenance treatment for opioid dependence to either minocycline (200mg/day) or placebo for 15 days. Upon inclusion, participants will be subjected to a pain assessment to evaluate baseline pain thresholds and tolerance: the Cold Pressor Test. An experimental treatment of either minocycline or placebo will then be initiated and maintained for 15 days. Additionally, at the beginning of Week 2 of treatment, participants will be given a Personal Digital Assistant (PDA) an HP iPAQ Pocket PC 2003 Pro that will administer Ecological Momentary Assessments (EMA). Using EMA, we can assess change in pain sensitivity, withdrawal symptoms and cognitive performance in the participants' natural environment, which increases the ecological validity of the study. Participants will be asked to return to the laboratory several times a week for the 15 consecutive days that they are taking minocycline in order to receive the study medication and to assess changes in pain thresholds and tolerance (i.e. to assess the presence, or lack thereof of hyperalgesia). Upon completion of experimental treatment, participants will be asked to return a final time to undergo pain measurement once more, to assess any changes in pain sensitivity after completion of minocycline.

B. Medication

Minocycline: In this study, minocycline will be administered at 200 mg/day, as a single dose, for 15 days, similar to the dosing in our previous studies (Sofuoglu et al., 2011; Sofuoglu et al., 2009). This is the usual daily dose of minocycline used in clinical trials for the treatment of

infections. Following oral administration, peak plasma levels of minocycline are reached within 1- 4 hours. The elimination half-life of minocycline ranges from 11 to 24 hours.
The most common side effects associated with minocycline include dizziness, vertigo, nausea and vomiting. Photosensitivity manifested by an exaggerated sunburn reaction has been has been reported rarely with minocycline. Patients who experience CNS side effects including light headedness, dizziness, or vertigo should be cautioned about driving vehicles or using hazardous machinery while on minocycline therapy. These symptoms may disappear during therapy and usually disappear rapidly when the drug is discontinued. Concurrent use of tetracycline may render oral contraceptives less effective. Subjects will need other forms of contraception. Minocycline is contraindicated in pregnant women and can be harmful to the fetus. To minimize these risks, subjects will have frequent outpatient visits, where they will receive take-home oral minocycline capsules sufficient to last until their next scheduled outpatient visit, and any adverse effects from the study medication will be monitored under the nurses' supervision. Female subjects will be tested for pregnancy before each test session. As minocycline should be taken on an empty stomach, subjects will be advised not to eat for one hour prior to coming to the clinic.

C. Study Procedures

Cold Pressor Test (CPT): Adapted from Eckhardt et al. (1998), the CPT has good predictive validity for the analgesic effects of opioids (Conley et al., 1997). For this test, two water coolers filled with either warm (100.04ºF/37.8ºC) or cold water (32.9-34.7ºF/0.5-1.5ºC) are used. To begin the CPT, participants first immerse their forearm into the warm-water bath for 2 min. Participants are then instructed to immerse their forearm into the cold water bath and report the first time they experience pain. They are also instructed to keep their arm submerged as long as they can but were permitted to withdraw their arm from the water if the pain gets uncomfortable. Latency to first pain sensations (threshold) and to arm withdrawal from the water (tolerance) is recorded. During testing and following arm withdrawal, heart rate, blood pressure is monitored. After arm withdrawal, the McGill Pain Questionnaire will be completed. Before, during, and after pain testing, the VAS will be completed.

EMA Procedures: Participants will be given a PDA at the beginning of Week 2. The Smartphone will be programmed to beep four times each day at random times (Random Assessment; RA) for one week.

EMA Assessments: Participants will be asked if they feel any pain "at this moment" on seven-point Likert scales (1=strongly disagree to 7=strongly agree). Craving for heroin (e.g., "At this moment, I am craving heroin") and difficulty concentrating will be assessed with single items. Several additional items will assess opioid withdrawal. At each time point, the PDA will administer the Sustained Attention to Response Test (SART).

1) Physiological

Heart rate and blood pressure: Heart rate and blood pressure will be measured throughout the session.

Breathalyzer: A breathalyzer reading will be obtained before the start of each session.

Blood Samples:

Serum Cytokines: Serum levels of pro-inflamatory cytokines will be assayed using electrochemiluminescence multi-array technology (Meso Scale Discovery, Gaithersburg, MD) as described in the methods of DellaGioia et al. (2013). These include Interleukin-1 beta (IL-1β), Interleukin-2 (IL-2), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Interleukin 12 p70 (IL12p70), Interferon gamma (IFNg), Tumor Necrosis Factor alpha (TNFα), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF). Serum samples will be obtained at baseline, and once more at the end of minocycline/placebo treatment. Statistical analysis will examine if serum cytokine levels change with medication treatment.

2) Cognitive Performance

For cognitive performance, we chose three tests that are likely to be sensitive to minocycline's effects in our subject population: the Rapid Visual Information Processing (RVIP), Sustained Attention to Response Test (SART), and the Digit Symbol Substitution Test (DSST).

The RVIP is widely used as a measure of sustained attention with a working memory component. In this task, subjects are asked to respond to any of three digit sequences in a continuous stream of digits lasting for 7 minutes. A white box appears in the center of the computer screen, inside which digits from 2 to 9 appear in a pseudo-random order at the rate of 100 digits per minute. Subjects are instructed to detect consecutive odd or even sequences of digits (e.g., 2-4-6, 3-5-7, 4-6-8, 5-7-9, etc.) and to register responses using a press-pad.

The SART (Robertson et al., 1997) is a Go No-Go task. It assesses the ability to withhold responses to an infrequently occurring target (No-Go trials). A total of 225 single digits (25 x 9 digits) are presented on a computer monitor for 250 ms each, immediately followed by a mask for 900 ms. Subjects must press a spacebar in response to every digit except the "3".

The DSST is a test of psychomotor performance, which measures motor persistence, sustained attention, response speed and visuomotor coordination. The task is to fill in blank spaces with the symbols that are paired with the number above the blank space as fast as possible for 90 sec. DSST was chosen because several studies have demonstrated its sensitivity to opioid effects (Cooper et al., 2012; Zacny and Lichtor, 2008).

3) Self-report

Intake Measures:

Structured Clinical Interview for DSM-IV (SCID) for DSM-IV Axis I disorders: This semi-structured interview based on DSM-IV (APA, 1994) and will be administered to diagnose Axis I disorders.

Session Measures:

Brief Pain Inventory – Short Form: BPI-SF is a self-report questionnaire that assesses severity of pain, impact of pain on daily function, location of pain, pain medications, and amount of pain relief in the past 24 hours or the past week (Cleeland and Ryan, 1994).

Opioid Withdrawal Symptom Checklist (OWSC): Withdrawal signs and symptoms will be assessed using a 22-item withdrawal instrument that has been reliably used to assess opiate withdrawal. This scale rates the following items from 0 to 4: lacrimation, nasal congestion, yawning, sneezing, coughing, throat clearing, restlessness, nausea/vomiting, gooseflesh, sweating, stomach cramps, muscle cramps, and feeling hot/cold.

Time-line Follow-Back Assessment Method (TLFB): Drug use for each day during the 30-day period prior to the study, throughout the period of study participation, and at the follow-up session will be obtained using this self-report measure. Participants are given a blank calendar covering the specified time interval, and are asked to retrospectively reconstruct their drug use over that time interval. The process is facilitated by establishing anchor points (e.g., holidays, anniversaries, major national events, etc.). It can be scored to provide the number of days on which various levels of drug use occurred. The time-line method has good test-retest reliability and good validity for verifiable events. It has been used in numerous studies to assess drug use habits.

Short-Form McGill Pain Questionnaire (SF-MPQ): The SF-MPQ will be used to capture the sensory and affective dimensions of the pain experience immediately following the CPT (Melzack, 1987). In this 15-item questionnaire, participants describe their experience of pain by choosing among a series of possible answers [none (score=1), mild (score=2), moderate (score=3), or severe (score=4)]. The items to describe the pain include 'Throbbing','Shooting', 'Stabbing', 'Sharp', 'Cramping', 'Gnawing', 'Hotburning', 'Aching', 'Heavy', 'Tender', 'Splitting', 'Tired,' 'Exhausting', 'Sickening', 'Fearful', and 'Punishing-Cruel'. Scores are added across all 15 items to generate a sum score, which ranging from 15 and 60.

Visual Analog Scale (VAS): The VAS will be used by participants as a secondary rating scale for subjective pain severity before, during, and after pain testing. This scale contains a horizontal line, anchored by verbal descriptors of "no pain" and "pain as bad as it could be". Participants will place a vertical line at the point that best indicates their present pain (Burckhardt & Jones, 2003).

Drug Effects Questionnaire (DEQ): Subjects will rate the following items from "not at all" to "extremely" on a 100 mm scale: "alert," "calm," "confused," "depressed," "confused," "high," "anxious," "sedated," "tired," "social," "self-confident," "talkative," "hungry," "feeling the drug strength," "feel good drug effects," "feel bad drug effects," and "want more drug." These items are commonly used to assess the subjective effects of drugs (Fischman and Foltin, 1991).

Profile of Mood States (POMS): This 72–item questionnaire consists of adjectives commonly used to describe momentary mood states, has been found to be sensitive to the mood-altering effects of drugs including amphetamine (de Wit and Griffiths, 1991; Levin et al., 1998), and is widely used as a research tool in behavioral pharmacology (Fischman and Foltin, 1991). Its reliability and validity have been extensively evaluated (McNair et al., 1971). The POMS rates eight mood states on a 5–point rating scale (0=not at all to 5=extremely): (1) Anger, (2) Anxiety, (3) Confusion, (4) Depression, (5) Elation, (6) Fatigue, (7) Friendliness and (8) Vigor. The POMS will be used during each session to measure the effects of minocycline on mood.

Systematic Assessment of Side Effects (SAFTEE): In order to monitor adverse events from the study medications, the SAFTEE will be administered before and after each session. This is a symptom checklist (Levine and Schooler, 1986) that has been used successfully in our previous studies to assess possible side effects of study medications. It includes information regarding severity of any presenting side effects, as well as the course of action taken by study staff in response.

4) Genetics:

DNA Extraction: The PAXgene blood DNA Kit (PreAnalytiX Gmbh) will be used for peripheral blood DNA extraction. The isolation procedure is easy to perform, and rapid, and results in high yield and high quality genomic DNA. Briefly, 8.5 ml of human whole blood is collected into PAXgene Blood DNA tubes, which contain a proprietary blend of reagents, optimized for isolation of high-quality genomic DNA. Genomic DNA is then isolated from peripheral blood using the PAXgene Blood DNA Kit. The extraction is performed in a single processing tube to minimize the risk of sample mix-up and cross-contamination. High yields of pure DNA are obtained that perform well in a wide range of downstream applications (e.g., PCR, genotyping, restriction enzyme digestion, Southern-blotting, and pharmacogenomic studies). Typically, 150-500 ug of genomic DNA (A260nm/A280nm: 1.7~1.9) can be obtained. In this study, we will examine genetic variation that may influence variations in opioid-induced hyperalgesia, as well as variations in minocycline's effects. These will include genes that are related to glutamate, GABA, dopamine, opioid receptors, as well as other targets.

Procedure:

After an initial scripted phone screening to determine obvious exclusions, potential participants will be screened in person, which will include discussion of basic inclusion and exclusion criteria and a brief description of the study. Details of the Cold Pressor Test pain assessment will be discussed. If potential participants are still interested, a full screening will be completed. A research assistant and a clinician will perform the initial screening. During this time, informed consent will be obtained along with a signed release to allow study personnel to monitor opioid maintenance, and laboratory examinations will commence. This comprehensive evaluation will include medical, psychiatric, and drug use histories, along with physical, psychiatric, and laboratory examinations. Laboratory examination will include a complete blood count, liver and thyroid function tests, serum cytokines, serum electrolytes, BUN, creatinine, PT, PTT, RPR, random glucose, urine analysis (including urine pregnancy for women), and urine toxicology screening and genetics. Women will be asked to report the first day of their last menstrual cycle, and note will be taken of any ongoing oral contraceptive prescriptions. In addition to this standard clinical evaluation of psychiatric and medical histories, mental status examination, physical examination, and laboratory assessment, potential participants will undergo a clinical diagnostic interview, the Structured Clinical Interview for DSM-IV Disorders (SCID).

All sessions will occur at the West Haven VA Connecticut Healthcare Campus. During an adaptation session, all participants will undergo a baseline urine toxicology screen. On test days, all participants will be asked to refrain from caffeine, nicotine, and from eating within one hour of arrival. Participants will be asked to come to the testing site for a total of ten times: one initial screening day (~ 3 hours), one day for adaptation (~ 1.5 hours), three testing days (~ 2 hours),

four outpatient days to receive medication and check in with research and medical staff (~1 hour), and one final day for follow-up (~ 0.5 hour). Participants will be asked to arrive at approximately the same time each morning, coordinating with attendance at their opioid maintenance clinic. A study nurse will confirm with their respective program that participants did not receive either methadone or buprenorphine that morning, and will call the program when testing is complete to permit dispersal of that day's methadone or buprenorphine dose. On the initial screening day and at the end of medication treatment, blood will be drawn to determine serum cytokine levels. Participants will undergo a variety of cognitive and self-report measures, as well as assessments to confirm restraint from illicit drug use and lack of adverse effects of medication. Prior to their daily methadone or buprenorphine dose and thus at trough plasma levels of opioid, participants will receive either minocycline (200mg) or placebo. Subsequently, all participants will perform the Cold Pressor Test. During all pain assessment sessions, heart rate, and blood pressure will be monitored. Participants will complete the McGill Pain Questionnaire and a VAS after the completion of pain assessment. On outpatient medication visit days, participants will have vital signs checked, undergo a urine screen, and receive their study medication for that day, as well as sufficient take-home minocycline/placebo capsules to last until their next scheduled visit. During their follow-up visit, approximately one week after completing minocycline/placebo, participants will undergo the same procedures as the adaptation session to monitor any changes in pain response following cessation of study medication.

Table 1. Experimental Design

		Week 1			**Week 2**			**Week 3**	**Week 4**
IS	**AS**	TS	Out-patient Session	Out-patient Session	TS	Out-patient Session	Out-patient Session	TS Discharge	**FU**

Abbreviations: IS: Initial Screening; AS: Adaptation Session; Out: Outpatient Session; TS: Test Session; FU: Follow-up

Table 2A. Schedule of Events: Adaptation Session

Time	**Measures and Events**
-60 min	Vital Signs; breathalyzer; urine screen
-45 min	OWSC; TLFB; BPI-SF; DEQ; POMS; SAFTEE
-30 min	RVIP, SART, DSST
-15 min	HR/BP; VAS
0-15 min	CPT; HR/BP; McGill; VAS; Discharge Lunch or Snack

Table 2B. Schedule of Events: Test Session (once per week)

Time	Measures and Events
-45 min	Vital Signs; breathalyzer; urine screen; blood sample*
-30 min	OWSC; TLFB; BPI-SF; DEQ; POMS; SAFTEE
-15 min	RVIP, SART, DSST
0 min	Minocycline/placebo administration, Snack
15 min	HR/BP; DEQ
30 min	HR/BP; DEQ
45 min	HR/BP; DEQ; VAS
60-75 min	RVIP, SART, DSST, CPT; HR/BP; McGill; VAS; SAFTEE; Discharge Lunch

Table 2C. Schedule of Events: Follow-Up (Week 4)

Time	Measures and Events
-60 min	Vital Signs; breathalyzer; urine screen
-45 min	OWSC; TLFB; BPI-SF; DEQ; POMS; SAFTEE
-30 min	RVIP, SART, DSST
-15 min	HR/BP; VAS
0-15 min	CPT; HR/BP; McGill; VAS; Discharge Lunch or Snack

Abbreviations: COWS: Clinical Opiate Withdrawal Scale; TLFB: Time-line Follow-Back; BPI-SF: Brief Pain Questionnaire – Short Form; DEQ: Drug Effects Questionnaire; POMS: Profile of Mood States; SAFTEE: Systematic Assessment of Side Effects; RVIP: Rapid Visual Information Processing; SART: Sustained Attention to Response Test; DSST: Digit Symbol Substitution Test; CPT: Cold Pressor Test; HR: Heart Rate; BP: Blood Pressure; VAS: Visual Analog Scale
*On the last test day only

11. Statistical Considerations

a. Statistical analysis.

The primary dependent variables will be pain sensitivity, cognitive performance, subjective drug effects, and physiological measures. Pain sensitivity will be assessed with the Cold Pressor Test (CPT). The cognitive performance will be assessed with the Digit Symbol Substitution Test (DSST) and a Go-No/Go task. Subjective drug effects will be assessed with the Drug Effects

Questionnaire (DEQ). The physiological responses will include heart rate, and blood pressure. The independent variable, Treatment, will be categorical with 2 levels: minocycline or placebo. The primary analysis will be done with repeated measures of variance. In these analyses, effects for treatment (minocycline or placebo) and session and the interaction between treatment and time will be included. Adjustments will be made (e.g., Bonferroni correction) for the multiple testing of the data.

b. Sample size and power analysis:

There are no previous studies that examined minocycline's effects on OIH, as told by the CPT, in opioid-maintained patients. For power calculations, we referenced a previous study that examined the effect of gabapentin on the CPT in MM patients (Compton et al., 2010). As such, to allow for an approximate 15% drop out rate, we plan to recruit 60 participants, with 30 in each condition. This will yield over 80% power with a moderate effect size (d=0.5) for the between-subjects comparison of minocycline or placebo effects on opioid-induced hyperalgesia.

12. Risks and benefits

Potential risks

1. Minocycline: The most common side effects associated with minocycline dizziness, vertigo, nausea and vomiting. Photosensitivity manifested by an exaggerated sunburn reaction has been has been reported rarely with minocycline. Patients who experience CNS side effects including light headedness, dizziness, or vertigo should be cautioned about driving vehicles or using hazardous machinery while on minocycline therapy. These symptoms may disappear during therapy and usually disappear rapidly when the drug is discontinued. Concurrent use of tetracycline may render oral contraceptives less effective. Subjects will need other forms of contraception. Minocycline is contraindicated in pregnant women and can be harmful to the fetus. To minimize these risks, subjects will be administered minocycline in the clinic daily and any adverse effects from the study will be monitored. Female subjects will be tested for pregnancy before each test session.

2. Blood Drawing: Subjects will have approximately 50 cc of blood drawn as a result of their participation in the study. Blood drawing can cause some pain and result in a hematoma.

Protection of subjects

a. Our inclusion and exclusion criteria will be applied by experienced professionals who will be carefully trained and monitored in order to accept only appropriate subjects into the study. Thus, effective screening will exclude subjects who would be placed at a greater risk. This is determined by the medical and psychiatric history, drug use history, the physical examination, and the laboratory studies done prior to beginning this research protocol.

b. Confidentiality will be protected by having records identified by code number only with the master list including names kept in a sealed envelope in a locked file in the Principal Investigator's office. Confidentiality in this study is of the utmost importance to us. All information obtained will be stored in coded form. The paper files will be kept in locked

file cabinets in Bldg. 36 and the electronic records will be kept in VA intranet (M-drive).

Risk Benefit ratio
Participation in the study may lead to continued successful treatment for opioid addiction, as well as a decrease in OIH, which will be beneficial to subjects participating in the study. Given the anticipated benefits to subjects and society, the moderate to low risk to subjects is reasonable.

13. Safety

The study will be monitored by a Data and Safety Monitoring Board (DSMB) because this population might be considered vulnerable due to their substance abuse. Risks associated with participating in this protocol are moderate. There is adequate surveillance and protections to discover adverse events promptly and keep their effects minimal. The Board will be composed of persons who are experienced in the conduct of clinical trials for the treatment of addictive disorders and who have appropriate expertise in substance abuse and psychopharmacology. There will be at least one physician on the board, who is not directly involved in this trial but who has the requisite expertise. In order for the DSMB to fulfill its mission of assuring the safety of human subjects and the scientific integrity of the study being conducted, the Board will review adverse event data no less than three times per year. During the meeting the DSMB will review a table of adverse events at these four-month intervals. Following each DSMB meeting, written minutes will be prepared and distributed, summarizing any recommendations including interim analyses, as described above. These written reports will insure accurate preparation of any protocol amendments that might be necessary. After each DSMB meeting, this written report will describe all recommendations including additional safety steps. All serious adverse events such as deaths, hospitalizations and unexpected toxicity will be reported to the IRB as well as our local hospital risk management committee under expedited reporting, i.e., immediately by telephone as the information is available to us, followed by written report within 48 hours. The procedures for the written reporting include written documentation using the clinical notes related to the adverse event and specific forms detailing the event with a sign-off by all appropriate supervisory personnel. Communication of recommendations and decisions are made back to the investigator in a timely manner. These reports will also circulate to the DSMB as the safety monitor.

Reporting of Adverse Events
An adverse event is any physical or clinical change or disease experienced by the subject at any time during the course of the study, whether or not considered related to the use of the study drug. This includes the onset of new illness and the exacerbation of pre-existing conditions. Subjects will be questioned and/or examined by the investigator or his/her designee for evidence of adverse events. The questioning of subjects with regard to the possible occurrence of adverse events will be generalized such as, “How have you been feeling since your last visit?” The presence or absence of specific adverse events will not be solicited from subjects.
All adverse events will be recorded in the subject’s medical and/or research records. The onset and end dates, severity, and relationship to the study drug will be recorded for each adverse event. The severity of the adverse event will be assessed according to the following grading system. Any action or outcome (e.g., hospitalization, discontinuation of therapy, etc.) will also be recorded for each adverse event.

Grading. For the severity of adverse events, the following definitions will be used:

• *Mild*: awareness of sign, symptom, or event, but easily tolerated,

• *Moderate*: discomfort enough to cause interference with usual activity and may warrant intervention,
• *Severe*: incapacitating with inability to do usual activities or significantly affects clinical status, and warrants intervention,
• *Life-threatening*: immediate risk of death

Attribution. The investigator will also assess the relationship of any adverse event to the use of the study drug, based on available information, using the following guidelines:
• *Definite*: Adverse event(s) will clearly be related to investigational agent(s) or other intervention
• *Probable*: Adverse event(s) will likely be related to investigational agent(s)
• *Possible*: Adverse event(s) may be related to investigational agent(s)
• *Unlikely*: Adverse event(s) will doubtfully be related to investigational agent(s)
• *Unrelated*: Adverse event(s) will clearly not be related to the investigational agents(s)

Definition of Serious Adverse Events. All serious adverse events, whether or not deemed drug-related or expected, will be reported by the principal investigator or designee to the IRB within 24 hours (one working day) by telephone. A serious adverse event is any event that: is fatal, is life-threatening (life-threatening is defined as the patient was at immediate risk of death from the AE as it occurred), is significantly or permanently disabling, requires inpatient hospitalization or prolongs hospitalization, is a congenital anomaly or birth defect.
Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject, and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
A written report will follow as soon as possible which includes a full description of the event and any sequelae. This includes serious adverse events that occur any time after the inclusion of the subject in the study (defined as the time when the subject signs the informed consent through discharge from the study (i.e., either subject completed or discontinued their study participation). The subject is considered discharged either after the completion of the last visit or contact (e.g., phone contact with the investigator or designee). Discontinuation is the date a subject and/or investigator determines that the subject can no longer comply with the requirements for any further study visits or evaluations (e.g., the subject is prematurely discontinued from the study).
All serious but unanticipated adverse events will be reported to the HSS and HIC within 48 hours; and serious anticipated adverse events will be reported immediately to the HSS and HIC whenever their magnitude or frequency exceeds expectations.
Serious adverse events will get reviewed immediately by the PI. Otherwise, this moderate risk study will be monitored quarterly by the PI and the members of the DSMB, and reported to the IRB if trends in AEs emerge.

14. Informed consent: Recruitment will be by word-of-mouth, referrals from area programs and by advertisement. A research staff member will interview opioid dependent individuals that are

interested in participating in the study over the phone. If subjects pass the initial screening for the study, they will then come into the clinic for a full screening evaluation. Upon arrival, research assistant will read the detailed consent form and will ask questions to make sure that the subjects understand the procedure and their rights, and informed consent will be obtained.

15. Confidentiality: Confidentiality in this study is of the utmost importance to us. All information obtained will be stored in coded form. The names of the subjects will be used in hospital records.

16. Location of Study: This study will be conducted at the West Haven VA Connecticut Healthcare System.

17. Payment: All participants will be compensated for their participation. Participants will be paid $30.00 for the initial screening and $50 for the adaptation session. Participants will also receive $50 for each of the three test sessions, and $20 for each of the four outpatient sessions as well as $50 for the follow-up visit. In addition, participants will be paid $20.00 a week to help off- set transportation cost, and $1.50 for each completed assessment on the PDA. Thus, participants can potentially receive a total of $482.00.

18. Source of Funds: This protocol is being funded by MIRECC.

19. Duration: The entire study will take approximately two years to complete.

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