Mechanisms of Sleep Disruption Hyperalgesia

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1. Abstract

Twenty percent of Americans suffer from chronic pain. Sleep disturbance is similarly prevalent and among the most common and disabling neurobehavioral problems associated with chronic pain. Hyperalgesia is a pathophysiologic feature of chronic pain syndromes. In this study, we propose to initiate a systematic investigation of the mechanisms by which sleep disruption incites hyperalgesia (HA). Addressing this knowledge gap, by identifying the mechanisms of sleep disturbance-induced hyperalgesia (SD_HA) has critical implications for the etiology, prevention and treatment of chronic pain. Preliminary data from our investigative teams implicate two untested and possibly interrelated candidate mechanisms: 1) inflammation and 2) opioidergic antinociceptive system impairment. We have found that experimental sleep restriction activates cellular and genomic markers of inflammation; based on non-human animal data, we hypothesize inflammation will heighten hyperalgesia. We have also shown that experimental sleep disruption impairs descending pain inhibition, causes spontaneous pain, and diminishes opioid analgesia. Despite these convergent data, whether and to what degree SD_HA is mediated by inflammation is unknown. We have assembled an interdisciplinary team from Johns Hopkins and UCLA to tackle the problem of SD_HA. We propose a translational experiment in healthy human subjects to determine the role of inflammation in SD_HA and study the effects of sleep disruption and inflammation on opioid analgesia. Using a mixed-model, randomized controlled experiment, all participants will undergo baseline sleep and experimental sleep disruption conditions. We will employ a novel sleep fragmentation manipulation developed by our group, as an analogue for the type of sleep loss most commonly associated with pain and insomnia—multiple nocturnal awakenings. Following undisturbed sleep and sleep disruption conditions, we will examine next-day hyperalgesia and analgesic response to either: (a) morphine or (b) placebo. Hyperalgesia and analgesia will be assessed using a heat-capsaicin pain model, which provides an index of spinally mediated hyperalgesia [secondary hyperalgesia (2º HA)], yet applied to the study of SD_HA. Candidate inflammatory markers will be measured at the: 1) genomic 2) cellular and 3) systemic levels of analysis, and will be evaluated as mediators of the effects of sleep disruption on hyperalgesia and opioid analgesia.

2. Objectives (include all primary and secondary objectives)

***AIM 1:* To examine the effects of experimental sleep disruption on spinal sensitization by evaluating laboratory pain responses in the heat-capsaicin pain model.**

Hypothesis 1.a. Relative to uninterrupted sleep, experimental sleep disruption will enhance primary hyperalgesia (1º HA) and 2º HA (an index of central neuronal sensitization).

***AIM 2:* To examine the effects of experimental sleep disruption on opioid analgesia.**

Hypothesis 2.a. Relative to undisturbed sleep, sleep disruption will diminish the analgesic effect of morphine on 1º HA, 2º HA (heat-capsaicin pain model), and cold pressor pain tolerance (CPT).

AIM 3: ***To determine the effects of sleep disruption on cellular and genomic markers of inflammation and characterize the role of inflammatory activity on laboratory pain responses and opioid analgesia.***

Hypothesis 3.a. Relative to undisturbed sleep, experimental sleep disruption will increase markers of inflammation across genomic, cellular, and systemic levels of analysis.
Hypothesis 3.b. Markers of inflammation will mediate the effects of experimental sleep disruption on 1º HA and 2º HA, and diminished morphine analgesia on 1º HA, 2º HA, and CPT.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Twenty to thirty-five percent of the world population suffers from chronic pain syndromes[1-3] at an estimated cost of $100 billion per annum in the US alone[4]. In addition, persistent pain profoundly impairs quality of life(5, 6) and increases risk for medical[7] and psychiatric morbidity[8]. Chronic pain is conceptualized as a disease of the nervous system, involving varying degrees of peripheral input and dysregulation of central nociceptive modulatory systems(9, 10). Central neuroplastic alterations amplify and maintain nociception leading to generalized hyperalgesia and clinical pain that can persist disproportionately to or in the absence of peripheral damage. Hyperalgesia, i.e. heightened responsivity to noxious stimuli, characterizes chronic pain syndromes and is critical to the pathogenesis and treatment of chronic pain[11]. The complex interactions between behavioral, environmental and neurophysiologic processes that promote and maintain hyperalgesia, however, are just beginning to be explored and remain poorly understood.

Insufficient sleep is one promising, modifiable behavioral factor with major relevance to the study of hyperalgesia and chronic pain. Sleep disruption due to chronic insomnia is the most common clinical cause of sleep loss with a prevalence between 10-15% [12-15], but a substantial number of individuals obtain insufficient sleep from self-imposed curtailment, lifestyle, and work-related factors[16]. The CDC reports that almost one third of US adults experience insufficient sleep for the majority of days in the preceding month[17].

Sleep disruption is one of the most disabling comorbidities reported by 50-88% of chronic pain patients(18, 19). Several controlled experiments demonstrate that sleep deprivation causes hyperalgesia[20-22] and longitudinal data indicate that sleep disruption and clinical pain reciprocally interact in a vicious cycle[23-27]. Exacerbations in pain predict decrements in sleep and poor sleep in turn predicts subsequent pain[28]. Sleep disturbance increases the risk for developing chronic pain after acute traumatic injury(29, 30) and enhances the odds of developing widespread pain(31, 32). Conversely, restorative sleep increases the likelihood of remission from chronic pain[33]. These findings, which are independent of psychological factors, such as negative mood, suggest that sleep disruption may play a critical role in the poorly understood transition from acute to chronic pain. **Since sleep disorders and insufficient sleep are highly prevalent and treatable, the potential impact of systematic research in this area is substantial and may enhance our understanding of chronic pain pathophysiology, as well as lead to novel pain prevention and management approaches.**

The mechanisms of sleep disruption-induced hyperalgesia (SD_HA) are unknown, but our previous work implicates functional alterations of supraspinal, descending pain modulatory sytems[34], in which opioid peptides play a critical mediating role[35-41]. Compromised pain

inhibitory capacity, psychophysiologically measured in humans using diffuse noxious inhibitory control (DNIC) testing paradigms, has been demonstrated in many idiopathic clinical pain conditions[42-48], such as fibromyalgia. One way to probe the mechanisms underlying sleep dependent impairment to these systems, with great potential for significance, is to determine whether sleep disturbance reduces the effectiveness of exogenously administered opioids.

Supported by preclinical studies[95;96] and our pilot data (see below), we propose to **determine whether sleep disruption interferes with opioid analgesia in humans (AIM2).** Such a finding would have broad implications for clinical practice and conceivably influence the care of millions of patients. Opioid receptor agonists are the standard treatment for moderate to severe acute pain. Approximately16% of all emergency room visits result in the prescription of opioid analgesics[49] and long-term opioid therapy is increasingly used to treat non cancer-related chronic pain[50]. Establishing the impact of disrupted sleep, a highly treatable condition, on opioid efficacy, therefore, could lead to more efficient, effective, and safer use of opioids to treat pain and potentially reduce risk of overuse and dependency. Inadequate pain management continues to be a major problem in the US[51], despite data demonstrating that poorly controlled pain increases the length of hospital stays and contributes to poor outcomes(52, 53). Positive findings from the proposed study, therefore, could potentially change clinical practice guidelines to include proactively identifying and treating sleep disturbance via behavioral and/or pharmacologic interventions before or in conjunction with opioid therapy to improve pain management outcomes.

Our focus on **inflammation as another key mediator of SD_HA** also has broad implications for chronic pain prevention and treatment. Sleep deprivation elevates pro-inflammatory substances, which are known to sensitize nociceptors and induce enhanced responsivity to noxious stimulation in animals. It is not known whether increased inflammation induced by sleep deprivation mediates hyperalgesia in humans. By investigating multiple levels of the inflammatory cascade, including intracellular cytokine production, genomic and molecular signaling pathways, our approach provides a finer grained understanding of the mechansims by which sleep disruption alters inflammatory processes. Beyond connecting alterations in inflammatory pathways to hyperalgesia in humans, this knowledge also has broader health implications for many chronic conditions such as cardiovascular disease(54, 55) and autoimmune disorders[56] in which rates of sleep disorders are high and chronic inflammation plays a substantial role in morbidity.

Lastly, we will evaluate possible **interrelationships between opioid efficacy and inflammation**, determining whether inflammation mediates (statistically) sleep disruption's attenuation of opioid analgesia. This hypothesis derives from recent animal studies(57, 58) and translation would have major implications for pain medicine, including the development of novel analgesics. If SD-induced inflammation does interfere with opioid analgesia, this provides a scientific basis for clinical reports that combining anti-inflammatory agents with opioid analgesia reduces post operative opioid consumption and enhances surgical outcomes[59].

Summary. The proposed project addresses two major public health epidemics—insufficient sleep and chronic pain. Each aim has the potential to change the clinical practice of pain prevention and management. The scientific knowledge gleaned by our detailed study of the mechanisms of SD_HA—including genomic and cellular dimensions of the inflammatory cascade, opioidergic function, and their interaction—has broad implications for some of the most costly and prevalent chronic diseases, including opioid addiction, cardiovascular disease, and rheumatologic disorders.

4. Study Procedures

a. Study Design

RESEARCH DESIGN AND METHODS

Design: As depicted in Figure 1, we have adopted a mixed model, randomized controlled, double-blinded, experimental design that combines the feasibility of a parallel, between-groups approach [Drug condition (morphine vs. placebo saline)] with the statistical power advantages and cost effectiveness of a within subjects approach. Sleep is the within subjects condition [Uninterrupted Sleep (US) vs. Forced Awakenings (FA)]. The US condition is 2, 8-hour nights of undisturbed sleep (US1 and US2), whereas the FA condition involves two consecutive nights (FA1 & FA2) of 8 forced awakenings plus a recovery night (not shown in diagram). Eligibility will be determined by three screening visits, including an adaptation polysomnography (PSG) assessment [Night1 (v3)]. Subjects are randomized (Morphine or Placebo) and will then complete two separate admissions (v4 & v5) at the JHBMC CRU to undergo the US and FA conditions and next day assessments. An optional testing session (see Optional Sleep Pain, Emotions, and Reward Study Procedures, below) will occur in the daytime after US1 and FA1. The order of sleep condition admissions will be counterbalanced and there will be a ≥two week washout period between admissions. The daytime pain testing protocol (Cap/QST & drug) will be performed twice, the day after US2 and the day after two nights of sleep disruption (FA2). The protocol includes blood sampling to quantify inflammatory markers, heat-capsaicin sensitization, quantitative sensory testing (QST), and drug administration. Five QST sessions will be conducted to assess HA and morphine analgesia (1 pre-drug and 4 post-drug; details provided below and in Fig. 3). Morphine/placebo will be administered through subcutaneous injection after the completion of QST1. There will be a 40 minute rest period following morphine/placebo administration to allow for peak analgesic effect to take place. Subsequently QST 2-5 (i.e., rekindling) will be conducted at 40 minute intervals.

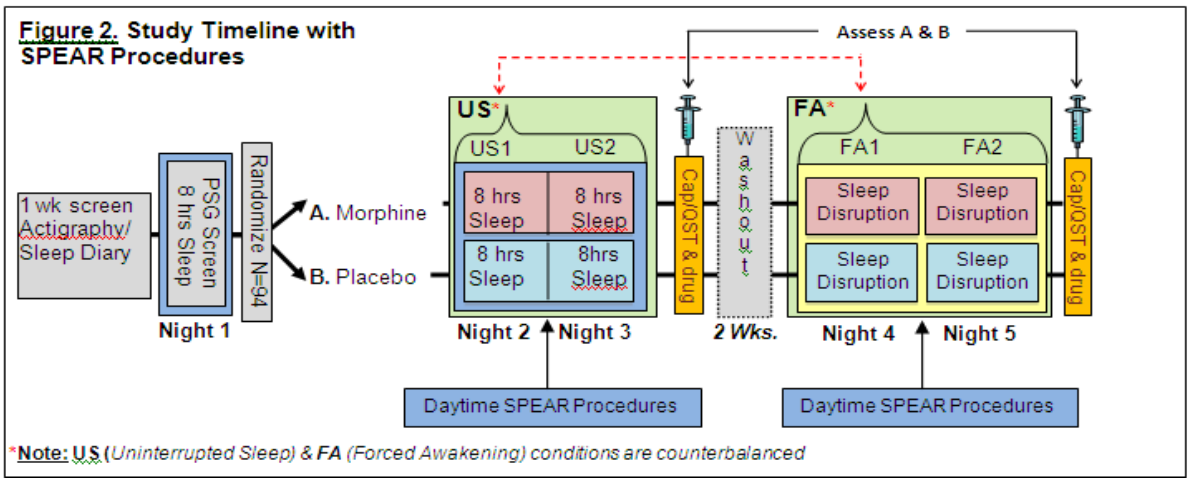
Optional Sleep, Pain, Emotions, and Reward Study Procedures

Participants will have the option to participate in the Sleep, Pain, Emotions, and Reward Study (SPEAR). The purpose of this optional study is to evaluate the influence of sleep continuity disruption on emotion reactivity and regulation, as well as motivation and reward processing. This study is embedded within the Mechanisms of Sleep Disruption Hyperalgesia Study, such that participants will undergo the same screening procedures. Eligibility criteria for the SPEAR study will be similar to the Mechanisms of Sleep Disruption Hyperalgesia Study. Subjects participating in SPEAR will complete an additional consent form for these procedures. Consent will be obtained in advance, along with the Parent study consent procedures. The procedures for participants in the SPEAR Study will differ from those associated with the Mechanisms of Sleep Disruption Hyperalgesia Study in the following ways:

1) On the day following Night 1 of the US condition (US1), and on the day following Night 1 of the FA condition (FA1), participants in the SPEAR study will complete a series of emotion, motivation, reward-related, and psychomotor vigilance tasks, as well as additional pain testing. Some of these tests will occur simultaneously. It is expected that the experimental procedures associated with SPEAR will last approximately 2-3 hours (see below for detailed procedures related to the optional SPEAR study).
2) Participants will have the option of participating in one magnetic resonance imaging (MRI) scan along with two positron emission tomography (PET) scans. We are including PET imaging in this protocol in order to evaluate the extent to which dopamine receptor 2/3 binding correlates with the emotion and reward-related behavioral data we are already collecting as part of the SPEAR substudy, which is a natural extension of our ongoing work.

A study design of the entire study (Mechanisms of Sleep Disruption Hyperalgesia + SPEAR) is provided in Figure 1 below:

Figure 1. Study Timeline When SPEAR Study is Embedded in Mechanisms of Sleep Disruption Hyperalgesia Study



ASSESSMENT PROCEDURES

Recruitment: We will recruit subjects via community flyers, print, and electronic media.

Screening (phone and in person Visit 1): A phone screen eliciting information relevant to the inclusion/exclusion criteria will be conducted, adapting our current screens for the proposed study. After passing the initial phone screen, subjects will complete a 2 hour visit at the JHU Behavioral Sleep Medicine Lab, which will include: informed consent, a urine toxicology screen, and anthropometry. We will administer the following standardized questionnaires: The Pittsburgh Sleep Quality Index [(PSQI)(60)], the Epworth Sleepiness Scale (ESS)(61), the Brief Symptom Inventory (BSI)(62), the Brief Pain Inventory (short form) (BPI)(63), and a Health History Form (HHF)(64). The ESS and PSQI, have well established cut-off criteria demarcating excessive daytime sleepiness(65) and poor sleep(60, 66). The BSI global scales are well normed indicators of general psychological distress(67). The HHF obtains detailed information related to medical disorders, pain history, medications and health behaviors (caffeine, exercise, etc.) that is supplemented by interview to establish eligibility. Subjects will complete the Structured Clinical Interview for DSM-IV Psychiatric Disorders (SCID-DSM-IV Patient Questionnaire)(68) to identify likely Axis I disorders, including substance abuse. Follow up administration of selected, full SCID-IV Axis I(68) modules will determine psychiatric eligibility. The Structured Interview for Sleep Disorders(69) will be used to exclude sleep disorders. The Time-line Follow-Back (TLFB) procedure(70) will obtain opioid use history. Subjects will be trained on the Sleep and Pain Diaries and Wrist Actigraphy procedures.

Screening Visit 2: Subjects will keep standard daily sleep(71-73) and pain diaries(74) for 1 week. Morning entries gather sleep continuity parameters. Evening entries query for daytime pain(63), fatigue, naps, medications, menstruation, and exercise. Subjects will continuously wear an ActiSleep Plus Monitor (or equivalent) wrist accelerometer, which provides objective estimates of circadian rhythm,(75) and sleep continuity(76, 77). Sleep diary and actigraphy data will be reviewed to determine that the sleep, pain, and other eligibility criteria are met. In addition, participants will have the option of providing a saliva sample to be used for future genetic analyses. Should participants accept this option, 1.0 mL of whole unstimulated saliva will be collected by passive drool using a saliva collection aid (e.g., Oragene DNA Self-Collection Kit), stored according to standard specifications. In addition, subjects will have a history and physical exam

and must test negative for pregnancy via urine pregnancy test (females) and recreational drugs and opioids. A complete blood count test, hepatic and kidney function panel will be performed. Drs. Tompkins, Strain, or Umbricht will make final medical eligibility determinations.

Subjects will be briefly familiarized with the pain testing procedures and capsaicin cream to further help determine eligibility. A thermode will be secured to the forearm with a Velcro strap and will be programmed to emit heat at a constant temperature (e.g., 45°C) for 5 minutes. The thermode will then be removed and .1% capsaicin cream will be applied to the area on which the thermode rested, and will be covered with a raised adhesive patch. Subsequently, the patch and capsaicin will be removed and the skin will be cleaned with an alcohol swab. Participants who find the pain from the capsaicin treatment to be intolerable, or who lack an adequate pain response, will be excluded from further participation in the study. These criteria are in place to avoid causing undue pain (e.g., those whose pain ratings are already quite high would presumably experience even more pain during subsequent quantitative sensory testing over the affected area) and to rule out those whose "baseline" pain scores are too low to assess opioid analgesia.

Screening Visit 3 [inpatient Clinical Research Unit (CRU)]: Twenty-four hours prior to, and during admission for Visit 3 (v3) and all CRU visits, subjects will refrain from taking analgesics, hypnotics, and other centrally acting (e.g., caffeine) or anti-inflammatory agents. Subjects will undergo a standard PSG, as described below, to rule out occult sleep disorders and provide an adaptation night. Subjects meeting ICSD Criteria(78) for a sleep disorder (e.g. sleep apnea) will be ruled out and referred for clinical care.

Randomization Plan. Subjects passing v3 will remain on the unit and be randomized to receive either morphine or placebo during both of their US and FA admissions (v4 & v5). The Research Pharmacy will generate the randomization list to maintain double-blinding. We will use a dynamic probabilities algorithm with minimization(79, 80) to balance groups on sex, obesity (BMI), and age, since these factors influence pain and inflammation. We will also balance on Chinese ethnicity, due to its association with morphine metabolism(81). To control for sequence effects, we will counterbalance US and FA conditions by randomizing subjects to 1 of 4 sequences: 1) Placebo/US first; 2) Placebo/FA first; 3) Morphine/US first and 4) Morphine/FA first. Subjects randomized to FA first, will remain inpatient for 3 consecutive nights [(v4) FA1, FA2, Recovery]. After two weeks washout, they will complete their US admission(v5). Subjects randomized to the US sequences first will complete the US admission [(v4) US1, US2] followed two weeks later by their FA admission [(v5) FA1, FA2, & Recovery].

Experimental Assessments.

General Inpatient CRU Procedures.To maintain experimental control, subjects will not leave the unit during each admission (v3-5) and will not be permitted to sleep outside their prescribed nocturnal schedule. Staff will continuously monitor, maintain, and regularly document wakefulness. Subjects will wear actigraphs at all times during v3-5 to provide an additional, objective procedural integrity check. Standardized meals and snacks will be provided by the CRU. Breakfast will be served at approximately 7:30 AM. Subjects will continue to complete sleep and pain diaries during v3-5 to provide indices of spontaneous pain and fatigue.

Questionnaires. Each inpatient evening, subjects will complete the **Stanford Sleepiness Scale**, to measure subjective changes in sleepiness(82) and the Positive Affect Negative Affect Schedule – Extended (83) to measure general affective dispositions and discreet emotions. At baseline only,

participants will complete a battery of questionnaires including the Temporal Experience of Pleasure Scale (84) to measure anticipatory and consummatory anhedonia, the Behavioral Inhibition/Behavioral Activation Scale (85) to measure approach-oriented motivation, the Trait Meta-Mood Scale (86) to assess attention to one's own mood, and the ability to regulate positive and negative moods, and the Monetary Choice Questionnaire to assess delay discounting (87). Research participants will also complete brief computerized cognitive (assessing attention, concentration, and memory) and motivation tasks following the experimental sleep manipulations.

Optional Sleep, Pain, Emotions, and Reward (SPEAR) Study Procedures

Participants will complete several tasks assessing perceptual (e.g., reaction time), emotional, and psychophysiological (e.g., facial electromyography; galvanic skin response) responses to the presentation of emotionally evocative images taken from the standardized and widely used bank of the International Affective Picture System (88). In addition, participants will complete tasks assessing their responses to the presentation of real and hypothetical monetary or food rewards, food reward questionnaires, and a task assessing psychomotor vigilance.

Participants will also undergo quantitative sensory testing (QST) procedures conducted in the parent project to assess responses to heat, pressure, and cold stimuli (e.g., pain threshold, tolerance, intensity). The heat and pressure pain threshold and cold pressor procedures are the same as those conducted in the Mechanisms of Sleep Disruption Hyperalgesia study, and are described in greater detail below. Additionally, heat stimuli will be presented simultaneously with cognitive, emotion and reward-related tasks.

Additionally, participants (N = 15) will have the option to participate in a PET imaging substudy, which will involve an MRI scan and two PET scans (one after each sleep condition).

MRI

A limited MRI of the brain (without contrast) will be obtained at JHOC for future anatomical co-registration with the brain PET scan. The MRI exam will take approximately 20-30 minutes. Incidental findings on MRI scans that are pathognomonic for an active disease or pathological process which requires medical intervention will be exclusionary. Other incidental findings, such as unidentified bright objects (UBOs), most venous vascular anomalies, benign cysts, heterotopic rests of neurons, and anatomical variants that are known to be prevalent in the general population of healthy people, will not be exclusionary. Unexpected findings of concern will be reviewed by a qualified neuroradiologist in consultation with the investigators to determine the subject's eligibility for participation in the study in light of these findings. If the MRI shows any clinically significant abnormalities, the participant will be notified.

PET Scans:

In the morning following the first night of each sleep condition (US and FA), subjects will be transported from the CRU at JHBMC to the Johns Hopkins PET Center for the scheduled PET scan.

Prior to the first PET scan, subjects will have a facemask constructed to facilitate maintaining the same position of the head for the PET scans. The facemask will be made prior to the first PET scan and will not require an additional visit. The facemask will take approximately 10 minutes to construct and it will be used during the scanning.

Before each PET scan a catheter will be inserted into a vein of one arm for the injection of the radiotracer.

After the catheter is placed the participant will be placed in the PET scanner. A 6 to 10 minute transmission (attenuation) scan will be performed before or after the PET scan. Participants will undergo a PET scan lasting approximately 90 minutes after the intravenous bolus injection of

approximately 20 mCi [^{11}C]raclopride. Dynamic PET data will be acquired during scanning. Continuous single lead ECG will be measured throughout the PET imaging. If during this period abnormal single ECGs are detected, 12-lead ECG will be recorded. After the completion of the PET scan, subjects will remain at Johns Hopkins under medical supervision. Subjects will be discharged if deemed safe to be discharged by the clinical investigator.

Nocturnal Polysomnography (PSG). PSGs will be conducted on all experimental nights (1-4) in private rooms. Lights out will occur at the subject's average bedtime per diary and actigraphy. Registered PSG technicians (RPSGT) will execute the PSG procedures according to standard technical guidelines(89). The recording montage uses the AASM(89) recommended placement for EEG, EOG, EMG and ECG. We will acquire electrophysiologic signals using an Embla N7000 polygraph. Respiratory function and effort will be measured via oronasal thermistor, nasal air pressure transducer, pulse oximetry, and abdominal and thoracic plethysmography belts. After Night1, we will abbreviate the montage for comfort, removing anterior tibialis EMGs and all respiratory sensors. Records will be scored according to AASM guidelines, by an RPSGT and reviewed by a physician, board certified in sleep medicine. Both will be blind to study hypotheses.

Experimental Sleep Conditions.

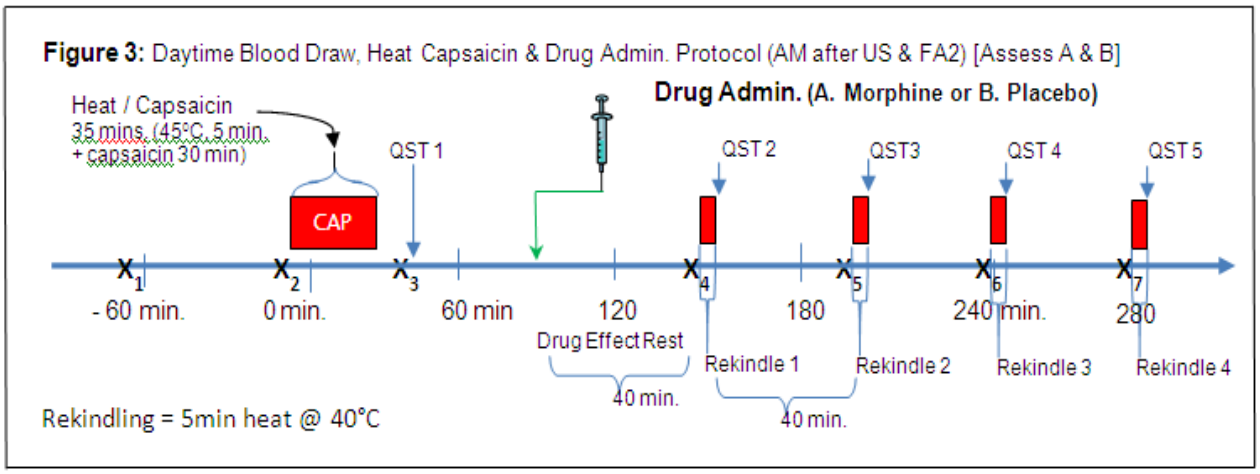
Forced Awakenings Condition.The FA condition will be conducted on two consecutive nights as previously described(34). An 8-hour sleep opportunity period starting from lights out is divided into eight, one-hour intervals. One of the intervals is randomly determined to be a 60 minute forced awakening, during which no sleep is permitted. Each of the remaining seven, 60 minute intervals are subdivided into tertiles (20 min. blocks). A forced, 20 min awakening is randomly scheduled to occur in either the 1st, 2nd, or 3rd tertile of each hour. During forced awakenings, staff keep subjects awake, either by voice or gentle shaking. Subjects sit up in bed, but lights remain off. Techs, monitoring PSG signals, inform the attendant if the subject starts to fall asleep. PSG monitoring is maintained for the entire sleep period. The maximum total sleep time possible, if subjects sleep 100% of the time when not forced awake (7, 20-min & 1, 60-min), is 280 minutes.

Uninterrupted Sleep Condition. An 8 hour period of undisturbed sleep is permitted.

Optional Sleep, Pain, Emotions, and Reward Study Procedures

A second night of undisturbed sleep will be added for participants who elect to participate in the optional study.

Blood Draws, Pain Testing & Drug Protocol. Figure 3 depicts the timing of blood draws, heat capsaicin procedures, quantitative sensory testing, and drug administration. This protocol will be implemented in the mornings after the US night and the second FA night (FA2). Drs. Smith and Campbell have experience conducting all of the pain testing procedures(90-93). Drs. Strain, Tompkins, and Umbricht have extensive expertise studying the effects of opioid agonists(94, 95). All pain testers complete a rigorous training process that includes inter-rater reliability testing (kappa>.80). Participants are permitted to terminate any of the procedures at any time. The following procedures will be conducted in an identical fashion on both visits. All assessments will be conducted by BSMP staff, who will be blind to subject conditions.



Blood Draws & Handling Procedures. Subjects will remain in a semi recumbent position during the procedure. Approximately 30 minutes after Lights On (i.e., wake), the nurse will insert a single port lumen peripheral catheter in an upper extremity vein, contralateral to the arm used for capsaicin testing. A continuous saline infusion at 250cc/h will be maintained. The line will be flushed with saline before and after all blood draws, and will remain installed until the final QST procedure is complete. As shown in Figure 7, seven blood draws will be obtained (3 pre-injection and 4 post-injection). Blood draws 1 and 2 will be obtained approximately 60 minutes apart, prior to the 35 minute heat capsaicin procedure, to provide a resting baseline. After the initial capsaicin procedure, all blood will be drawn prior to each rekindling. Blood will be immediately transported to the Rheumatology Disease Research Core for immediate preparation by the RDRC tech.

Heat-Capsaicin and Rekindling Procedures. We will utilize procedures similar to established protocols(96, 97). Heat will be delivered using a computer driven peltier heating element (Medoc TSA II). The procedure involves a 35 minute sensitization period. A 10.24 cm^2 treatment site, the size and shape of the thermode will be randomly assigned and marked on either a lower or upper, non overlapping surface of the ventral forearm opposite the arm from which blood is drawn. Lower and upper treatment sites will be counterbalanced across admissions. The thermode is secured on the skin with velcro straps and heated at 45°C for 5 minutes. Pain ratings will be collected once every minute on a 0-100 VAS ("no pain", "most extreme pain imaginable"). An open square, raised adhesive frame patch (internal dimensions same as thermode) is immediately applied to the borders of the treatment site and Capsaicin cream (0.1% Capsaicin)(90) is evenly spread onto the skin and permitted to absorb for 30 minutes. This induces moderate (Mean = 48.25$\pm$ 27.93), but well tolerated pain. The raised frame prevents spillage or leakage of capsaicin outside the treatment site. Capsaicin is removed, blood draw 3 is obtained, and QST1 is performed as described below. Following morphine/placebo injection and the subsequent 40 minute rest, the treated skin will be re-kindled a total of four times at 40 min intervals by heating the treatment site with the thermode at 40°C for 5 min. Following each rekindling episode, a series of QST procedures to quantify 1º and 2º Hyperalgesia will be conducted. Studies have demonstrated excellent test-retest stability in hyperalgesia measurements over 4 rekindlings(96, 97, 97). The timing associated with these procedures may vary as a function of experimental demands.

Quantitative Sensory Testing (QST) Procedures. All tests will be conducted in the order described.

Primary Hyperalgesia. Heat–Pain Threshold(HPTh) will be assessed using an ascending method of limits paradigm prior to application of heat capsaicin, after the 35 minute sensitization period and after each rekindling. The thermode is secured to the treatment site and the temperature gradually increased (rate of rise = .5ºC/sec) from a pre-set baseline (31ºC), until the subject indicates when

the stimulus "first feels painful" (threshold) by pressing a button, which stops and records the temperature(98). We will conduct two trials at each QST session, the average of each serves as the index of 1°HA(97, 99).

Secondary Hyperalgesia. The area of 2°HA to mechanical stimulation will be quantified with a standard von Frey hair by stimulating along eight linear paths around the treated site in steps of 5mm at 1 second intervals(96). Stimulation starts well outside the hyperalgesic area and continues towards the treated area until the subject reports a change in sensation. The border is marked on the skin with a pen and traced to acetate paper. The degree of 2°HA is quantified by calculating the surface area using a planimeter (Planix).

Mechanical Temporal Summation**.** Temporal summation of pain will be assessed using repetitive mechanical stimuli. The assessment of temporal summation involves rapidly applying a series of identical noxious stimuli and determining the increase in pain across trials; animal studies have suggested that temporal summation occurs centrally in second-order neurons in the spinal cord as a consequence of sustained C-fiber afferent input(100-102). For temporal summation of mechanical pain, pain ratings in response to a single punctate noxious stimulus will be compared to pain ratings in response to a sequence of identical punctuate noxious stimuli(103). A weighted pinprick stimulator with a flat contact area will be used to deliver, to the ventral surface of the arm, either a single pinprick stimulus or a train of 10 pinprick stimuli repeated at a constant rate. Following the single stimulus and the 10-stimulus train, the subject is asked to give a pain rating. Single pinprick stimuli are alternated with the trains of 10 stimuli. Similar procedures using this type of weighted pinprick stimulator have been used in diabetic neuropathy,(104) healthy subjects,(105) and to assess temporal summation(106, 107).

Pressure Pain Threshold. An electronic algometer (e.g., Somedic) will be used to assess responses to noxious mechanical pressure (108, 109). Pressure is increased steadily at a constant rate until the subject indicates that the stimulus "first feels painful".

Cold Pressor Pain ToleranceTesting (CPT). Subjects will immerse their capsaicin treated side hand in a cold water bath (4°C) for as long as possible, up to an un-informed 5 minute time limit. When the sensations become intolerable, subjects remove their hand and the duration of submersion is recorded as the index of CPT(110). We decided to include CPT as a primary outcome for Aim 2 because it is widely used in analgesia studies, and we have found it sensitive to sleep-related effects on alfentanil analgesia. It is highly unlikely that CPT will interfere with the heat capsaicin procedures as the pain-suppressive effects of such a task return to baseline within approximately five minutes(111).

We will perform some of the QST assessments simultaneously.

Optional Sleep, Pain, Emotions, and Reward Study Procedures

The QST procedures conducted as part of the optional SPEAR study include heat and pressure pain threshold, and CPT, as described above. Additionally, in a separate placebo analgesia test, participants will be presented with visual cues (e.g., different colored dots) prior to delivery of a thermal stimulus. Subjects will be conditioned to expect more or less pain associated with thermode activation based on the presentation of visual cues. The actual temperature of the thermode will vary. Subjects will be asked to rate pain intensity and unpleasantness on a 0-100 scale in response to the stimuli. Deception is necessary for the effective assessment of placebo analgesia, which requires that participants' expectations be manipulated to compare the difference in pain ratings at the same stimulus intensity under different conditions of expectation. Placebo analgesia is thought to be regulated by aspects of the reward system, including opioid and dopamine neurotransmission, and is therefore an ideal task to include in this substudy, which explicitly seeks to understand the range of behavioral reward system responses to sleep deprivation.

Participants will consent to "authorized deception" in order to protect their ability to make informed consent as much as possible without disclosing the exact moment in which the deception will occur, which would invalidate the placebo analgesia task. Participants will be debriefed following the completion of the study and informed in explicit terms that the task in which they were presented with different colored dots prior to thermal stimulation included thermal stimuli that did not, in fact, change in temperature. We will inform them that it was necessary to provide misleading information because we are interested in knowing if being sleep deprived alters the function of expectation in modulating the experience of pain. We will inform participants that this information will further refine our understanding of sleep, pain, and analgesia. Finally, we will give participants the option to withdraw their data after learning of the nature of deception.

Drug Protocol. Morphine procedures have been developed based on Dr. Strain's research and the literature(112). No food or non-clear liquids will be permitted for approx. 4 h. (clear liquids 2 h.) prior to injection. A CRU pharmacist will prepare the saline or morphine doses onsite. A registered nurse (blind to drug condition) will administer the drug via subcutaneous injection. For safety reasons, the registered nurse will be aware that the syringe could contain either saline or morphine.. Drs. Strain, Tompkins, or Umbricht will be available by pager to provide oversight.

Physiologic Monitoring and Safety. A nurse will monitor heart rate, blood pressure, SPO2, and respiration rate during the procedures. The CRU maintains an anaphylaxis kit, and supplemental oxygen. A standing order from Dr. Strain, Tompkins, or Umbricht for Naloxone will be available to fully reverse the effects of morphine in the case of an unlikely untoward event. An Ambu bag is also available to mechanically assist ventilation. Due to the effects of sleep deprivation and morphine after FA2, subjects will complete a third night of recovery sleep on the unit. To minimize burden after the US condition, subjects will be transported home and instructed not to engage in certain activities for the next 12 h (e.g., cooking, driving, alcohol).

Inflammatory Mechanisms. Given our preliminary data(113, 114), we will focus on inflammatory biology dynamics using a vertically integrated mechanistic approach to examine upstream signaling pathways, cellular production of proinflammatory cytokines, and circulating levels. Circulating levels and cellular markers of inflammation will be assessed by assaying plasma inflammatory biomarkers [(IL-6, TNF-α, IL-1ra (surrogate for IL1-β)] in duplicate by ELISA and lipopolysaccharide (LPS) stimulated monocyte production of IL-6 and TNF-α by flow cytometry(113). We will focus on this select set of markers based on our theoretical focus on inflammation, our preliminary data, evidence that these markers are associated with pain responses in animals(115), impaired morphine efficacy in mice[58], and sleep disturbance(114, 116, 117). Because IL1β detection can be unreliable in humans due to low concentrations, we will utlilize IL-1ra as a surrogate marker for IL-β. For this reason, IL-1ra will be a secondary marker. We will use ELISA methods because we have found that multiplex approaches are less sensitive for quantifying circulating cytokines(118). Inflammatory signaling will be measured by assay of activation of NF-κB using intranuclear staining and flow cytometric analyses(114). Molecular approaches will examine the impact of cytokines on target cell gene expression using highly sensitive RT-PCR quantitative analyses of cytokine-regulated gene products in PBMC. We developed and routinely perform these approaches at the Cousins Center for Psychoneuroimmunology at UCLA to capture the both effects of altered cytokine concentrations as well as changes in cellular sensitivity to cytokine activity.

In vivo cytokine levels. Plasma levels of proinflammatory cytokines from frozen samples will be quantified, using Quantikine High Sensitivity human IL-6, TNF-α, and IL-1RA immunoassay kits (R&D Systems, MN). IL-6 and TNF assays have comparable intra and inter assay coefficients

of variation of 4% and 10%, respectively. For IL1ra, the intra and inter assay coefficients are 2% and 4%, respectively.

Intracytoplasmic cytokine expression(113, 119). These assays focus on monocyte cell populations as they are the primary source of IL-1, IL-6 and TNF-α. For cell preparation and intracellular immunostaining of monocytes, we adapted the protocol of Nomura et al.(113). Briefly, heparinized blood is aliquoted and co-stimulated with LPS (0.1- 1 (g/ml) to activate monocytes. Monocytes are stained with anti-CD14-PerCP and either anti-TNF-PE or anti-IL-6-PE (BDIS). The percentage of cytokine-secreting (PE positive) cells among CD14-PerCP positive population is determined with counting of 12000 CD14+ events via flow cytometry.

Molecular Impact of Proinflammatory Cytokines. We have found that mRNA levels for HMG-1, IRF-4 and IRF-7 are sensitive to IL-1β, IL-6, and TNF-α signaling, and are also known to increase significantly during in vivo inflammation, thus, serving as sensitive indicators of proinflammatory cytokine activity even if cytokine protein levels remain below the limits of detection in plasma. To assess changes in indicator gene expression, total RNA samples will be extracted from 10 mL of PBMC (Qiagen PAXgene Blood RNA tubes with RNEasy extraction) and assayed using the real-time RT-PCR services of the IBC. In this process, total RNA is reverse transcribed at 50ºC for 30 minutes using target-specific primers (Applied Biosystems Inc. TaqMan Gene Expression Assays) in conjunction with the Qiagen 1-step RT-PCR enzyme mix and a BioRad iCycler real-time PCR thermal cycler. Following reverse transcription, samples are heated to 95ºC for 15 minutes to denature the reverse transcriptase and liberate hot-start DNA polymerase, which then amplifies reverse transcripts over 40 cycles of universal real-time PCR cycling conditions (15s at 95ºC, 60s at 60ºC). Fluorescent probe signals resulting from DNA amplification are quantified by iCycler software and subsequently normalized to GAPDH and β-actin housekeeping genes to control for total number of cells assayed. We will use the same approach to assess mRNA levels IL-1β, TNF-α, IL-6 and their receptors (IL-1R, TNFRII, IL6R, gp160).

Nuclear Signaling of Proinflammatory Cytokines(114). We have found that sleep restriction induces activation of a number of transcription factor families including the pro-inflammatory NF-κBκ/Rel 235 family. This transcription family has been implicated as critical regulators of gene expression in the setting of inflammation. NF-κB is clearly one of the most important regulators of pro-inflammatory gene expression, and activation of this cellular inflammatory signaling will be measured as previously described(120). Briefly, nuclear extracts will prepared from isolated PBMC and binding of activated NF-κB p65 to its consensus DNA sequence will measured using TransAM NF-κB p65 ELISA (Active Motif, Carlsbad, CA). In addition, intranuclear signaling will be evaluated by flow cytometry for evaluation of resting (i.e., constitutive) and stimulated levels. PBMC will be left unstimulated or stimulated for 15 minutes at 37ºC with10 ng/ml TNFα for induction of NF-κB. PBMC will be fixed, permeabilized for nuclear staining, and then incubated with phycoerythrin-labeled monoclonal antibodies specific for the active/phosphorylated forms of NF-κB (p65). Constitutive levels of signaling are determined by mean fluorescence intensity (MFI) in unstimulated cells, and the ability of cells to initiate new signaling is determined by comparing MFI in stimulated vs. unstimulated PBMC.

Maintaining Integrity of Assays Between Sites. Using protocols developed by Dr. Irwin, the Hopkins RDRC, will receive fresh blood samples from the CRC, prepare them according to procedures briefly described above, freeze at -80ºC and ship to UCLA for analysis. This includes: 1) plasma processing; 2) stimulation of whole blood with LPS and fixation for intracytoplasmic cytokine expression protocol; 3) preparation of PBMC pellets for isolation of DNA/RNA, and 4) PBMC isolation, stimulation with TNF- α, and fixation for intranuclear signaling protocol. To ensure fidelity to protocol, Dr. Irwin will travel to Hopkins twice in Year 1 to work with Dr. Soloski to train the dedicated RDRC tech. As part of this process, test samples will be performed. All of these protocols were developed for the purpose of freezing plasma and cells for future analyses, and

include fixation where appropriate to preserve cellular integrity. The protocols are routinely performed in Dr. Irwin's laboratory, with frozen samples stored for weeks (cellular assays) to months (plasma, DNA/RNA pellets) with excellent results. All samples will be coded and de-identified to maintain blinding.

Feasibility and Attrition: Since our team has previously executed a similar sleep deprivation study, tested opioid protocols, and routinely used quantitative sensory testing, we are confident that we can successfully execute the proposed study. In order to maximize retention, our three visit screening process and rigorous exclusion criteria minimizes attrition after randomization, ensures safety, and minimizes confounds. In our previous sleep deprivation study[34], 20% ruled out at screening v1; 10%, at v2, and 5% at v3 (PSG screen). Once randomized, however, **only 11% of subjects dropped out** of the 7 night protocol. The proposed 5 night protocol reduces subject burden and may reduce drop-outs. Using these numbers, we plan to conduct: 139 v1s, 111 v2s and 100 v3s. We plan to **randomize N= 94** and estimate 15% dropout after randomization to achieve **80 full completers.** To maximize retention, subjects will be remunerated up to $1,050 for their effort ($25 v1, $25 v2, $150 the PSG screen, $300 for US Condition, $350 for FA condition, and $200 bonus). We have included a $200 bonus to incent subjects to return and complete the second admission. In our prior study, we recruited 32 female full completers and an additional 12 males in 2 years and found no differential drop out between sleep deprivation and control groups. We are, therefore, confident that our plans to obtain **80 full completers** in 5 years are feasible.

Optional Sleep, Pain, Emotions, and Reward Study Procedures
Participants who are eligible and elect to participate in the SPEAR study will be compensated an additional $300 for daytime testing procedures, including MRI and PET scanning. They may earn up to an additional $23.50 based on performance on the motivation and reward tasks (participants will not be informed of the total amount they can earn in these tasks in order to minimize expectancy effects; however, they will be informed that they can earn some additional money based on their performance on the tasks).

b. Study duration and number of study visits required of research participants.

   The study will last approximately 5 weeks and involves a total of four visits.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

   This is not a clinical trial. We have adopted a randomized placebo control experimental design, however, which requires the subjects and the pain testing technicians to be blind to the subject's group assignment to minimize expectancy effects and technician bias that could comprise the causal conclusions from being drawn. A CRU pharmacist will prepare the saline or morphine doses onsite. A registered nurse (blind to drug condition) will administer the drug. For safety reasons, the registered nurse will be aware that the syringe could contain either saline or morphine.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

   N/A

e. Justification for inclusion of a placebo or non-treatment group.

Although this is not a clinical trial, the causal nature of the research questions necessitate that we control for placebo/expectancy effects. If subjects were unblinded, it would be difficult to draw causal conclusions about whether sleep deprivation alters the effect of opioid efficacy because the results would be biased by the subject's expectancy that pain ratings should diminish when given an opioid. Similarly, if technicians are aware whether a patient is receiving morphine, they may inadvertently convey information about how they expect the subject to respond. This would compromise validity.

f. Definition of treatment failure or participant removal criteria.

Participants failing to continue to meet any of the inclusion/exclusion criteria will be removed from the study and an appropriate clinical referral provided. For example, subjects developing a psychiatric disorder or symptoms requiring treatment (e.g., mania, suicidality, and psychosis), major medical illnesses or serious injuries, which would preclude further participation, will be withdrawn. Subjects who have severe side effects to morphine will be withdrawn. Subjects who consistently fail to show up for screening sessions will be withdrawn. Subjects who refuse the sleep deprivation procedures may be withdrawn.

5. Inclusion/Exclusion Criteria

GENERAL INCLUSION CRITERIA FOR ALL PARTICIPANTS:

1) Healthy, 18-48 year olds meeting Research Diagnostic Criteria for Normal Sleepers(121); 2) a stable sleep phase within 21:00 and 10:00; 3) total sleep time >6.5 and ≤8.5 hours/night; sleep efficiency ≥85%, Epworth Sleepiness Scale <10; 4) Pittsburgh Sleep Quality Index <5); 5) non-smokers/nicotine users; 6) low caffeine users (≤ 2 cups, q.d.).

PET Inclusion Criteria

1) Have clinical laboratory test results within the reference ranges for the population or results within acceptable deviations that are not considered by the investigator to be clinically significant.
2) All subjects and their partners of childbearing potential must commit to use two methods of contraception, one of which must be a barrier method, from the time of screening and throughout the study and until follow-up.
3) Have sufficient venous access.

GENERAL EXCLUSION CRITERIA FOR ALL PARTICIPANTS:

1) BMI ≥35; 2) lifetime history of chronic pain (>6 months); 3) acute pain; 4) significant medical or psychiatric morbidity within 6 months or lifetime history of: bipolar disorder, psychotic disorder, serious, recurrent major depression, serious posttraumatic stress disorder or seizure disorder; 5) respiratory, hepatic, renal or cardiac conditions that would contraindicate opioid use; 6) lifetime history of alcohol or substance abuse or dependence; 7) lifetime history of opioid use > 36 doses or > 7 days consecutive use; 8) prior adverse reaction to general anesthetics/opioids or capsaicin; 9) clinically significant abnormal complete blood count or comprehensive metabolic profile; 10) positive toxicology screen for opioids or recreational drugs; 11) pregnant or lactating women; 12)

Table 1. Cumulative Enrollment & Scientific Activities

Year	Q1	Q2	Q 3	Q4
1st	Start up	Startup	Active N=5	N=10
2nd	N=15	N=21	N=27	N=33
3rd	N=39	N=45	N=51	N=57
4th	N=63	N=69	N=75	N=81
5th	N=87	N=94	Analysis Manuscripts	Analysis Manuscripts

significant preadmission psychological distress (T-scores >64 on the Brief Symptom Inventory global scales); 13) significant lifetime history of serious head injury that is judged to influence pain processing or sleep systems. These rigorous criteria are designed to ensure safety and minimize major confounds influencing pain, inflammation(122), sleep, or opioid efficacy. We are confident of feasibility based on our prior work with similar criteria(34, 123). See enrollment plan, Table 1.

PET Exclusion Criteria

1) Have participated in other research protocols in the last year such that radiation exposure would exceed the annual limits.
2) Pregnant or nursing women.
3) History of head trauma with prolonged loss of consciousness (>10 minutes) or any neurological condition including stroke or seizure (excluding childhood febrile seizure) or history of migraine headache.
4) History of any clinically relevant hematological, hepatic, respiratory, cardiovascular, renal or CNS disease or other medical condition that is capable of constituting a risk factor when taking the study drug.
5) Abnormal vital signs, ECG or clinical laboratory evaluations which are considered clinically significant by the clinical investigator.
6) Suffer from claustrophobia and would be unable to undergo MRI or PET scanning.
7) Clinically significant abnormal MRI.
8) Subject has implanted or embedded metal objects, prostheses, or fragments in the head or body that would present a risk during the MRI scanning procedure, or have worked with ferrous metals either as a vocation or hobby (for example, as a sheet metal worker, welder, or machinist) in such a way that might have led to unknown, indwelling metal fragments that could cause injury if they moved in response to placement in the magnetic field.

Rationale for Eligibility Criteria. The normal sleeper profile is typically used in insomnia research (e.g.,(124) and must be evident both at intake (retrospective) and as an average profile (2-weeks of baseline diaries and actigraphy)). The RDC criteria for normal sleep rules out individuals meeting criteria for sleep disorders, including sleep apnea and periodic limb movement disorder, etc. The Visit 3 PSG screening will be used in the diagnostic process to rule individuals with occult sleep disorders based on standard ICSD criteria using objective PSG derived indices of sleep disordered breathing (respiratory disturbance index <15 and apnea/hypopnea index <10), periodic limb movements, etc. Because we are seeking to experimentally determine the effects of sleep deprivation on hyperalgesia, individuals with sleep disorders are precluded from participation as this would confound the findings.

Adults over 48 are excluded because of age-associated reductions in slow wave sleep(125), increased risk of sleep disorders, and comorbid medical factors might confound results. Reduced slow wave sleep has been shown to influence pain sensitivity(126).

Severely obese individuals with BMIs above 35 are excluded because they are at high risk for exclusion, due to sleep apnea and other co-morbidities (127).

Seizure disorder, bipolar disorder, and psychotic disorders are ruled out because sleep deprivation may exacerbate these conditions. Similarly, we require subjects to report psychological distress within normal limits because sleep deprivation is a stressor, which could unduly impact an individual with very high levels of distress. Individuals with a significant psychiatric disorder within the past 6 months are excluded because these conditions can be associated with prolonged sleep architecture changes even with symptom remission.

Respiratory, hepatic, renal, or cardiac problems, which would either significantly alter morphine metabolism or which could be detrimentally impacted by morphine effects are exclusions. Morphine lowers respiratory drive and therefore can pose significant hazard to individuals with conditions such as asthma.

Individuals with alcohol and substance abuse disorders are excluded because opioids have significant abuse potential. We are studying relatively opioid naïve subjects because opioid exposure and long-term use can alter opioid receptor sensitivity and regular use can lead to tolerance.

Restrictions on nicotine and heavy caffeine usage are required due to known effects on pain testing and/or inflammation. Heavier caffeine usage can cause withdrawal symptoms when discontinued, which could confound hyperalgesia testing. These criteria are commonly used in the pain testing literature and we have not found them to pose a recruitment problem(93).

A normal CBC is required because infections, etc can significantly alter sleep and sleep need, which might skew results.

PET-related eligibility criteria is designed to minimize risks associated with MRI and PET imaging, as well as to ensure that the brain-based comparisons across subjects are not influenced by abnormal neurological profiles.

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

Selection of Capsaicin, Dose, and Administration. Capsaicin was chosen because its application in the context of a continuous thermal probe provides a unique opportunity to measure both primary and secondary hyperalgesia in a manner that cannot be as effectively accomplished without its application. The coupling of heat with capsaicin permits the study of primary and secondary hyperalgesia without causing tissue damage. Further, it uniquely affords the opportunity for multiple rekindlings of thermal pain over time, which is a crucial element of the experimental design. The dose chosen (0.1% Capsaicin) has been previously validated (85) and safely used in other studies from our laboratory.

Selection of Morphine, Dose (.08 mg/kg), Blinding, and Administration. We considered a variety of opioid agonists, doses, and routes of administration. We selected morphine for several reasons: 1) it is the prototypical mu opioid receptor agonist without clear sex differences in efficacy(81, 128); 2) prior animal studies of cytokines blocking opioid analgesia used morphine(57, 58, 129, 130) and 3) morphine is efficacious in reducing 1^o and 2^o HA in the heat capsaicin model(131). We selected the .08 mg/kg dose, which is lower than some controlled studies(132), to minimize possible side-effects, which might increase drop out and because several studies demonstrate efficacy of a single .08 mg/kg dose on a wide range of laboratory pain tests, including CPT(92, 133). In consultation with the CRC, we decided to administer the drug via subcutaneous injection.

Selection of radioligand for PET imaging. The current protocol employs an IND for [^{11}C]raclopride (IND # 32,914). The sponsor- investigator, Dr. Dean Wong, has had the raclopride IND since 1989, and the IND was more recently amended to include AMP. [^{11}C]raclopride is now widely employed throughout the world as a radiotracer for measuring dopamine D_2 receptors with high specific activity (radiotracer alone) and low specific activity (added non-radioactive mass saturates some of the receptors). More recently, however, [^{11}C]raclopride has been used to measure intrasynaptic dopamine release following various perturbations; specifically, high specific activity raclopride radiolabels D_2 receptors, but can be competitively displaced by endogenous intrasynaptic dopamine. Dopamine release into the synapse, in turn, can be modulated by psychological challenges such as

cocaine craving cues, video games, etc (Drobes and Tiffany, 1997; Wong et al, 2006). Thus, [^{11}C]raclopride provides a unique tool for measuring not only D_2 receptors (high and low specific activity), but also for measuring the intrasynaptic dopamine tone (high specific activity alone).

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.
N/A

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.
N/A

7. Study Statistics

a. Primary outcome variables.

Selection of Independent Variables and Primary Outcomes. The primary independent variable in all analyses is the within-subject comparison of SD versus US. We will also evaluate whether sleepiness (SSS), fatigue (diary), positive and negative affect (PANAS-X) and spontaneous pain (diary) are associated with outcomes and include these variables as covariates in secondary analyses when indicated. Primary outcomes were chosen based on evidence from our prior work and the literature, described above. A summary of primary outcomes by aim is presented in Table 2.

Table 2: Summary of Outcome Measures By Aim

AIM	CONSTRUCT	MEASURE
Aim 1-3	1°HA♦ peripheral sensitization 2°HA♦ spinal sensitization	1°HA-Heat pain threshold (°C), treated skin 2°HA-Surface Area (cm^2), mech. HA untreated skin
Aim 2 & 3	Pain Tolerance	Duration (sec) of hand submersion in 4°C Water
Aim 3	Inflammation - Systemic - Cellular - Genomic - Genomic Signaling	 Circulating (plasma) IL-6, TNF-α , IL-1RA** Stimulated & Unstim. PBMC levels of IL-6 & TNF PBMC mRNA conc. of IL-6**, TNF-α**, IL-1β** Stimulated & Unstim. PBMC levels of NF-κB

♦ Heat Capsaicin Testing; ** secondary outcomes

b. Statistical plan including sample size justification and interim data analysis.

Analytic Plan ***Hypothesis 1.a.(Sleep disruption enhances HA):*** We will use multilevel longitudinal mixed effects models to test the null hypothesis that baseline and sleep-deprived indices of *1° & 2°* hyperalgesia are equal. We will also test for differences in linear time trends by condition.

Hypothesis 2.a. (Sleep disruption diminishes morphine analgesia): Similar models will test the null hypothesis of no effect of sleep deprivation on the analgesic effects of morphine on hyperalgesia and cold pain tolerance. We will also test for differences in linear time trends.

***Hypothesis 3.a. (Sleep disruption increases activation of markers of inflammation)*:** We will first compute area-under-the-curve-ground (AUC-G) and -increase (AUC-I) values following standard formulas(134) for the inflammatory markers. AUC allows us to simplify the analysis of the repeated measures data to increase power(134). AUC-G represents the total amount of inflammation during the pre-drug administration segment (blood draws1-4), whereas AUC-I represents the level of increase in inflammation in response to heat capsaicin (blood draws 3-4), relative to the within-condition baseline levels (blood draws 1 & 2). Mixed effect models will test the difference in marker AUCs between sleep conditions.

Hypothesis 3.b. (Markers of inflammation mediate the effects of sleep disruption on: 1) HA and 2) diminished opioid analgesia): Following the MacArthur approach(135) we will first establish relationships between the markers and both hyperalgesia (HA) and sleep disruption (SD). Thus, these analyses will only be conducted if tests of Hypothesis 3.a. indicate a relationship between SD and activity of a specific marker. For these markers, we will then test the relationship between marker activity and HA using mixed effects models. When significant, interactions between SD and marker activity will be taken as evidence of *mediation* rather than *moderation,* as should a main effect of marker activity after controlling for this interaction and the main effects of SD(135).These analyses use only data from the pre-drug administration segment ;therefore, the placebo and opioid groups are combined. Mixed effects models will test the relationship between marker activity and diminished opioid analgesia, allowing for correlation within individuals across sleep conditions and pre- and post-drug segments. Statistical significance of the main effect of marker activity, the interaction between drug condition and marker activity, or the interaction between drug, marker, and SD will be taken as evidence of a mediating effect of marker activity.

Power Analysis & Sample Size. We calculated sample sizes via Monte Carlo simulation(136, 137). Continuous variables were standardized to express regression coefficients as effect sizes. Power for a given sample and effect size was calculated as the percentage of datasets (1000 simulations) for which either: 1) the p-value for a wald test or 2), the p-value for a likelihood ratio test for nested models was lower than the alpha specified for the model. Based on the simulations described below**, we determined that a sample size of N=80 will be adequate to accomplish all of our Aims.**

Aim 1: (Sleep disruption enhances HA). To account for correlation between measurements within individuals across sleep conditions, a parallel process model was parameterized for MPLUS(136, 137) with the baseline mean outcome set to 0 and the SD mean outcome set to a range of values representing various effect sizes. Linear trends within sleep condition are not anticipated(96, 97), so slopes (and variance of slopes) were simulated as 0. With an N=80, we estimate 80% or greater power for effect sizes of 0.40 or greater. In our preliminary heat-capsaicin pilot (see section 4.A.1.), poor sleep was associated with an effect size of 0.57 on 2^{o}HA. This correlational data, in a naturalistic sample of healthy subjects, is likely to underestimate the effect of our sleep disruption condition on HA.

Aim 2: (Sleep disruption diminishes morphine analgesia). Similar to Aim 1, a parallel process model (this time with 4 processes, one per segment and sleep condition) was parameterized for MPLUS. As above, all slopes and random slope variances were simulated as 0. The difference in drug effect as a function of sleep condition was based on Steinmiller's study of the effect of codeine on daytime sleepiness (effect = 0.9)(138) and our pilot data showing the effects of poor sleep on alfentanil vs. placebo (see 4.A.2; effect size = 1.9). Since the hypothesis test involves 4 separate parameters, power was calculated via a likelihood ratio test with 1 degree of freedom comparing an unconstrained model to one in which the parameter function was constrained to be 0. Simulations showed that with an N=80, we have 80% power for effects of 0.7 or greater. Therefore, we are sufficiently powered to detect similar effects reported in the literature and in our pilot data.

Aim 3: (Sleep disruption increases activation of markers of inflammation). Because several inflammatory markers are being investigated, we will employ a more conservative alpha value of 0.01. This simulation was parameterized similar to Aim 1, but with main effects of both marker activity and sleep deprivation and their interaction and with a single mean outcome for each sleep condition. With N=80, we have 80% power to detect main effect sizes for marker activity of $\geq$0.56. Based on our prior work (see section 4.A.4), with the exception of the mRNA markers (effects =.2 to .36), the effects for 1 night of sleep restriction on all other markers range from .56 (cellular

production of IL-6/TNF) to .76 (NF-κB). Because our sleep manipulation is much more robust (two nights of FA), we expect to be well powered to detect effects on all inflammatory markers. To be conservative however, and to further reduce multiple comparison error, we will consider the mRNA markers as secondary outcomes.

c. Early stopping rules.

We will report all adverse events and evaluate on a case-by-case basis whether the occurrence of a particular adverse event has implications for discontinuing the study to ensure participant safety and well-being.

8. Risks

a. Medical risks listing all procedures, their major and minor risks, and expected frequency.

Listed below are all major procedures and associated risks. All risks are determined to be minimal. Please refer to the Method section for detail. All of these standardized procedures have been conducted with participants both in earlier studies from our laboratory, and in other laboratories around the world.

Administration of Morphine (.08 mg/kg). Morphine is an opioid receptor agonist, which has high affinity for mu receptors, and is widely used in the treatment of moderate to severe pain. Previous literature has demonstrated the effects and side effect profile of the current route and dose of morphine(133). We are using a routine dose based on body weight that is lower than is commonly use in emergency departments to treat acute musculoskeletal pain(139). This substantially minimizes possibility of toxicity and reduces potential side effects.

Major risk factors of morphine toxicity are respiratory depression (diminished respiratory drive), and possible death. Additional serious side effects are anaphylaxis, seizures, falling or unsteadiness, confusion, unusual changes in mood or behavior, slow heart rate (bradycardia), difficulty passing urine, hypotension, severe constipation, slowed cognition, and psychomotor impairment.

Minor risks and the most common side effects include: nausea, vomiting, lightheadedness, dizziness, euphoria, sweating, constipation, sleepiness, and pruritis, and mild allergic reaction(s) (i.e., rash, hives, itching, wheezing).

Sleep Disruption/Deprivation: The most common risks associated with sleep deprivation include: sleepiness, poor concentration, slowed reaction times, irritability, negative mood, and a diminished capacity to operate a motor vehicle or perform work. Sleep deprivation lowers seizure threshold, and rarely, may trigger manic episodes in individuals with bipolar disorder.

Topical Capsaicin: Capsaicin is the pungent ingredient of hot chili peppers and has been contacted and ingested by the vast majority of individuals. In some cultures it is consumed in large quantities without serious risk. Our group has tested well over 100 subjects using topical capsaicin at this dose, combined with heating the skin at the temperatures proposed and with some subjects undergoing administration of capsaicin 4 times over the course of a protocol. During these hundreds of administrations, there have been no serious adverse events. The most common adverse reactions are application site erythema and pain, which is usually mild to moderate. Pruritus is also common. Less frequently, temporary papules may develop. Intense pain can be experienced if capsaicin

comes into contact with mucous membranes or compromised skin (open sores/wounds). Mild sensitivity to warm/hot temperate at the site of application may persist for a couple days.

Heat-Pain Testing: There is an extremely small chance that the heating device might produce a burn, but the risks associated with the relatively small amount of heat applied to the skin (maximum of 122 degrees Fahrenheit or 50 degrees Celsius) are very low. The Medoc device has safety features built into both the hardware and software. The risks associated with use of this equipment are less than those associated with common household appliances. We and others across the world have previously used the proposed laboratory pain procedures in a number of previous studies with healthy adults and chronic pain patients. No serious adverse effects were reported.

Mechanical Temporal Summation: There is a very slight chance that the probe might superficially puncture the skin for individuals with "thin skin."

Pressure Pain Threshold: There is a very slight chance of mild transient bruising associated with the use of an algometer (< 5% of cases).

Cold Pain Tolerance Task**:** There are no significant risks from immersion of a hand in cold water by a healthy individual for the 5 minute maximum time permitted. The task could exacerbate Reynaud's syndrome, therefore these patients are excluded.

Intravenous Catheterization/Blood Draws: Some discomfort may be associated with the drawing of blood samples, and there is a very small chance that phlebotomy may cause hematoma, infection, anemia, and/or minor pain at the puncture site. However, these procedures are routinely performed without incident in medical settings, and catheters will be installed by qualified nursing staff.

Subcutaneous Injection: Some pain or discomfort around the injection site may be associated with the injection of drug or placebo.

Saline Placebo: There are no risks associated with saline itself. Minimal risks associated with subcutaneous injection are described above. Injections will be administered by qualified nursing staff.

Polysomnography (Sleep Monitoring): There are minimal risks associated with routine polysomnography testing. Very rarely, individual may experience a mild allergic reaction to electrode gels/paste.

Physical Exam and Structure Diagnostic Interviewing: There are no known or reported medical risks associated with undergoing a physical exam or the proposed diagnostic interviews performed by a qualified professional.

Urine Sample: There are no risks to collecting a sample of urine. Strict confidentiality of the sample & results will be maintained.

Actigraphy: There are no risks associated with wearing a wrist actigraph.

Questionnaires: Minimal risks associated with completing the questionnaires are subject fatigue and the possibility of minor psychological distress associated with answering sensitive questions regarding psychological functioning.

Optional Sleep, Pain, Emotions, and Reward Study Procedures

Emotion-Related Tasks: Some of the images presented during the emotion-related tasks are graphic, including images of violence, mutilation, death, and erotica. The graphic nature of these images is similar to what is permitted in R-Rated movies. It is possible that some participants may be distressed after being exposed to these images. However, the risks associated with these images are expected to be minimal based on the fact that they have been standardized on thousands of individuals and included in hundreds of research studies conducted with similar populations.

Electromyography and Galvanic Skin Response Monitoring: There are minimal risks associated with routine electromyography and galvanic skin response testing. Very rarely, the individual may experience a mild allergic reaction to electrode gels/paste.

Motivation, Reward-Related, and Psychomotor Vigilance Tasks: There is a minimal risk that participants may experience fatigue and/or boredom while completing some of these tasks.

Additional Pain Testing: See risks described above under Heat Pain Testing, Pressure Pain Threshold, and Cold Pain Tolerance Task.

Placebo Analgesia: The primary risk associated with this task pertains to deception. Research deception may cause:

1. Infringement of the autonomy of research subjects
2. Distress and lack of trust in research
3. Negative emotional reactions

MRI: MRI does not involve exposure to ionizing radiation and has no known serious risks. Individuals with pacemakers, aneurysm clips, shrapnel or other unallowed implanted metallic devices will be excluded from study. All subjects complete the standard MRI screening questionnaire prior to MRI study. Participants may experience claustrophobia before entering the MRI machine and during the performance of the MRI while lying in the MRI machine. If participants are markedly bothered by claustrophobia, they will be eliminated from the study. Participants may experience fear and discomfort during the MRI due to the noise of the machine. To decrease the noise of the MRI, participants will be asked to insert ear plugs before entering the MRI.

Skull x-ray. As indicated in the MRI procedures, participants may be asked to undergo two skull x-rays to determine the participant has not metal in their skull. This is a standard safety procedure employed by the Johns Hopkins MRI staff to verify it is safe for the participant to enter the MRI area. If participants undergo the skull x-ray there will be a radiation exposure of 0.01 rem to the participant.

Radiation: As [^{11}C]raclopride is a radiotracer, participants will be exposed to radiation. The amount of radiation exposure to a participant in this protocol will be approximately 0.94 rem. This total radiation exposure is within allowable radiation exposure limits (not to exceed 5 rem per year).

Radiotracer: [^{11}C]Raclopride will be administered during the two scans with high specific activity [^{11}C]Raclopride. The risks of adverse pharmacological events are negligible. Less than 15µg will be administered for each scan. The risks associated with much higher levels (therapeutic) may include transient sleepiness, restlessness, mild muscular spasms or dystonias (more severe but still

transient muscular spasms). The administered dose is lower than the usual therapeutic dose which should make the risk of getting any of the above mentioned side effects less likely. Should any of these side effects occur they are easily treatable and reversible. Tardive dyskinesia, a sometimes irreversible syndrome involving involuntary movements of the face, mouth, or other parts of the body, is an uncommon side effect typically seen in patients taking Raclopride or similar drugs for a prolonged period of time. At all times, participants will be monitored by a physician who will take appropriate measures to remedy these rare effects.

PET scanner. Participants may experience discomfort while lying on the PET scanner table for the scans. Participants will be repositioned and cushioned with padding if they feel discomfort.

Thermoplastic facemask. The thermoplastic facemask may cause the participant some discomfort. If the mask is too tight, we will remold it or reposition the participant. They may experience some skin irritation when the facemask is created.

b. Steps taken to minimize the risks.

All personnel involved in study procedures will be fully trained in the protection of human subject as required by our IRBs. If information gathered during either study indicates that an individual is in need of emergent psychiatric care (e.g., expressed suicidality). Drs. Smith, Buenaver, and Campbell are licensed psychologists, and one of the three will immediately assess the needs of the participant who will be referred for psychiatric care at Johns Hopkins or an appropriate community referral. During any phase of the study, subjects who report psychological distress related to their participation will be provided a referral to a mental health professional who will provide a one-session consultation. Described below are specific protections against risks by procedure.

Administration of Morphine (.08 mg/kg). As described, individuals with respiratory (e.g., asthma), hepatic, renal, cardiac, or other medical conditions that would contraindicate morphine administration will be excluded. Blood tests verifying liver and kidney function will be conducted and reviewed by one of the study physicians (Dr. Strain,Tompkins or Umbricht) prior to implementing the procedure. Subjects with a history of allergic reaction to opioids or anesthesia are excluded. We are also excluding individuals with alcohol and substance abuse disorders because opioids have significant abuse potential. An appropriately licensed CRU pharmacist will prepare the morphine doses onsite. A registered nurse will administer the drug as prescribed by Dr. Strain, Tompkins, or Umbricht. For safety reasons, the registered nurse will be aware that the syringe could contain either saline or morphine. The subject will remain in a recumbent position during the procedure to prevent falls. Drs. Strain, Tompkins, or Umbricht will be available by pager to provide any medical oversight as needed. A nurse will monitor heart rate, blood pressure, SPO2, and respiration rate during the procedures. The CRU maintains an anaplylaxis treatment kit and a supplemental oxygen tank. Sufficient naloxone will be available onhand. An Ambu, CPR bag is also available to facilitate mechanical ventilation if needed. The CRC also has an AED. The protocol will include a standing naloxone order signed by Dr. Strain, Tompkins, or Umbricht sufficient to fully reverse the adverse effects of morphine should a subject experience respiratory distress or other morphine-related complications. Nurses will be trained under the direction of Drs. Strain and Tompkins. The CRC has established a standard emergency protcol to ensure the safety of subjects undergoing opioid administration.We are using a routine dose of morphine based on body weight that is typically lower than is commonly use in emergency departments to treat acute musculoskeletal pain(139). This substantially minimizes possibility of toxicity and reduces potential side effects. Drs. Strain and Tompkins are both very experienced physicians who specialize in and

routinely care for patients with opioid dependence. They have considerable expertise with clinical and research protocols involving opioid administration and naloxone/naltrexone blocade. Dr. Umbricht is also very experienced in these domains and will be available as study physician in the event that both Drs. Strain and Tompkins are unavailable. Subjects receiving morphine under sleep deprivation conditions will be required to remain on the Unit for a recovery period (overnight) prior to disharge to prevent accidents due to impairment caused by morphine plus sleep deprivation. After the undisturbed sleep condition, subjects will return home the same day, but will not be permitted to drive and instructed not to engage in potentially hazardous activities for the next 12 hours, e.g., cooking, driving, operating heavy machinery, or drinking alcohol or other depressants.

The nursing staff is well trained to assist subjects experiencing side effects such as nausea and emesis. As described, food and drink is limited and restricted prior to injection to minimize these side effects. Subjects will complete the 6-item drug effect VAS and an adjective checklist (36 items), after injection to gather data on frequency and severity of side effects. Dr. Strain has used these measures in his opioid pharmacology work to study side-effects of opioid medications. This will be reported regularly to the IRB and our data safety and monitoring committee for review. These measures were specifically designed to capture common and uncommon side effects of morphine and opioid medications.

Sleep Disruption/Deprivation: Because sleep deprivation lowers seizure threshold and may trigger manic episodes in individuals with bipolar disorder, individuals with these conditions are excluded as described. All subjects undergoing sleep deprivation will remain under surveillance on the medical research unit for a recovery night sleep to prevent accidents that might be caused by drowsiness. Because sleep deprivation is a stressor that can impact mood, we have excluded subjects with major psychopathology or acute psychological distress.

Topical Capsaicin: Prior to capsaicin application, we will ensure that the skin is not compromised at the site of administration. Participants are instructed that they may discontinue any of the pain procedures at any time should they become too intense or unpleasant. Ice will be available for application, which dramatically reverses the heat-capsaicin pain if subjects wish to discontinue. Further, when the protocol is finished, we will cover the application area with a large band-aid and give participants several additional band aids so they can keep the area covered in order to avoid accidentally rubbing it on any other bodily areas that could be potentially painful (e.g., rubbing their eye(s), mucous membranes).

Heat-Pain Testing: All pain testing equipment will be tested prior to use to ensure it is functioning properly. Johns Hopkins IRB requires that all devices are approved by bioengineers for safety and proper functioning before approval of any research protocol. Thermodes and devices touching participant's skin are sterilized before and after use with an appropriate agent. The thermal sensory analyzer has built-in safety features to prevent burning. The temperature to be used will not cause burn. For all of the standardized laboratory pain procedures, participants are repeatedly informed that they may terminate any of the procedures at any time, and participants are monitored continuously by the technician. In the event that a subject is injured in any way by any of the study procedures, Dr. Tompkins, Strain, or Umbricht will evaluate the condition and, if appropriate, refer the participant for medical attention and notify the IRB, as appropriate. Across hundreds of research participants with various conditions, we have had no adverse events deemed greater than mild (even this level AE occurs in < 1/100 cases) related to the use of these devices.

Cold Pain Pain Tolerance Task**:** There are no significant risks from immersion of a hand in cold water by a healthy individual for the 5 minute maximum time permitted. The task could exacerbate Reynaud's syndrome, therefore these patients are excluded.

Blood Samples. There is a very small chance that phlebotomy may cause hematoma, infection, anemia, and minor pain at the puncture site. However, these procedures are routinely performed without incident in medical settings, and catheters will be installed by qualified nursing staff according proper sterile technique.

Subcutaneous Injection: Subcutaneous injection is routinely performed without incident in medical settings. This route of administration was selected over intramuscular injection because it is better tolerated by patients (140). A qualified nursing professional will administer the subcutaneous injection according to proper techniques.

Saline Placebo: There are no risks associated with saline itself. Minimal risks associated with the routine IV catheterization procedure are described below. Catheters will be installed by qualified nursing staff.

Polysomnography testing. Very rarely, individuals may experience a mild allergic reaction to electrode gels/paste. The CRU has appropriate medicine (e.g., cortisone cream) to ameliorate discomfort as needed.

Physical Exam and Structure Diagnositic Interviewing. There are no known or reported medical risks associated with undergoing a physical exam or the proposed diagnostic interviews performed by a qualified professional.

Urine Sample. There are no risks to collecting a sample of urine. Strict confidentiality of the sample & results will be maintained.

Actigraphy. There are no risks associated with wearing a wrist actigraph.

Questionnaires. Minimal risks associated with completing the questionnaires and cognitive testing are subject fatigue and the possibility of minor psychological distress associated with answering sensitive questions regarding psychological functioning. Subjects are instructed that they do not need to disclose any information that may be too uncomfortable. A Certificate of Confidentiality has been obtained to further protect participant privacy and ameliorate subjects' concerns regarding sensitive information.

Emotion-Related Tasks: Participants will be made fully aware of the types of graphic content included in the International Affective Picture System images at the point of consent, and then again prior to engaging in the task. They will have opportunities to decline participation or discontinue participation at any time. Potential participants with severe or recurrent psychopathology will not be eligible to participate, so it is unlikely that participants will become severely distressed as a result of viewing these images.

Electromyography and Galvanic Skin Response Monitoring: Very rarely, individuals may experience a mild allergic reaction to electrode gels/paste. The CRU has appropriate medicine (e.g., cortisone cream) to ameliorate discomfort as needed.

Motivation, Reward-Related and Psychomotor Vigilance Tasks: To mitigate the unlikely potential for excessive fatigue and/or boredom, subjects will be reminded that they may discontinue any task at any time.

Additional Pain Testing: See risk minimization steps described above under Heat Pain Testing, Pressure Pain Threshold, and Cold Pain Tolerance Task.

Placebo Analgesia:
Risk 1: Infringement of participant autonomy. This risk is sensitively minimized when prospective subjects are given an "authorized deception" disclosure that alerts them to the use of deception in the research during the informed consent process. We inform research participants about the use of deception, the fact they have the opportunity to decline to participate, and that they will learn the specific nature of deception at the end of their study participation.

Risk 2: Distress and lack of trust in research. Revealing the truth about the study during the debriefing process may potentially result in participant's distress who can be upset at learning how they were deceived. However, based on literature on deception in placebo research and our experience, no lack of trust in research have been observed.

Risk 3: Negative emotional reactions. No negative reactions or lasting negative consequences from learning the details regarding the use of deception have been observed. We believe this has been mitigated by the authorized deception part of informed consent.

From the perspective of researchers, the authorized deception may influence the placebo-induced analgesia by reducing beliefs and certainty about the treatment. This point deserves future research.

PET and MRI procedures: Before and during PET scans, the physiological and psychological status of each participant will be closely followed. Before and during each PET scan, blood pressure, heart rate, and rhythm strip will be carefully monitored.

A physician will monitor the physiological and psychological status of the participants before, during, and after inventions until the participant returns to the baseline level. The physician will institute the appropriate medical and psychological interventions when they are needed.

If an acute clinical problem is encountered in the course of the study, the participant will be referred for treatment as indicated.

c. Plan for reporting unanticipated problems or study deviations.

Any protocol deviations and/or adverse events will be systematically documented by Dr. Michael T. Smith, the PI, and reported immediately to the Johns Hopkins IRB. Case Report Forms (CRFs) will identify study participants by their initials and a unique study participant identifier. The participant's name will not be attached to any data. CRFs will be stored in a locked office, which is only accessible to the study coordinator and authorized personnel. The principal investigator will provide an interim report of all adverse events to the IRB at the time of continuing review.

The FDA and IRB will be notified of serious adverse events associated with [^{11}C] raclopride, in accordance with the FDA and IRB reporting requirements.

All adverse events, unanticipated problems, or study deviations will be reported per the current JHM IRB guidelines pertaining to problem/event reporting posted on the JHM IRB website.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

Some of the personal health information gathered during the screening process or during treatment is sensitive. The laboratory blood testing will identify the presence of illegal drugs (if detectable), which might have consequences if they were released to others. Every effort will be made to maintain privacy and confidentiality. A number of procedures will be in place to prevent a breach of confidentiality. All potential subjects will be fully informed of their rights pertaining to disclosure of PHI in accordance with HIPAA regulations. The proposed research will be subject to the strict rules of confidentiality maintained at Johns Hopkins Medical Institutions. Confidentiality will be maintained by assigning participants a study number and numerically coding all data. One hard copy file linking the code number with identifying information will be kept in separate locked file with direct access available to the PI only. All records and research data will be kept in locked filing cabinets or computers. Information about urine toxicology screening results will be handled by Dr. Smith and the project coordinator and will be kept separately from the study clinical record in a locked file maintained by Dr. Smith. Only summaries of group data will be reported in any publications or presentations, with no identification of individuals. These precautions should serve to minimize legal risks to participants. The PI is a psychologist, licensed in the state of Maryland, and is bound by state laws on confidentiality. Further, Johns Hopkins is committed to protecting the rights of potential research participants. In light of this, the Hopkins IRB has developed a training program on protection of human subjects that is required for all Hopkins personnel engaged in research involving human subjects.

e. Financial risks to the participants.

There are no financial risks or costs to study subjects for any of the procedures.

9. Benefits

a. Description of the probable benefits for the participant and for society.

All participants will receive, at no cost, a comprehensive evaluation of their sleep and mental health status, including a screening polysomnographic sleep study, which might identify an occult sleep disorder such as sleep apnea. These individuals will be informed of any disorders identified and referred for appropriate clinical care. Other than mentioned above, there is no other benefit to participation for subjects in the study, but the risks to participation are minimal and justifiable.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

<u>Subject Compensation</u>

Visit 1	=	$25
Visit 2	=	$25
PSG Screen	=	$75
US Condition	=	$300

FA Condition	= $350
Completion Bonus	= $275
Total	**= $1,050**

Subjects will be remunerated up to $1,050 for their effort ($25 v1, $25 v2, $75 the PSG screen, $300 for US Condition, $350 for FA condition, and $275 bonus). We have included a $275 bonus to incent subjects to return and complete the second admission. For participants who leave the study early for any reason, they will receive partial compensation pro-rated as a function of their participation.

Optional Sleep, Pain, Emotions, and Reward Study Procedures
Participants who are eligible and elect to participate in the SPEAR study will be compensated an additional $300 for daytime testing procedures: $100 for each PET session, $25 for each QST and reward testing session, and a $50 completion bonus.

Thus, the total amount a participant could earn for participation if they are eligible and participate in all procedures associated with the Mechanisms of Sleep Disruption Hyperalgesia study and the SPEAR study is $1,350. In addition to the participation payments, participants may earn a small amount of additional money (up to $23.50) as part of the motivation tasks. This amount will not be advertised or included in screening or consenting because it could create expectancy effects that could bias results. However, participants will be informed that they can earn some additional money based on their performance on the tasks.

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

There are no costs to study participants.

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