

Pain sensitization and habituation in a model of experimentally- induced insomnia symptoms

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PART B STUDY DESCRIPTION

TITLE OF PROTOCOL	Pain sensitization and habituation in a model of experimentally-induced insomnia symptoms		
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B1. PURPOSE OF PROTOCOL

Sleep is believed to have a protective effect on health, given findings that sleep deficiency, such as insomnia, results in suboptimal well-being⁸ and adverse health consequences²⁵. At least 40% of individuals suffering from symptoms of insomnia (e.g., difficulty initiating sleep, disrupted sleep, or waking up too early) also suffer from co-morbid chronic pain^{28;39}. Though it was traditionally believed that the experience of pain causes insomnia symptoms, recent evidence has demonstrated that **insomnia itself is a strong predictor of pain both in general as well as in chronic pain populations**¹².

Despite strong evidence for a sleep-to-pain directionality²⁰, the mechanisms by which insomnia amplifies the experience of pain is not yet understood. The **goal of this project is to understand pain amplification in response to insomnia from a mechanistic perspective**. Given the high prevalence of chronic pain and coexisting insomnia, there is a critical need to invest in research designed to identify these mechanistic pathways. **This research is fundamental for the future development of novel strategies targeting specific mechanisms to prevent or reduce pain exacerbated by insomnia.**

To gain mechanistic insights into the insomnia-pain relationship, we developed a **model of repeated exposure to experimentally-induced insomnia symptoms** characterized by: (1) Induction of insomnia symptoms, including delayed sleep onset, sleep disruption with frequent nighttime awakenings, and advanced sleep offset. (2) Repeated induction of such simulated insomnia episodes, which allows for the investigation of a key feature of many biological systems, i.e. the ability of systems to adapt to a repeated challenge.

Using this model, we will investigate **two mechanistic candidates – inflammation and pain inhibition**; both are sensitive to sleep deficiency and are important for pain processing. **(1) Inflammation:** Pro-inflammatory markers (interleukin [IL]-6 and prostaglandin [PG]E2) increase in insomnia and other forms of sleep deficiency²⁶, while the newly discovered anti-inflammatory lipid mediators (resolvins) decrease. Inflammatory markers also play an important role in pain processing: Pro-inflammatory markers result in sensitization of the nociceptive system (i.e., increased responsiveness to noxious stimulation)⁴⁵, while the anti-inflammatory lipid mediators are known for their active role in the resolution of inflammation and pain^{24;47}. **(2) Pain inhibition:** The ability to inhibit pain is deteriorated in insomnia disorder²¹ as well as in many chronic pain conditions^{38;49}. The capability to inhibit pain has been shown to determine the process of habituation to pain⁶ (i.e. decreased response to repeated noxious stimulation)^{11;40}, which appears to serve as a critical mechanism counter-balancing the process of sensitization and protecting against the development of chronic pain^{32;36}.

Hypothesis: Repeated exposure to insomnia symptoms increase vulnerability to chronic pain by promoting two processes: (1) sensitization of the nociceptive system via a progressive increase of the inflammatory response and (2) decreased habituation to pain via a progressive deterioration of the pain-inhibitory response.

This hypothesis will be tested by implementing a novel model of repeated exposure to experimentally-induced insomnia symptoms, consisting of 3 consecutive 4-day episodes, each starting with 3 nights of sleep characterized by typical insomnia symptoms (delayed sleep onset, sleep disruption, early morning awakening), followed by a single recovery night of 8 hours of sleep. This model mimics sleep patterns that are typical in insomnia populations^{31;41} as well as chronic pain populations^{28;29}. Twenty-six healthy women and men will be studied in an intra-individual balanced design, with each participant undergoing 2 18-day-in-hospital conditions (total number of days include baseline and additional recovery nights), one consisting of repeated exposure to insomnia symptoms induction and the other serving as control condition with 8 hours of sleep per night. Frequent blood and urine sampling as well as administration of a complex pain testing battery will be obtained and utilized to evaluate the following aims:

Aim 1 will investigate the **inflammatory response** to repeated exposure to experimentally-induced insomnia symptoms. We postulate that there will be a progressive increase in pro-inflammatory markers (IL-6, PGE2) and decrease in anti-inflammatory lipid mediators (resolvins), leading to **sensitization of the nociceptive system**, as manifested by lower pain thresholds to pressure and heat, as well as increased temporal summation of pain (an index of central sensitization).

Aim 2 will investigate the **pain-inhibitory response** to repeated exposure to experimentally-induced insomnia symptoms. We postulate that there will be a progressive deterioration in the capability to inhibit pain (measured by the conditioned pain modulation test, resulting in **less habituation to pain** (as measured by pain intensity ratings to daily pain supra-threshold stimuli).

Aim 3 will investigate the **ability of the inflammatory and pain-inhibitory system to recover** from the effects of experimentally-induced insomnia symptoms. We postulate a progressive impairment of systems to return to baseline upon repeated exposure to insomnia symptoms.

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

Chronic pain affects over 100 million adults in the United States – more than those suffering from heart disease, diabetes, and cancer combined¹⁴. Chronic pain is highly co-morbid with insomnia^{28;39}, such as difficulties falling asleep, staying asleep, or early morning awakening. It has been well-established that the relationship between insomnia (as well as other forms of deficient sleep, including short sleep duration) and pain is bi-directional in nature¹². Not only does pain result in insomnia²¹, but insomnia and other forms of deficient sleep reciprocally amplify pain. In fact, insomnia symptoms more strongly predict the frequency or intensity of pain symptoms than pain predicts insomnia symptoms¹² in both the general population and clinical pain populations. Similarly, experimentally-induced sleep restriction or disruption for several days leads to increased pain symptoms and pain hypersensitivity^{17;37}.

Despite strong evidence for sleep-to-pain directionality, the **mechanisms** underlying how insomnia initiates and amplifies the experience of pain and increases vulnerability to chronic pain over time are not understood. Given that one-third of the population experience insomnia symptoms²⁷, there is a critical need to invest in research designed to identify these mechanistic pathways. This research is fundamental for the future development of novel strategies targeting specific mechanisms to prevent or reduce pain exacerbated by insomnia.

The goal of this project is to understand the mechanisms through which insomnia amplifies pain. We will test two promising mechanistic candidates – inflammation and pain inhibition – using our novel **model of repeated exposure to experimentally-induced insomnia symptoms**. Our model is characterized by: (1) Induction of **insomnia symptoms**, including delaying sleep onset, sleep disruption via frequent nighttime awakenings, early morning awakening. (2) **Repeated induction** of insomnia episodes, i.e., after a period of sleep recovery, the next experimentally-induced insomnia episode starts. With our model of repeated exposure to experimentally-induced insomnia, we are able to investigate a key feature of many biological systems: the ability to adapt to repeated challenges,

e.g., the response of a system may decrease or increase over time, rather than remain static¹⁵. Studying single exposure to, or episodes of, deficient sleep do not have the ability to elicit information on system response changes – information that is likely of critical importance in gaining mechanistic insights. For example, the failure to habituate to the repetition of painful stimulation is not evident during the exposure to a single episode of sleep restriction, but emerges during repeated exposures to episodes of sleep restriction (see Preliminary Data Section, Figure 7).

Based on preliminary data, mechanistic insights of how insomnia may amplify pain will be gained by focusing on **two processes** (see conceptual model in **Figure 1**):

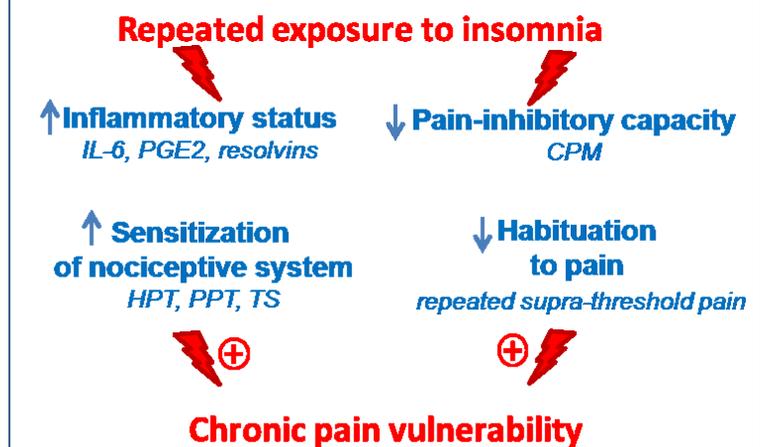
- (1) **Inflammation (Aim 1): Pro-inflammatory markers**, in particular IL-6, PG-E2, result in **sensitization** of the nociceptive system, as demonstrated by an increased response to

noxious stimulation of peripheral and/or central nociceptive neurons⁴⁵. These sensitizing markers have been shown to robustly increase over the course of experimental sleep restriction in association with increased pain reporting^{16;18}, and are elevated in patients with insomnia^{7;43}. **Anti-inflammatory markers** are the newly discovered lipid mediators resolvins, which are biosynthesized from the omega-3 fatty acids²³. These mediators are part of an active biochemical program that enables the resolution of inflammation as well as pain, thus acting as anti-nociceptive agents²⁴. Importantly, our preliminary data show that resolvins of the E series (RvE3) are detectable in human plasma and are sensitive to sleep restriction (see **Figure 5**).

In summary, in Aim 1, we postulate a progressive increase in proinflammatory markers and opposite decrease in anti-inflammatory markers over the course of repeated exposure to experimental insomnia, leading to sensitization of the nociceptive system. This sensitization process will manifest in lower pain thresholds, as well increased temporal summation (TS) of pain (i.e., pain increases with the duration or repetition of a noxious stimulus, see also Appendix 3 for details), which is frequently used as an index of central sensitization of pain transmission neurons in the dorsal horn⁴⁶.

- (2) **Pain inhibition (Aim 2): Pain** is modulated and controlled by central, top-down pain-facilitatory and inhibitory circuits, which can dramatically increase or reduce (respectively) the intensity of a sensation³⁰. The capability to inhibit pain has been shown to contribute to the phenomenon of **pain habituation**. Specifically, painful stimuli administered on a daily basis result in substantially decreased pain ratings^{11;40} (habituation), and this effect has been linked to the activation of pain-inhibitory circuits⁶. The ability to habituate to daily aches and pains likely counteracts the process of sensitization and has been suggested to serve as a protective mechanism against the development of chronic pain^{32;36}. We postulate here that the failure to habituate to repeated (day-to-day) painful stimulation over the course of repeated exposure to a model of experimental insomnia is driven by a progressive deterioration in the capability to inhibit pain, which has previously been shown sensitive to short-term sleep disruption³⁷ as well as to insomnia disorder²¹.

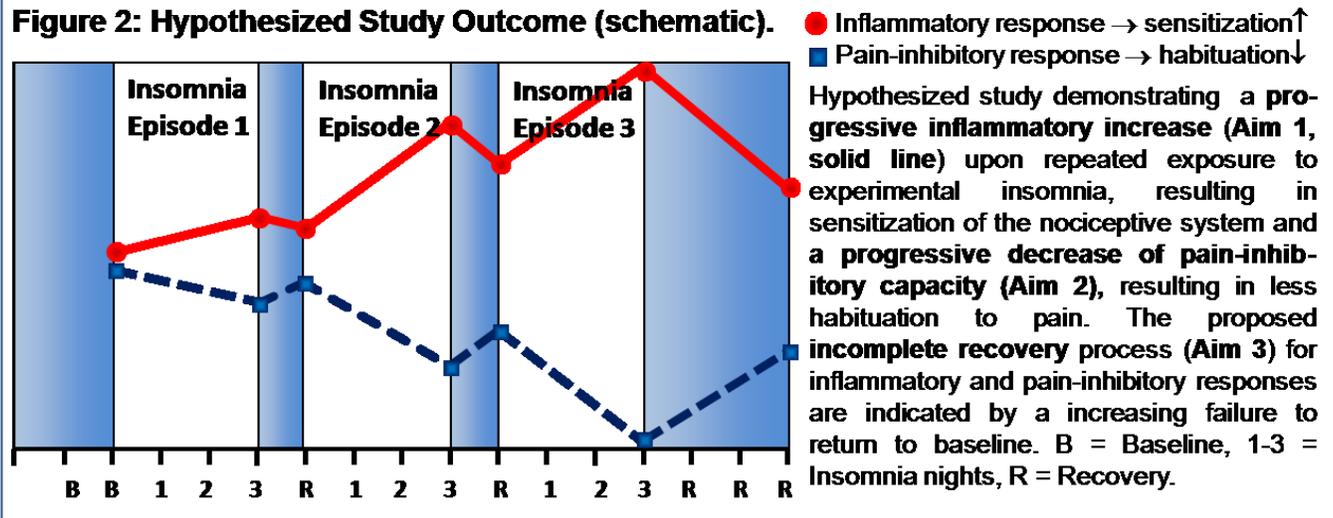
Figure 1: Conceptual model of mechanistic pathways underlying the insomnia-pain relationship



In summary, we propose two processes by which repeated exposure to experimentally induced

insomnia promotes chronic pain vulnerability: (1) Increased sensitization of the nociceptive system via inflammatory dysregulation; (2) Decreased habituation to pain via failure of the pain-inhibitory system.

Do we recover from the effects of repeated exposure to experimental insomnia episodes (Aim



3)? Is a single night of good sleep in-between episodes of deficient sleep sufficient to reverse inflammatory and pain-inhibitory system changes? Or are more nights required? This is an important question designed to assess the elasticity or plasticity of systems in their attempt to return to normal functioning after exposure to sleep deficiency. **If these changes are more or less difficult to reverse by good sleep, this finding may urge to focus on the prevention of such changes, rather than treatment after change occurred.**

Recovery from deficient sleep has been rarely studied, but we and others have shown that after a single episode of sleep restriction or total sleep deprivation, the inflammatory marker IL-6 (plasma and mRNA levels) do not normalize after one night or even two nights of recovery sleep⁴². Furthermore, preliminary data from our ongoing study suggest that the expression of IL-6 by monocytes never fully returns to baseline during brief recovery sleep periods occurring during a longer period of sleep restriction (see **Figure 3**). From this perspective, (and as depicted in **Figure 2**), we expect that during a single night of recovery sleep (typical for insomnia^{31;41}) between successive episodes of experimental insomnia, the ability of inflammatory systems markers to return to baseline will progressively decline, and their sensitizing actions will prevent normalization of pain measures (i.e., pain thresholds and temporal summation of pain). Similarly, we assume that recovery of the pain-inhibitory system will not be fully achieved during a single night of good sleep in-between experimental insomnia episodes, leading to a situation where the next exposure to insomnia ‘hits’ an incompletely recovered system. As depicted in **Figure 2**, we expect that the ability of the pain-inhibitory system to return to baseline will progressively decline during successive recovery sleep periods, leading to less habituation to pain over time, as manifested in a failure to experience supra-threshold pain as less intensive across day-to-day exposure.

Summarizing the importance and implications of the knowledge gained:

- (1) While it has long been known that insomnia is highly comorbid with chronic pain conditions, we still do not have a good understanding of the underlying mechanisms. To our knowledge, this proposal will be the first to investigate the mechanistic roles of two processes that we hypothesize to underlie insomnia-induced pain processing abnormalities: (a) Inflammatory-induced sensitization of the nociceptive system and (b) pain-inhibitory-induced habituation.
- (2) Considering the high life-time prevalence of insomnia and chronic pain, as well as their high comorbidity, mechanistic knowledge is essential for the development of specific targets to prevent or ameliorate pain amplification due to insomnia. As it is unlikely that we will eradicate insomnia in

the population, targeting the mechanisms connecting insomnia and chronic pain could mitigate the consequences of insomnia. A mechanism-specific strategy for the prevention of pain amplification could be the administration of anti-inflammatory agents before or in the very beginning of exposure to insomnia.

(B) INNOVATION

- (a) The major innovative aspect of this proposal is the approach, which is designed to investigate the **mechanisms** (inflammatory-induced sensitization, pain-inhibitory induced habituation) of pain amplification using a model of repeated exposure to experimentally-induced insomnia symptoms. While research has long focused on the characterization of the sleep-pain association, it is time to shift to the investigation of mechanisms underlying this association.
- (b) A novel aspect of this proposal is testing mechanisms within a **model of repeated exposure to experimentally-induced insomnia symptoms**, which closely resembles sleep-wake patterns that are increasingly common in the general population and highly comorbid with chronic pain conditions: How repeated exposure to insomnia symptoms affects the inflammatory and the pain-inhibitory systems, thereby leading to the proposed sensitization of the nociceptive system and less habituation to pain, is currently unknown. This approach has high ecological validity, which will facilitate the translation of mechanistic findings directly into clinical applications.
- (c) Another new aspect of this proposal is the investigation of the **time course and inter-dependencies of the inflammatory and pain-inhibitory systems responses** upon repeated exposures to experimentally-induced insomnia symptoms. To date, both candidates have not been studied simultaneously or over an extended time period. Given the adaptive nature of biological systems, changes in the response magnitude of both systems upon repeated challenges may elucidate valuable mechanistic information that cannot be obtained by studying single episodes of sleep deficiency.
- (d) The current proposal will address the **ability of systems to recover** from recurrent episodes of experimentally-induced insomnia, an area that has been frequently neglected. The extent to which recovery sleep is able to reverse changes induced by insomnia may elucidate valuable information for the development of prevention and treatment strategies.

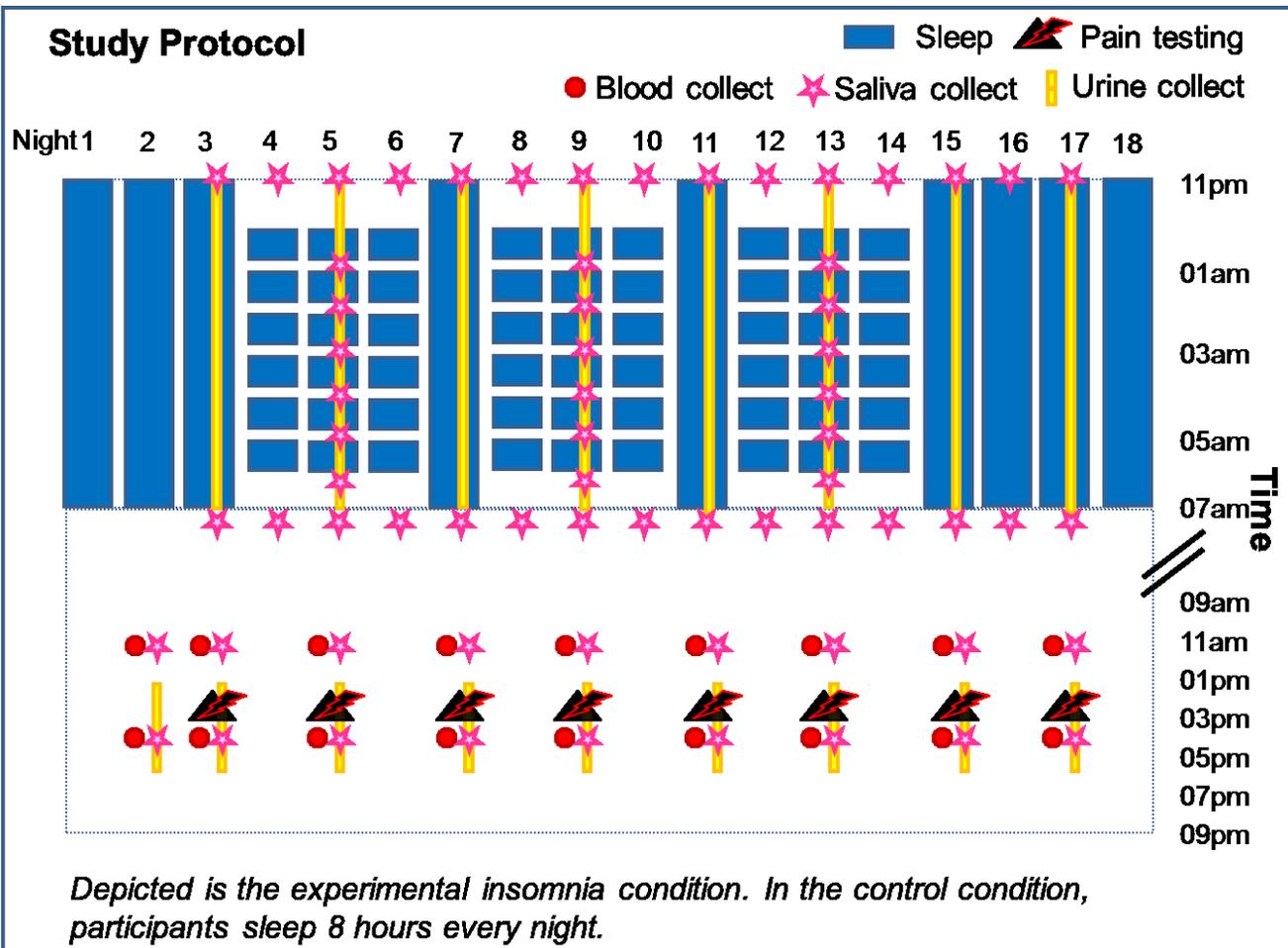
To summarize, this proposal has various innovative aspects in the investigation of pain amplification in response to insomnia: (1) Taking a mechanistic approach (inflammatory-induced sensitization, pain-inhibitory induced habituation to pain), rather than continuing to characterize the general association between insomnia (or sleep deficiency in general) and pain. (2) Studying inflammatory and pain-inhibitory systems changes within a real-life model consisting of repeated exposure to experimentally-induced insomnia symptoms, rather than studying changes within short-term models of sleep restriction or deprivation of a few days. (3) Investigating the ability of system changes to recover from repeated exposure to experimentally-induced insomnia symptoms, rather than concentrating only on the effects of deficient sleep.

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

Proposed Research Plan.

The overarching hypothesis of this research is that repeated exposure to experimentally-induced insomnia symptoms increases vulnerability to chronic pain by promoting two processes: (1) sensitization of the nociceptive system via a progressive increase of the inflammatory response and (2) decreased habituation to pain via progressive deterioration of the pain-inhibitory response.



This proposal will implement a research design consisting of 3 repetitions of a 4-day episode with 3 nights of experimentally-induced insomnia symptoms followed by 1 night of recovery sleep, thereby resembling frequently observed sleep patterns in individuals suffering from insomnia. I.e., after an interval of 1 to 3 nights of poor sleep, individuals experience a better night of sleep, likely resulting from a buildup of sleep pressure during the insomnia episode^{31,41}. The protocol will end with 4 nights of recovery sleep, in order to statistically model time to obtain full recovery of the inflammatory and pain-inhibitory system. The study will take place under controlled in-laboratory conditions in the Clinical Research Center, in order to ensure maintenance of sleep-wake schedules and minimize the influence of confounding variables such as food or fluid intake, light exposure, body posture and motor activity. Each protocol includes frequent blood draws, urine collections, and the administration of a complex pain testing battery on 8 out of the 18 in-hospital days.

Study Design.

This study will employ an intra-individual balanced 2 x 18-day in-hospital protocol (experimental insomnia vs. control sleep condition, see **Figure 8**). The first 2 nights of each 18-day protocol will be adaptation nights, and the 3rd night will serve as the baseline night, with a sleep opportunity of 8 hours (23pm-7am) on all 3 nights. In the experimental insomnia study arm, insomnia symptoms will be induced during the next 3 nights, followed by 1 night of 8-hour recovery sleep (for more details, see model description under Procedures). During nights with insomnia symptom induction, sleep onset will be delayed by 1 hour (midnight 12am), the sleep period (12-6am) will be disrupted by hourly 20 min long awakenings, and sleep offset will be advanced by 1 hour (6am). This episode of 3 nights of experimental insomnia followed by 1 night of recovery sleep will be repeated three times. After the last experimental insomnia episode, participants will have 4 recovery nights. In the control arm of the study, participants will receive an 8-hour sleep opportunity for all 18 nights of the protocol. These 2 arms of the study will be separated by an interval of at least 1 month in order to allow recovery from blood sampling. Monthly separation was chosen to control for potential effects of menstrual phase in women. Depending on the bed occupancy space, participants will stay in a private room in the CRC. If a private room is not available, participants will stay in a double occupancy room with an individual of the same sex (assigned at birth)

Visit 1. Initial screening. All potential participants will have the study explained to them in detail by the PI or another senior member of the research team. If interested, potential participants will sign the informed consent and nursing staff in the Clinical Research Center (CRC) will take vital signs and collect a blood sample. A short battery of screening tests and questionnaires will also be collected in order to assess health status (see **Appendix 1** for blood tests and questionnaires). Potential participants will then be introduced to the web-based, secured diary recording system (REDCap²²) and given an actigraphy watch to take home to estimate sleep-wake habits, for 2 weeks (see **Appendix 1**). These data will be used to verify nightly sleep duration between 7 to 9 hours to be used for eligibility.

Visit 2. Medical history, physical, and sleep screening. During this visit, the study physician will meet with the study participant for a physical exam and review of blood test results. Anemia and abnormal thyroid results are the most frequent exclusionary medical findings in our healthy volunteers. Participants who remain eligible following the medical screen will be instrumented for polysomnographic recording (PSG) for a sleep screening night in the CRC, in order to rule out any possible sleep disorders (see detailed exclusion criteria in Protection of Human Subjects).

Visit 3 and 4: Two 18-day in-hospital stays (experimental insomnia and sleep control condition). Throughout the 18-day **experimental insomnia condition** (see **Figure 8**), participants will start with 3 nights of adaptation/baseline nights, before undergoing 3 episodes of 3 nights of insomnia symptom induction followed by 1 night of 8 hours of sleep. Four recovery nights will follow the last experimental insomnia episode. During this 18-day period, participants will have 8 days of intensive recording (on the baseline night, every 2nd and 4th day of each episode, as well as the 3rd recovery night at the end of the protocol, see also **Figure 'Study Protocol'**). These intensive measurement periods will include PSG recordings, blood sampling, and urine collection during daytime (7am-11pm) and nighttime (11pm-7am) periods. Emotional and physical well-being will be assessed on computerized visual analog scales every 4 hours throughout waking periods (see **Appendix 2**) and a pain testing battery will be administered at 2pm (see **Appendix 3**). For the experimental insomnia nights, the sleep opportunity will be from 12am-6am (rather than 11pm-7am) with hourly 20min-awakenings. However, participants will remain in bed in a semi-supine position during the wakeful nighttime periods (11pm-12am, 20-min awakening bouts, 6am-7am), in order to maintain consistent postural and physical activity inputs across all study nights and conditions. Lights

will be less than 20lux during wakeful nighttime periods, which is still bright enough to read or play board games with the attending research assistant. These environmental precautions are taken to minimize changes in conditions that can be attributable to body movement or the time-keeping hormone, melatonin. The **sleep control condition** will also be conducted in the CRC. Because the study environment and protocol requirements themselves will have a considerable effect on various behavioral and inflammatory outcome variables, we need to compare the results of our insomnia induction protocol against a sleep control condition. These environmental (CRC)-related effects have occurred in multiple previous studies where we have observed decreases in inflammatory markers while staying in the CRC during control sleep periods^{16,18}.

At least seven days before entering each 18-day in-hospital stay, participants will be asked to follow the study sleep schedule (bedtime 11pm-7am), which will be verified by electronic sleep diary/actigraphy data. The week prior to the 2nd 18-day visit, blood tests used at screening will be repeated to ensure values are in the normal range. Throughout both protocols, participants will be maintained on a balanced diet (NA⁺ and K⁺ controlled) and regimented fluid intake in order to maintain body weight/composition throughout the study. Meals and fluids will be served at regular hours. To prevent sedentary conditions and maintain constant activity levels, participants will take a 10-15 min walk within the CRC or outside on hospital property every couple of hours throughout the waking periods of the protocol (except during induced nighttime awakenings). We will also encourage participants to follow their pre-study exercise habits through a daily opportunity to visit the hospital gym on the non-intensive recording days. Following any gym visits, participants will be allowed *ad libitum* water for up to 1 hour. Participants may have visitors during daytime periods, as well as have access to email and phone, in order to minimize disruptions to their social networks and prevent social isolation. Each study arm will be separated by an interval of 2 months or more. This will allow us to study women during the same menstrual phase in both protocols and provide sufficient time to recovery from residual effects of blood sampling.

In case of last-minute cancellations of the 18-day in-hospital stays, we will attempt to fill the open slots with new participants. We will use adaptation day 1 of the study for screening purposes (i.e., initial and medical/overnight screening), and if fulfilling screening criteria, the participant will continue the following day with adaptation day 2 of the study.

Visit 5: Follow-up. Seven to 14 days following their final discharge from the CRC (after completing both study protocols), participants will return to the CRC to have a final blood draw to ensure that any potential activation of the blood markers have returned to baseline and to review their study experience.

Participants.

We anticipate screening 612 subjects in order to have 34 participants with an attrition rate of 32% complete both 18-day in-hospital study protocols. We typically screen 18 potential participants for every one that enters the in-hospital part of the study. In our experience with 2 25-day in-hospital stays separated by a monthly interval, we have an attrition rate of 32%. As such, 34 participants will need to enter the hospital phase to have 26 participants complete 2 18-day in-hospital protocols.

Procedures.

Model of repeated exposure to experimentally-induced insomnia symptoms. The hypothesis will be tested within an experimental insomnia model characterized by (1) induction of insomnia symptoms for 3-day long episodes, and (2) the repeated nature of such insomnia episodes, i.e. after a night of good sleep, the next episode will start. Participants will undergo 3 of those 3-day episodes of insomnia symptom induction, each followed by 1 night of 8 hours of sleep. Such patterns are

commonly experienced in individuals suffering from insomnia, where intervals of 1 to 3 nights of poor sleep are followed by a night of good sleep, likely due to the buildup of sleep pressure during the interval of poor sleep^{31;41}. The advantage of this approach is that it mimics sleep-wake patterns frequently found in the general population for a number of reasons, and in chronic pain populations in particular^{28;39}. In contrast to previous studies, mostly using single episodes of sleep restriction or deprivation, this approach will facilitate the translation of findings directly into clinical applications.

In this model, we will induce 3 typical insomnia symptoms: (a) a delay in sleep onset by 1 hour (from 11pm to 12am); (b) disruption of the sleep period (interval between 12am to 6am) by hourly 20min-awakenings, totaling 6 nighttime awakenings; (c) advancement of sleep offset by 1 hour (from 7am to 6am). To force nighttime awakenings, a computer-generated signal will be used (and will be backed up by human intervention if the computer is not successful). Specifically, an acoustic tone of 1000Hz will be generated by a computer located near the bedside (Aswin Software). The tone will start with a sound intensity of 40dB and will increase in 10dB-increments every 10sec to a maximal volume of 90dB. Participants will signal awakening by pushing a mouse button located within reach that will stop the acoustic signal. Immediately thereafter, a research assistant will enter the room, switch on the light (less than 20 lux), and help participant maintain wakefulness while staying in bed. If the acoustic signal does not wake up the participant after maximal tone intensity is reached (after one minute), the research assistant will enter the room and wake up the participant by calling her/his name, and if this is not effective, by gently shaking the participant.

During the 20 min-awakenings at night, participants will be asked to rate their emotional and physical well-being on computerized VAS, and will interact with the attending research assistant while staying in bed, until a computer-generated tone signals the next sleep opportunity. As in the control group, which has an undisturbed sleep opportunity of 8 hours for every night, participants will remain in bed between 23pm to 7am to control for body position and activity as well as light exposure.

Polysomnographic recording (PSG). Sleep will be recorded using the Embla system N7000 (Medcare US, Buffalo) on the screening night visit to ensure that participants are free from sleep disorders. The Embla system will also be used to collect sleep data on the 8 intensive recording days of each study run (baseline, every 2nd and 4th night of each episode, 3rd recovery night at end of protocol). The PSG montage will follow standard criteria and sleep EEG will be manually stage-scored on a 30 second epoch basis³. The main purpose for sleep data collection is the quantification and comparison of various sleep indices in insomnia symptoms induction and control nights (e.g., sleep duration, sleep latencies, percent time spend in different sleep stages). For explorative analysis (not specified in aims), spectral analyses will be conducted on EEG data using Remlogic software (Medcare US, Buffalo). The homeostatic response will be calculated for each episode, by calculating change from baseline for the two homeostatic markers N3 (slow wave sleep) and delta power, and comparing between episodes. We will explore whether the robust and stable homeostatic response that has been observed within exposure to a single episode of deficient sleep¹ will increase with episode repetition. Spectral analysis will be performed by the PI, who is experienced in this analysis¹⁹.

Blood Sampling. On the baseline day (Day 3), as well as on the 8 intensive recording days of each 18-day study run, blood samples will be drawn at 1100am and after pain testing, around 1550 (50 min after pain testing) per simple needle stick. The amount of the blood collected at each blood draw will range between 10ml and 50ml. The total amount of blood taken over each 18-day protocol will not exceed 550ml.

IL-6 will be measured in plasma in our laboratory, using a high sensitivity enzyme immunosorbent assay (ELISA, Quantikine ® HS, R&D Systems, Minneapolis, MN). We will measure IL-6 twice throughout the 8 intensive recording periods. IL-6 will be also measured in vitro as the capacity of monocytes to express IL-6. In brief, whole blood will be stimulated with lipopolysaccharide (LPS,

100pg/ml), incubated with fluorescence-conjugated antibodies (CD14, CD45, IL-6), and IL6-positive monocytes will be quantified using flow cytometry (Gallios™ Beckmann-Coulter Inc, Flow Cytometry Core at BIDMC).

Resolvins are the newly discovered anti-inflammatory lipid mediators that are actively involved in the resolution of inflammation and pain²⁴. Resolvins of the E series are produced from eicosapentaenoic acid (EPA)²³, and possess potent pro-resolving actions that include counter-regulation of cytokines and reduction in pain in animal models³⁵. Resolvins of the E series will be measured in the Lipid Mediator Metabolomics Core at the Brigham and Women's Hospital Boston (Director Dr. Charles Serhan), using liquid chromatography-mass spectrometry (LC-MS MS). Assessment will be performed twice per intensive recording day at 1100am and after pain testing.

PGE2 metabolite will be measured in collections of nighttime urine (11pm-7am) and daytime urine collected during/after pain testing (2pm-5pm) during the 8 intensive recording days. Urinary PGE2 will be analyzed in our lab using an EIA (Cayman Chemical, Ann Arbor, MI), and levels will be adjusted for urinary creatinine levels.

Creatinine will be measured in night- and daytime urine collects and used for the adjustment of PGE2 levels.

Cyclooxygenase 2 (COX-2), is the enzyme involved in PGE2 synthesis and is the target of the pain-relieving nonsteroidal anti-inflammatory drugs (NSAID)³³. COX-2 will be measured in vitro by the capacity of monocytes to express COX-2 after stimulation with lipopolysaccharide (LPS, 100pg/ml) and incubation with fluorescence-conjugated antibodies (CD14, CD45, COX-2), using flow cytometry (Gallios™ Beckmann-Coulter Inc, Flow Cytometry Core at BIDMC). Though not part of the aims, COX-2 measures may corroborate proposed findings of a role of PGE2 in experimentally-induced insomnia symptoms. COX-2 expression will be measured in the 1100am blood sample obtained on during baseline and on the 8 intensive recording days.

Opioid peptides: Beta-endorphin will be measured in leukocytes using flow cytometry.

Cortisol will be measured in plasma and saliva and assayed in the Harvard Catalyst Central Lab, using the Access Chemiluminescent Immunoassay (Beckman Coulter Fullerton, CA).

Norepinephrine: Norepinephrine will be measured in urine and plasma/serum collects and assayed in the CORE lab on the HPLC system.

DNA/RNA extraction from blood/saliva:

RNA will be extracted from blood by RNA extraction method using the QIAGEN RLT buffer or another RNA stabilizing reagent. Blood drawn at 1100am during the 4th and 14th days will be used for RNA extraction in the laboratory. Samples will be stored in -80°C freezer for future genetic analyses.

DNA will be extracted from saliva using the saliva DNA isolation kit from Norgen Biotek Corporation (product# RU 45400). Saliva samples obtained from participants at 1100am during baseline and every heavy recording day will be used for DNA extraction in the laboratory. Samples will be stored in -80°C freezer for future genetic analyses.

Cardiovascular markers: Radial artery blood pressure (BP) will be assessed by averaging three to five measurements of BP taken at 3 min intervals following standard American Heart Association (AHA) guidelines and using Dinamap automated oscillometric device (GE Medical SIT Inc., Milwaukee, WI) every 4h through the waking periods of the protocol, as well as during the disruption intervals at night. The arterial waveform in a finger of the non-dominant hand will be derived on a beat-to-beat basis by digital photoplethysmography (Portapres, TPI, Brussels, Belgium). The Portapres system will be used between 0830 and 0930 for autonomic tests on pain testing days.

Autonomic tests: To determine the effects of repeated fragmentation sleeps on autonomic system, participants will undergo valsalva's maneuver (VM) test, fast breathing test and mental stress test on 6 days' mornings. The tests will begin at 0930h, following portapres instrumentation and 3

consecutive brachial BPs, 10min baseline (BL) will be obtained to determine spontaneous baroreflex sensitivity and heart rate variability. Following BL, participants will be instructed to breath under a metronome control at a rate of 15breaths/min for 5min to obtain standardized HRV. Then participants will perform 3 VMs: we will place a nose clip on the subjects, after a normal inspiration, a sterilized mouth piece will be placed in the mouth, and a pressure gauge will be held in front of the subject, so they can read the pressure gauge. Subjects will forcefully exhale until the scale reads 40mmHg and they will hold that pressure constantly for 15sec. After the Valsalva strain, subjects will rest and breathe normally for 1min (recovery). The VM will be repeated two more times, thus a total of 3 VMs will be performed. Mental stress test will be elicited via serial subtraction for 5min. The total time of autonomic test will be about 40min.

Questionnaires:

At the beginning of each 18-day in-hospital stay, participants will be given several questionnaires that assess general well-being, emotion regulation, as well as response reactivity to challenges:

- Perceived Stress Scale (PSS14, Cohen et al., 1983)
- Fear of Pain Questionnaire (FPQ, McNeil et al., 1998)
- Pain Catastrophizing Scale (PCS, Sullivan, 2009))
- Life Orientation Test (LOT-R; Scheier et al., 1994)
- Affective Style Questionnaire (ASQ, Hofmann and Kashdan, 2010)
- Ford Insomnia Response to Stress Questionnaire (FIRST, Drake, 2004)
- State Trait Anxiety Inventory (STAI, Spielberger, 1970)

Cognitive function testing battery:

During the experimental insomnia symptom induction nights, executive function will be measured by the Stroop color-word test (for a detailed description, see Lezak, 1995).

At several study days, the Psychomotor Vigilance Test (PVT, 10min) will be used to measure reaction time every 4 hours throughout the waking periods of the protocol, starting 2 hours after lights on, after sleep inertia is dissipated.

Assessment of emotional and physical well-being. Every 4 hours during daytime wake periods, as well as after each nighttime awakening during the experimental insomnia induction nights, participants will rate intensity of mood and spontaneous pain symptoms using computerized visual analogue scales (AsWin, programmed by Martin Rivers & Associates, see **Appendix 2**). The test battery requires approximately 5 minutes per administration. Though *spontaneous* pain is not the focus of this grant, we will explore whether processes of inflammatory-induced sensitization and pain-inhibitory induced habituation to evoked pain may relate to the experience of spontaneous pain.

Pain testing battery: Pain sensitivity and pain inhibition tests will be administered at 2pm throughout the 8 intensive recording days. Throughout the 1 hour testing period, the participant will remain in a seated position in a comfortable chair. The sequence of laboratory events is as following (see **Appendix 3** for a detailed description of tests, as well as²¹).

- (1) Pain sensitivity tests: (a) Heat pain thresholds (HPT), (b) Pressure pain thresholds (PPT)
- (2) Pain modulation tests: (a) Temporal summation of heat pain (TS). (b) Conditioned pain modulation (CPM)
- (3) Cold pressor test (CPT)

We plan to apply heat stimuli using the Pathway Model CHEPS (Medoc, Minneapolis, MS), which has the advantage of a rapid and flexible heating rate required for TS testing (see Budget Justification). Pressure stimuli will be applied using an electronic pressure algometer (Somedic Sales AB, Hörby, Sweden). For heat pain applications at the foot (being applied in TS), we will use a temperature-controlled water bath (Techne® water baths, Bibby Scientific US, Burlington, NJ). The proposed

inflammatory-induced sensitization should manifest in lower HPT and PPT, as well as increased TS of pain (used as an index of central sensitization). The proposed failure of the pain inhibition system (measured through CPM), should result in a lack of habituation to pain, as measured by heat pain supra-thresholds throughout the 8 intensive recording days of the experimental insomnia induction or control sleep protocol.

Pain testing details:

(1) Pain sensitivity tests:

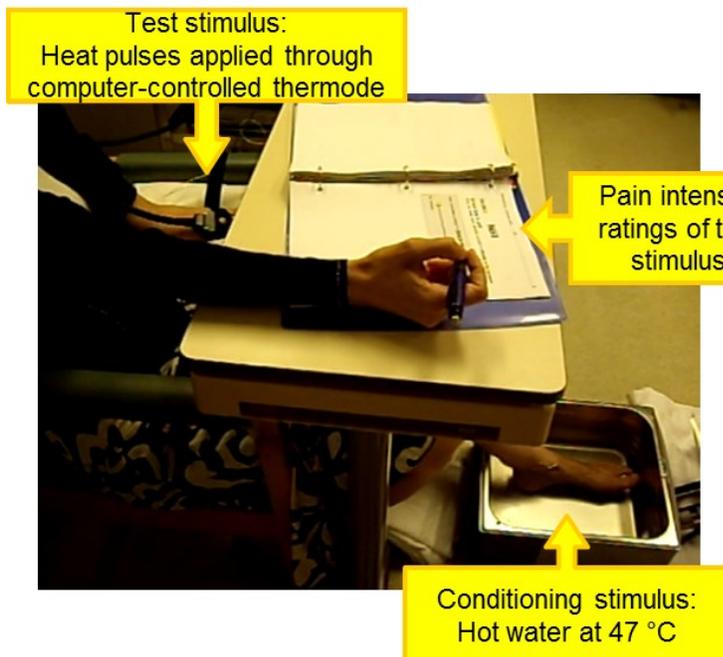
- (a) Heat pain thresholds (HPT, 10min) will be assessed using a precise, computer-controlled thermode (Somatosensory Analyzer, Medoc, Minneapolis, MS) for generating and recording responses to thermal stimuli. The thermode will be attached to the forearm volar skin of the arm not used for blood draws, via a Velcro strap, to the palm. From a baseline temperature of 32°C, the thermode is heated at a rate of 0.5 °C/sec. The participant is instructed to click a computer mouse with their free hand, as soon as heat pain is perceived. To improve accuracy, each threshold will be measured 4 times. The inter-stimulus interval is 15 sec, and the average will be used for further analysis.
- (b) Pressure pain thresholds (PPT, 10min) will be obtained with use of an electronic pressure algometer (Somedic Sales AB, Hörby, Sweden), and assessed at the posterior neck, trapezius muscle, and middle phalanx of the middle/ring finger, and pressure will be increased at a rate of 30 kPa/s. Participants will be instructed to press a control button when they experienced the first sensation of pain. A series of four pressure-pain stimuli will be applied with 15 sec intervals to each side, and the average of these stimuli will be calculated.

(2) Pain modulation tests:

- (a) Temporal summation (TS, 15min) of pain is frequently used as an index of central sensitization of dorsal horn neurons⁵⁶, and will be assessed using the Somatosensory Analyzer (Medoc, Minneapolis, MS). Four sequences each consisting of ten brief consecutive heat pulses will be applied via a thermode to the forearm volar skin of the arm not used for blood draws, with a pulse-to-pulse interval of 2.5 sec. The temperature used to assess TS will be tailored to each person's tolerance level. The first test sequence of this individually-tailored procedure will have a target temperature of 48 °C and an inter-pulse baseline temperature of 32 °C. Depending on whether the participant can tolerate the initial 10-pulse sequence, the target temperature of the second sequence will be increased or decreased, respectively, by 1.5 °C (i.e., increased to 49.5 °C or decreased to 46.5 °C). In the third sequence, the target temperature will be again increased or decreased by 1.5 °C, depending on whether the participant could tolerate the second sequence or not. The inter-sequence interval will be 2 min at a temperature of 32 °C. The thermode will be moved systematically between sequences, starting at the thenar eminence for a practice trial, and sequentially moving cephalad on the volar aspect of the forearm along the innervation of C5–6 for the remaining three trials. This sequence was designed to prevent testing on previously stimulated skin areas (see also²⁵). During each test sequence, participants will be prompted to rate the intensity of the 1st, 4th, 7th, and 10th thermal pulse using a 0–100 mm visual analogue scale (VAS), presented on separate data sheets for each rating. The participant will be instructed to say 'STOP' as soon as the sensation is no longer tolerable at any point during the testing. Temporal summation of pain will be defined as an increase in perceived pain intensity across the 10-pulse sequence, such that the last heat pulse intensity in a sequence is perceived as more painful than the first. For statistical analysis, TS will be calculated by subtracting the first rating from the last rating in the 10-pulse sequence. Highest tolerable temperature will be used as the conditioning stimulus in the conditioned pain modulation (CPM) paradigm.
- (b) Conditioned pain modulation (CPM, 20min), assesses the principle of 'pain inhibits pain', and

is a measure of the pain-inhibitory capacity of the central nervous system. For this protocol, *the test stimulus* will be the TS sequence at highest tolerable temperature, applied to the outer volar surface of the non-dominant forearm, along C8-T1 innervations. Immersion of the contralateral foot into a painfully hot water bath (47 °C, Techne® water baths, Bibby Scientific US, Burlington, NJ) will be the *conditioning pain stimulus* that is intended to activate the pain-inhibitory circuits and thereby decrease the perceived pain of the forearm test stimulus (see **Figure below** for setup).

In total, four CPM trials will be performed: two trials using a hot water bath (47 °C) and two baseline trials without a water bath. For each hot water bath trial, the participant's foot will be first submerged in the water bath. After 20sec of immersion, the 10-pulse temporal summation sequence will be applied to the forearm and the participant will be prompted to rate the pain intensity of the 1st, 4th, 7th, and 10th stimulus using the VAS. There will be a two-minute rest period between all trials during which the thermode will be systematically moved from the distal to proximal sites along the C8-T1 innervated skin in order to avoid re-stimulation of the previously sensitized skin. For statistical analysis, ratings of the two 10-pulse sequences



Setup for Conditioned Pain Modulation (CPM) testing:

Twenty sec after foot immersion into hot water bath (47 °C, the conditioning stimulus that is supposed to activate descending pain-inhibitory circuits), the participant rates the intensity of heat pulses (test stimulus, individually tailored to highest tolerable temperature) applied to the forearm.

under the conditioning stimulus (hot water) will be averaged, and contrasted against ratings from the baseline (no-water) condition. A decrease in pain intensity ratings under the hot water condition compared to the no-water condition will be used as an index of the pain-inhibitory capacity.

(3) Cold Pressor Test (CPT):

The Cold Pressor Test (CPT): This test requires the insertion of the hand into an ice-cold water bath (Techne® water baths, Bibby Scientific US, Burlington, NJ) held at a temperature of 3°C. Participants are instructed to leave their hand in the water bath for at least 1 minute and are told that they may remove their hand earlier if the pain gets unbearable. Maximum immersion time will be 3 minutes. Pain intensity and unpleasantness will be rated every 10 sec during the hand immersion, as well as every 10 sec for 2 minutes and 30 seconds post-testing to capture recovery slopes.

Statistical Considerations

Sample size: 34 subjects (including a 32% discontinuation rate) will run in balanced order through the two protocols, each consisting of a 18-day in-hospital stay (separated by an interval of 1 month or more) in order to complete the study with 26 subjects. **Power calculations** for the inflammatory response (aim 1) were estimated using findings coming from sleep restriction models on IL-6 (plasma levels and expression by monocytes). For the pain-inhibitory response (aim 2), we estimated power using the effects of a single episode of sleep disruption on the pain-inhibitory capacity³⁷. For the recovery response (aim 3), we used data from the effects of recovery sleep on IL-6 positive monocytes, as well as on the habituation to pain in the cold pressor test paradigm (data obtained in currently ongoing protocol). For all 3 aims, optimal power will be reached with a sample size of N=26 (see **Table 1**).

Randomization: Block randomization will be used with block sizes of 10, 8, 8, and 8. Each block will have equal numbers of females and males and equal numbers of sequences (1. insomnia induction – control sleep, 2. control sleep – insomnia induction). Randomization will be prepared by the study statistician and the participant will be informed of the sequence on study day 1.

Statistical Analysis:

Table 2 shows the primary outcome variables for aim 1-3, as well as secondary outcome variables used for explorative analysis (not part of aims).

Some of the variables will be measured at different time scales and reduced to the following summary variables: Subjective estimations of emotional and physical well-being assessed every 4 hours during the waking periods (and after each nighttime awakening during insomnia induction nights) of the protocol will be averaged to nighttime and daytime frequency and intensity measures.

Table 2: Outcome variables			
Primary outcomes			Secondary outcomes
Aim 1	Aim 2	Aim 3	Explorative
Inflammatory: IL-6, PGE2, resolvins	Pain-inhibitory: CPM	IL-6, PGE2, resolvins, CPM	COX-2, cortisol
Sensitization: HPT, PPT, TS	Habituation: Intensity ratings of HPST	HPT, PPT, TS, HPTS	Emotional/physical well-being
<i>HPT= heat pain threshold; PPT=pressure pain threshold, TS=temporal summation; HPST=heat pain supra- threshold, CPM=conditioned pain modulation, COX=cyclooxygenase</i>			

Table 1: Power calculations.

Variable	Δ Response	Power based on paired Student t-test (α=0.05) – N=26
IL-6 (pg/mL). Response to 10 days of restricted sleep to 4h/night compared to 8h control sleep.	1.95±1.59 pg/ml	>0.80
IL-6 positive monocytes (%). Response to three cycles of sleep restriction (3 x 5 nights of 4 hours of sleep/night), compared to 8h control sleep (ongoing protocol R01 HL 105544, see also Figure 3).	38±66 %	>0.80
Pain inhibition impairment (CPM test, %). Response to 3 days of experimental sleep disruption, compared to control sleep (estimation from graph) ³⁷ .	47±54 %	>0.80

Habituation to pain (CPT test, sec). Response to 3 cycles of sleep restriction (3 x 5 nights of 4 hours of sleep/night), on the ability to habituate to cold pain, compared to 8h control sleep (ongoing protocol R01 HL 105544, see also Figure 7).	-15±25 sec	>0.80
Habituation to pain after recovery sleep (CPT test, sec). Response to 2 recovery nights following 3 cycles of sleep restriction on the ability to habituate to cold pain, compared to 8h control sleep (ongoing protocol R01 HL 105544, see also Figure 7).	-24±28 sec	>0.80

Assessment of Aim 1: Inflammatory response and its relation to indicators of sensitization of the nociceptive system upon repeated episodes of experimentally-induced insomnia symptoms:

Outcome variables will be measured on 8 out of the 18 in-hospital protocols (baseline, 2nd experimental insomnia/control night and 4th recovery night of each episode, and 3rd recovery night at end of protocol). The analysis will focus on episode-wise comparison of outcome variables between experimental insomnia and control sleep conditions. Although several forms of linear mixed model will be used, our main analysis will focus on daily summary measures (mean, max, min, slope, AUC, etc.) The models will be constructed as follows: the study has two within factors (measurements over several days and measurements within the same day). Data will be used as it is or the difference between the two experimental conditions by episode will be used in the analysis. The main method of data analysis will employ linear mixed model (repeated measures analysis).

By defining appropriate contrast we can evaluate (i) the difference between experimental insomnia nights and recovery sleep nights for the entire study period, (ii) the difference between days 3 and 6 (day6 minus day3), 7 and 10, and 11 and 14 separately. We expect that each of the contrasts will yield a significantly positive value. These comparisons will show if IL-6 values drops or increases from experimental insomnia nights to recovery sleep nights. Similarly, from the same model, by defining appropriate contrasts, we will evaluate the difference between days 6 and 7, 10 and 11, and 14. These contrasts are expected to be negative. Time will also be used as a continuous variable to test the trend and quadratic effect, and if the above contrast does not suggest strong episode-wise effect but mild increment (or decrease) from one episode to the next. We may also use piece-wise regression with a knot at each episode (and also with just one knot to capture the effect of prolonged recovery towards the end of the experiment). As mentioned earlier, many of the exploratory analysis will be guided by plots and summary assessment of the data. Other inflammatory marker (PEG2, resolvins) will be analyzed in a similar fashion.

Sensitization variables (HPT, PPT, TS) will be analyzed similarly and are expected to mimic the time course of inflammatory variables. Correlation between inflammatory markers and sensitization variables will be evaluating using the Altman-Bland method². The linear mixed model described above can be used to obtain the necessary estimates to compute this correlation.

Assessment of Aim 2: Pain-inhibitory response and its relation to indicators of habituation to pain upon repeated episodes of experimentally-induced insomnia symptoms. Analysis will follow the strategy similar to one outlined in Aim 1. To evaluate co-variation of the pain-inhibitory response with indicators of habituation we will first obtain average values of each value for each day, and use the Altman-Bland correlation approach described earlier.

These data will be analyzed using linear mixed model for doubly repeated measures using SAS GLM procedure as described in:

http://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#statug_glm_sect054.htm.

Assessment of Aim 3: Recovery of the inflammatory and pain-inhibitory response upon

repeated episodes of experimentally-induced insomnia symptoms. The proposed incomplete resolution of inflammatory and pain-inhibitory responses during the recovery nights and the progressive impairment of these responses across insomnia induction episodes will be evaluated using a mixed model approach and utilizing four time points: baseline, and recovery at the end of 1st, 2nd, and 3rd insomnia episode. Recovery response will be further evaluated by comparing effects of a single night of recovery sleep to a set of 3 nights of recovery sleep at the end of the protocol. Data analysis for this aim is similar to data analysis described for Aim 1, but a different set of contrasts need to be defined. Our conjecture is that during the recovery IL-6 drops but does not reach to the level of previous recovery. To test this we will evaluate contrasts that compare IL-6 values at days 3 and 7, 7 and 11 and 11 and 15. Finally, the difference between day 15 and 17 is assumed to be larger than any other difference between sleep restriction and recovery days. To test this we will evaluate the differences between other recovery days (average of days 7, 11, and 15) and day 17. All analysis will be performed using SAS statistical package (version 9.3).

C. Subject Selection

Human Subjects Involvement and Characteristics

A total of 34 healthy adults (18-35 yrs, 17 women, including a discontinuation rate of 32%) will be enrolled. Subjects of all racial/ethnic backgrounds will be eligible to participate (see Planned Enrollment Report for expected distributions). Participants will be accepted into the study if they are in good health, do not have an active medical disorder or history of any medical disorders (including sleep disorders), as determined by a medical history, physical screening, and overnight sleep study (PSG, as per methods). Participants from the Boston area, who meet these strict medical eligibility criteria, will be recruited to participate in the 2 x 18-day in-hospital study. Once participants enter the study, they will stay in a patient room at the Clinical Research Center of Beth Israel Deaconess Medical Center. Nurses will check vital signs daily and will assess general emotional and physical well-being at regular intervals throughout each study day. The study physician will also perform an in-person visit with each participant every day, in order to ensure participant's well-being.

Inclusion Criteria:

- Women and men between the ages 18-35 years.
- Non smoking
- Body mass index (BMI) between 18.5 and 30 kg/m².
- For female participants: regular menstrual cycles, no significant discomfort during pre-menses/menses.
- Daily sleep duration between 7-9 hours, verified by REDCap electronic sleep diary.
- Sleep onset within one hour of 11pm (to ensure normal entrainment).
- Blood chemistry in the normal range.
- Medical examination finding within normal range.

Exclusion Criteria:

- Active infection/disease.
- History of neurological, chronic pain, immune, cardiovascular, liver/kidney, or metabolic disorder or Raynaud's syndrome within 6 months prior to study start.
- Active or chronic psychiatric disorders within 6 months prior to study start, including major depressive disorders, bipolar disorders, panic disorders, post-traumatic stress disorders (PTSD), thought disorders, and substance abuse/dependence disorders.
- In psychotherapy or any other behavioral interventions at study start (eg acupuncture for insomnia)

- Sleep disorders.
- PSG screen results of apnea-hypopnea index (AHI) of >5 events/hour based on PSG screening night, periodic leg movement index (PLMI) >15/hour based on PSG screening night; sleep efficiency <80% based on PSG screening night; restless legs syndrome, circadian rhythm disorders, and nightmare disorders determined by diagnostic interview.
- Pregnant/nursing.
- Regular medication use other than oral contraceptives.
- Donation of blood or platelets 3 month prior to study start.
- Systolic BP measurement <140/90 Hg.
- Use of anti-inflammatory medication one week prior to study start
- Positive urine toxicology screen.
-

Sources of Materials:

Blood, urine, and saliva samples from participants will be assayed for markers involved in the inflammatory and stress response.

Inclusion of Women:

All women of child bearing age who do or do not take oral contraceptives and who are not pregnant or nursing are eligible for the study.

B4. POSSIBLE BENEFITS

This research will not benefit participants directly. However, it will provide important information regarding the effects of repeated exposure to insomnia symptoms on mechanisms responsible for pain amplification. This research is fundamental for the future development of novel strategies targeting specific mechanisms to prevent or reduce pain exacerbated by insomnia

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

Side effects related to lack of sleep and maintaining wakefulness for an extended period of time include fatigue, frequent mood changes, increased bodily discomfort such as mild back pain, stomach pain, or headache, as well as nausea and vomiting.

There is a minimal risk of infection associated with drawing blood from a vein. There are no known risks of testing heat and pressure pain thresholds, and heat and cold pain tolerance. Repeated application of noxious heat pulses can be associated with irritation of the skin for up to 48 hours. There are no known risks of wearing BP or PSG equipment. The Portapres device, used to measure beat-to-beat blood pressure, involves two small cuffs worn on two fingers. The pressure of the inflating finger cuff may cause temporary discomfort, tingling or numbing to the finger, and if that happens we will loosen or switch the cuff to a different finger. It is not unusual to experience cool and blue finger tips when the cuff is inflated, and we may keep your hand warm with a warm pad. No lasting finger discomfort has been reported from using this device, but a single case of prolonged mild numbness has been reported.

Participants will be extensively screened before being accepted into the study. We will exclude participants with a history of immune, cardiovascular, pain-related, gastrointestinal, significant psychiatric disease, significant allergies, or conditions where extreme vasoconstriction can occur, such as Raynaud's syndrome. Participant screening will include blood tests for CBC and differentials, as well as thyroid hormone, and blood glucose; urinary toxicology screens will also be conducted. We

will exclude participants who smoke or take regular medications other than hormonal birth control medication. The medical history and physical will be conducted by the study physician. If the study physician is not available, the medical research officer will conduct the screenings. To ensure the participants' safety while undergoing shortened and disrupted sleep, as well as to assist the participants in following the protocol and maintaining alertness outside of all scheduled sleep periods, research staff will accompany the participant at all times throughout their stay in the CRC. All research assistants are trained to watch for signs of drowsiness and to circumvent sleep spells by initiating changes in activity, for example switching from watching a video to playing a board game with the participant if s/he appears drowsy. The study physician (or medical research officer) will check on all participants daily while they are admitted to the CRC, and serve as the doctor on-call throughout each participant's stay. Should participants need medical assistance, the monitoring research assistant will notify the ward nurses, who will address any immediate medical needs and also immediately notify the study physician and the PI. In the event of a medical emergency, the CRC is located in a critical care hospital, and a code team is available at all times.

Risk/benefit ratio: This work has the potential to understand the mechanisms by which insomnia may amplify pain. Preventive or treatment strategies may emerge in part due to the results of this research.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment

Participants will be recruited via internet postings, flyers, bulletin advertisements, subway advertisement, and local newspapers. When participants contact the research office, they will participate in preliminary screening via telephone and/or email. Participants will receive a REDCap survey asking general health questions as well as questions regarding their sleep. If it appears that an individual may qualify to participate in the study, s/he will be invited to come to the Clinical Research Center (CRC) where s/he will go through the informed consent process and complete questionnaires designed to assist in determining eligibility. During the initial visit to the CRC, nurses will also take vital signs and collect a blood sample. These results will be reviewed by the study physician prior to the potential participants' 2nd CRC visit, during which participants will undergo a medical history/physical and overnight sleep screening. If an eligible individual is still interested in study participation, s/he will be scheduled to participate in the two in-hospital portions of the study.

Consent

The PI and/or senior research team member will interview each subject and obtain informed consent from interested participants, after the participant has the study explained to them in detail and has had any questions answered.

Subject Protection

While none of the subjects in this study will be drawn from a population vulnerable to coercion or undue influence, the following measures to ensure subject protection will remain in place. Participants will be extensively screened before being accepted into the study. We will exclude participants with a history of immune, cardiovascular, pain-related, gastrointestinal, significant psychiatric disease, significant allergies, Raynaud's Syndrome, or a history of substance abuse. Participant screening will include blood tests for CBC and differentials, as well as thyroid hormone, and blood glucose; urinary toxicology screens will also be conducted. We will exclude participants who smoke or take regular medications other than hormonal birth control medication. The medical history and physical will be conducted by the study physician. To ensure the participants' safety while undergoing shortened and disrupted sleep, as well as to assist the participants in following the protocol and maintaining alertness outside of all scheduled sleep periods, research staff will accompany the participant at all times throughout their stay in the CRC. Research assistants will also accompany study

participants during all times that they are outside of the CRC (i.e., for outside-walks). All research assistants are trained to watch for signs of drowsiness and to circumvent sleep spells by initiating changes in activity, for example switching from watching a video to playing a board game with the participant if s/he appears drowsy. The study physician will check on all participants daily while they are admitted to the CRC, and serve as the doctor on-call throughout each participant's stay. Should participants need medical assistance, the monitoring research assistant will notify the ward nurses, who will address any immediate medical needs and also immediately notify the study physician and the PI. One week prior to medical screening of the study, participants will not be permitted to take any medications (except birth control medication). In the event of a medical emergency, the CRC is located in a critical care hospital, and a code team is available at all times.

If a participant reports to have suicidal ideation (positive answer to item 9 of the PHQ-9), the physician will be called to evaluate the condition of the subject which may result in bringing the subject to the emergency room.

B7. STUDY LOCATION

Privacy and Physical Setting

Every effort to maintain subject privacy will be made. When interested participants contact the study office, a research assistant will return their call from a private office, in order to maintain confidentiality. Emails sent to and received by study participants will be based on a study email address, and direct mail will only be sent with the participant's express permission and provision of a physical mailing address. Participants will only be contacted by phone at the number they provide to study staff.

For their screening visits, participants will present to the CRC, a central shared clinical research floor in the main part of the hospital. Presence in this area of the hospital does not provide any specific information for an outside observer who witnesses the participant presenting for screening. All in-person screening, including collection of physical data from participants, will take place in a private room, behind closed doors, in the CRC. The in-hospital portion of the study will be conducted in a private or shared room within the CRC. Participants will be outside of their private/shared rooms during walk periods (although will still remain in the CRC).

Whenever possible, participant information will be identified only with a 'participant identification number' and not with actual names. The exception to this is clinical data acquired in the CRC, which, in accordance with hospital policy, is identified with first and last name. As soon as it is feasible, this name identification is converted to a numeric identifier to maximize confidentiality.

B8. DATA SECURITY

All possible precautions will be taken to ensure data security. Data collected from participants will be identified using a subject number, rather than a name, whenever possible. All patient hard copies of materials containing patient information will be stored in a locked file cabinet in a locked office on a secured (swipe-card access) research floor of the hospital. Electronic data will be maintained on a secure server behind the BIDMC firewall. All computers in the laboratory require use of a login before accessing any information, and data are encrypted. Similar to hard copy materials, laboratory computers are located in locked rooms on a secure (locked) research floor of the hospital.

B9 Multi-Site Studies

Is the BIDMC the coordinating site? Yes No

Is the BIDMC PI the lead investigator of the multi-site study? Yes No

B10 Dissemination of Research Results

Descriptions of the data will be presented at national and international conferences and will be submitted to peer-reviewed journals. To encourage the greatest possible dissemination of our work we will, whenever possible, describe our observations in open access journals or through open access options in more traditional journals. We will also deposit copies of manuscripts accepted for publication in Pub Med Central to ensure unfettered public access to our work.

In addition, we plan to use the following dissemination strategies to reach out to various communities:

- BIDMC Active Tools & Media, which includes videos, podcasts, E-Letters, blogs, online chats, social media (Facebook, Twitter, YouTube) reaches employees as well as patient communities and informs about latest research results and medical advances.
- BIDMC Bulletin is a bi-monthly print publication that is distributed across the campus and shares news about employees – research, awards, and more.
- Community Connect to Research at Harvard Catalyst is part of the Public Communication Initiative and disseminates research findings and more to the public.
- Research Days at colleges and universities (e.g., Soma Weiss Research Day, Harvard Research Day) in the Boston area, at which our graduate and undergraduate students present their work on study-related projects.
- ClinicalTrials.gov