

BASIC SCIENCE

Sleep Deprivation and Recovery Sleep Prior to a Noxious Inflammatory Insult Influence Characteristics and Duration of Pain

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Study Objectives: Insufficient sleep and chronic pain are public health epidemics. Sleep loss worsens pain and predicts the development of chronic pain. Whether previous, acute sleep loss and recovery sleep determine pain levels and duration remains poorly understood. This study tested whether acute sleep deprivation and recovery sleep prior to formalin injection alter post-injection pain levels and duration.

Methods: Male Sprague-Dawley rats ($n = 48$) underwent sleep deprivation or ad libitum sleep for 9 hours. Thereafter, rats received a subcutaneous injection of formalin or saline into a hind paw. In the recovery sleep group, rats were allowed 24 h between sleep deprivation and the injection of formalin. Mechanical and thermal nociception were assessed using the von Frey test and Hargreaves' method. Nociceptive measures were performed at 1, 3, 7, 10, 14, 17 and 21 days post-injection.

Results: Formalin caused bilateral mechanical hypersensitivity (allodynia) that persisted for up to 21 days post-injection. Sleep deprivation significantly enhanced bilateral allodynia. There was a synergistic interaction when sleep deprivation preceded a formalin injection. Rats allowed a recovery sleep period prior to formalin injection developed allodynia only in the injected limb, with higher mechanical thresholds (less allodynia) and a shorter recovery period. There were no persistent changes in thermal nociception.

Conclusion: The data suggest that acute sleep loss preceding an inflammatory insult enhances pain and can contribute to chronic pain. The results encourage studies in a model of surgical pain to test whether enhancing sleep reduces pain levels and duration.

Keywords: allodynia, formalin, Hargreaves, inflammation, insufficient sleep, nociception, rodent model, surgical pain, von Frey

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Significance

Disrupted sleep is a risk factor for chronic pain. This preclinical study filled a gap in the knowledge by demonstrating that previous, brief sleep manipulations altered long-term levels and duration of pain caused by a noxious inflammatory insult. Sleep deprivation enhanced cutaneous mechanical hypersensitivity (allodynia) caused by a subdermal injection of formalin, whereas allowing a sleep recovery period after sleep deprivation significantly reduced the levels, duration and extension of allodynia caused by formalin. These data lend additional support to the notion that sleep loss can contribute to chronic pain, suggest that enhancing sleep prior to a noxious insult may protect from the development of chronic pain, and represent the foundation for future studies to elucidate central mechanisms for sleep-pain interactions.

INTRODUCTION

Sleep disorders are highly prevalent and insufficient sleep is increasingly recognized as a public health problem.¹ An estimated 50 to 70 million adults in the United States suffer from a sleep disorder¹ and 30% of employed adults reported a significantly shorter sleep duration than the average basal need.² Altered sleep continuity and sleep architecture are also common in hospitalized patients,^{3,4} and virtually all patients in the intensive care unit have severely disrupted sleep.⁵ Insufficient sleep is associated with increased morbidity and mortality. Disrupted sleep compromises immune function,^{6–8} and is linked to poor cardiovascular,^{9,10} metabolic,^{11–13} mental health,^{14–16} and pain^{17,18} outcomes.

Disrupted sleep and pain are prevalent, frequently associated problems with reciprocal negative effects; pain alters sleep, and sleep disruption worsens pain perception. The bidirectional nature of the interaction between sleep and pain is supported by several studies in animal models of chronic pain,^{19–25} healthy (pain-free) volunteers,^{26–33} and patients with different types of chronic pain.^{34–39} Importantly, longitudinal studies suggest that altered sleep during hospitalization for a traumatic injury or surgery is a risk factor for subsequent development of chronic pain.^{18,32,40} Furthermore, a recent meta-analysis of current literature demonstrated that insomnia symptoms increase the risk of developing future chronic pain, and revealed that sleep disturbance is a stronger predictor of pain than pain is of sleep disturbances.⁴¹

Based on the foregoing lines of evidence, the current pre-clinical study was designed to test two hypotheses concerning the long-term effect of acute sleep deprivation and recovery sleep on subsequent pain caused by an inflammatory insult. The first hypothesis was that acute sleep loss prior to a noxious inflammatory insult worsens post-insult pain levels. The second hypothesis was that allowing time for recovery sleep prior to an inflammatory insult diminishes the negative effect of preceding sleep deprivation on pain levels. To test the causal relationship between altered sleep and post-insult pain levels, rats underwent total sleep deprivation, received a subcutaneous microinjection of formalin (a model of persistent pain),^{42–44} and nociceptive levels were assessed during a 21-d period subsequent to formalin injection.

MATERIALS AND METHODS

Animals, Conditioning, Chemical Suppliers, and Solutions

All the experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Academies Press, 8th Edition, Washington, DC, 2011) and the recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.⁴⁵ The procedures using animals were approved by the University Committee on Use and Care of Animals. Adult male Sprague-Dawley (CrI:CD®(SD)) rats ($n = 48$) weighing 250 to 350 g

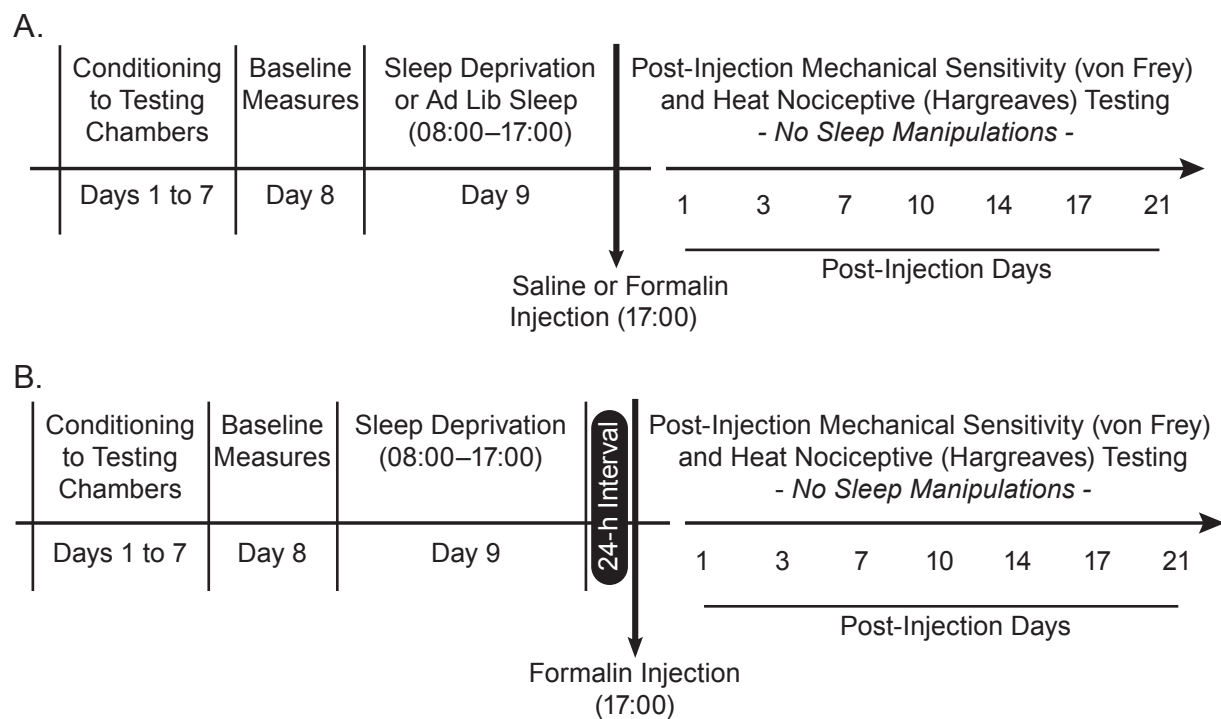


Figure 1—Experimental design and timeline used for quantifying the effect of sleep deprivation (**A**) and recovery sleep after sleep deprivation (**B**) on post-injection nociceptive levels using the formalin pain model in rats.

at arrival were purchased from Charles River Laboratories (Wilmington, MA, USA). Rats were group housed in a 12-h light:dark cycle (lights on at 08:00) with free access to food and water, and were allowed 7 d after arrival for acclimation to the novel housing environment. Thereafter, rats were conditioned to the testing chambers and handled for a minimum of 1 w before experiments began. Two days prior to the experiments, rats were individually housed in environmentally enriched cages until the end of the experimental protocol. Rats were monitored daily looking for signs of distress or discomfort (i.e., absence of grooming, feeding behaviors or exploratory activity, immobility, lethargy, scruffy hair, and porphyrin stain), as well as for early detection of infection in the injection site. Body weight was measured once per week and individual weight curves were used to reveal any abnormal deviation from the expected healthy weight range throughout the experimental period.

Formalin (10%) was obtained from Thermo Fisher Scientific (Waltham, MA, USA). The formalin solution was diluted to 5% in sterile saline (0.9%; vehicle control) and filtered using a 0.22- μ m syringe filter (Fisher Scientific #05-713-386) immediately prior to every series of injections.

Experimental Design

After obtaining baseline measures of mechanical sensitivity and thermal nociception, rats ($n = 38$) were randomly assigned to one of the following four groups in a between-subject study design: (1) *ad libitum* sleep + saline, (2) *ad libitum* sleep + formalin, (3) sleep deprivation + saline, and (4) sleep deprivation + formalin injection. All experiments began at 08:00. On the day of the experiment (Figure 1A), rats were either allowed

to sleep as needed or sleep deprived for 9 h (08:00 to 17:00). Thereafter (17:00), rats received an injection of formalin or vehicle into a hind paw. Measures of mechanical sensitivity and thermal nociception were obtained from each rat on day 1, 3, 7, 10, 14, 17 and 21. No additional sleep manipulations were performed after the injection.

A fifth group of rats ($n = 10$) was sleep deprived for 9 h (08:00 to 17:00), allowed a 24-h recovery period, and then injected with formalin (next day at 17:00) into a hind paw (Figure 1B). Measures of mechanical sensitivity and thermal nociception were obtained from each rat on day 1, 3, 7, 10, 14, 17 and 21. No additional sleep manipulations were performed after formalin injection. Measures of post-injection nociceptive levels in these rats were compared to their respective baseline levels, as well as to the sleep deprivation + formalin group.

Sleep Deprivation and Formalin Injection

Total sleep deprivation (i.e., rats were deprived of nonrapid eye movement [NREM] sleep and rapid eye movement [REM] sleep) was accomplished by mild auditory and tactile stimulation.¹⁹ Rats were maintained awake by tapping on the side of the cage and gently stimulating the whiskers or the tail using a pencil-sized paintbrush; stimuli were applied every time a sleep posture was observed. After a 9-h period of undisturbed sleep or total sleep deprivation, rats were gently restrained and received a subcutaneous injection (50 μ L) of saline (vehicle) or 5% formalin into the right hind paw. The injections were performed using a 26-gauge needle inserted into the dorsal surface between the medial toes and advanced 2 to 3 mm proximally under the skin before formalin was injected.^{42,44,46–48}

Published concentrations of formalin used in rats range from 1% to 5%.^{42,46–50} The concentration of formalin (5%) used in this study was selected according to the following criteria: (1) the variability of the nociceptive response is inversely correlated with the dose of formalin,⁴⁹ (2) to date, 5% has been the only concentration tested for assessing the time course of late-hyperalgesia (day 1 to week 4) in rat,^{42,47} and (3) all the concentrations within the cited range exert supra-threshold nociceptor stimulation.

Nociceptive Testing

Nociceptive measures obtained from days 1 to 21 post-injection started between 14:00 and 14:30 and were conducted by an investigator blinded to the treatment condition. In each group of rats that started the 21-day protocol, the order of the tests used for assessing mechanical sensitivity and thermal nociception was alternated between testing days; the order was reversed when the next group of rats entered the protocol. This approach was used to eliminate a potential systematic confound caused by a repeated sequence of tests performed in all the rats throughout the study. Rats were allowed 20 to 30 min to habituate to the experiment chambers before each nociceptive test. Room temperature and relative humidity were recorded prior to each testing session. Mechanical sensitivity was assessed using the von Frey test (ascending method) expressed as the threshold for paw withdrawal in grams.^{51–53} Rats were placed in individual Plexiglas chambers on an IITC von Frey mesh stand (Life Science Inc., Woodland Hills, CA, USA). For each determination, six von Frey filaments (2, 4, 6, 8, 10 and 15 g of pressure) were applied in ascending order to the plantar surface of the rat hind paw until bent (Touch Test Sensory Evaluator, North Coast Medical Inc., Gilroy, CA, USA). After the filament bent, it was held for 5 sec or until a withdrawal response occurred. The filaments were applied to the central region of the plantar surface avoiding the foot pads, only when the rat was standing on all four paws. No stimuli were applied if the rat was walking, grooming, or sniffing. Each filament was presented to a hind paw five times with a 30-sec interval between stimuli. Thereafter, the same procedure was repeated to stimulate the contralateral hind paw. A response was considered positive when the hind paw was completely removed from the platform. If withdrawal did not occur during five applications of a particular filament, the next filament in the series was applied. The mechanical threshold was defined as the lowest tension that evoked at least three responses out of five applications.

Thermal hyperalgesia was measured using the Hargreaves' paw withdrawal latency (PWL) method.⁵⁴ As described in detail previously,¹⁹ rats were placed in the testing chambers on a temperature-controlled (30°C) glass floor (IITC Plantar Analgesia Meter, Life Science Inc.). To test for thermal nociception, a light beam was aimed at the plantar surface of a hind paw. The light was then switched to active intensity and a timer was simultaneously started at the onset of the stimulus. As soon as the withdrawal of the paw away from the heat source was observed, the light beam and the timer were deactivated and the latency to withdrawal in seconds was recorded. Four PWL values were obtained from each paw by alternating the stimulus

between the hind paws. The PWL in response to the thermal stimulus was expressed as a percentage change from pre-formalin baseline values (percentage of maximum possible effect [%MPE]⁵⁵) using the following equation: %MPE = (post-injection PWL – baseline PWL) / (cut-off time – baseline PWL) × 100. A cut-off time of 15 sec for the thermal stimulus was chosen to ensure no tissue damage. Positive or negative %MPE values indicate, respectively, longer (i.e., decreased nociception) or shorter (i.e., increased nociception) latencies relative to baseline measures.

Statistical Analyses

Statistical evaluation of the data was performed with input from the University of Michigan Center for Statistical Consultation and Research. Data analyses were performed using Statistical Analysis System (SAS) version 9.3 (SAS Institute, Cary, NC, USA) and PRISM v6.0c (GraphPad Software, La Jolla, CA, USA). All data were tested for normality. Data are reported as mean ± standard error of the mean, and $P < 0.05$ was considered statistically significant. Differences in mechanical sensitivity and thermal nociception between *ad libitum* sleep + saline, *ad libitum* sleep + formalin, sleep deprivation + saline, and sleep deprivation + formalin groups were assessed using a linear mixed model.

Measures of mechanical threshold in *ad libitum* sleep + formalin versus sleep deprivation + formalin groups (injected and non-injected paw) were averaged across time (day 1 to 21 post-injection) and differences were assessed using an unpaired *t*-test. The synergy index was calculated using the mean mechanical threshold per group to determine whether there was a synergistic interaction^{56–58} between sleep deprivation and formalin-induced pain. The index was obtained using the following equation: $S = [RR_{11} - 1] / [(RR_{10} - 1) + (RR_{01} - 1)]$, where RR_{11} represents the relative risk of both factors (sleep and formalin) present, and RR_{10} or RR_{01} indicate that one factor is present and the second one is absent.⁵⁹ A synergy index of 1 means no interaction, whereas > 1 means that there was a synergistic interaction. Differences in the time course of mechanical sensitivity and thermal nociception, between injected and non-injected paw, in the sleep deprivation/recovery + formalin group were evaluated using a two-way analysis of variance (ANOVA) test. *Post hoc* tests comparing the means were adjusted for multiple comparisons using a Tukey multiple comparisons test. Differences in the time course of mechanical sensitivity in sleep deprivation + formalin versus sleep deprivation/recovery + formalin groups (expressed as percent from mean baseline values) were evaluated by regression analysis, and by a two-way ANOVA followed by a multiple comparisons Tukey procedure. Last, mechanical thresholds in *ad libitum* sleep + formalin, sleep deprivation + formalin, and sleep deprivation/recovery + formalin groups were averaged across time and the means were assessed using a one-way ANOVA followed by unpaired *t*-tests with Bonferroni correction.

RESULTS

The injection of formalin caused a typical biphasic response including nociceptive behaviors such as elevating, licking, and shaking the injected paw. These pain behaviors (not quantified)

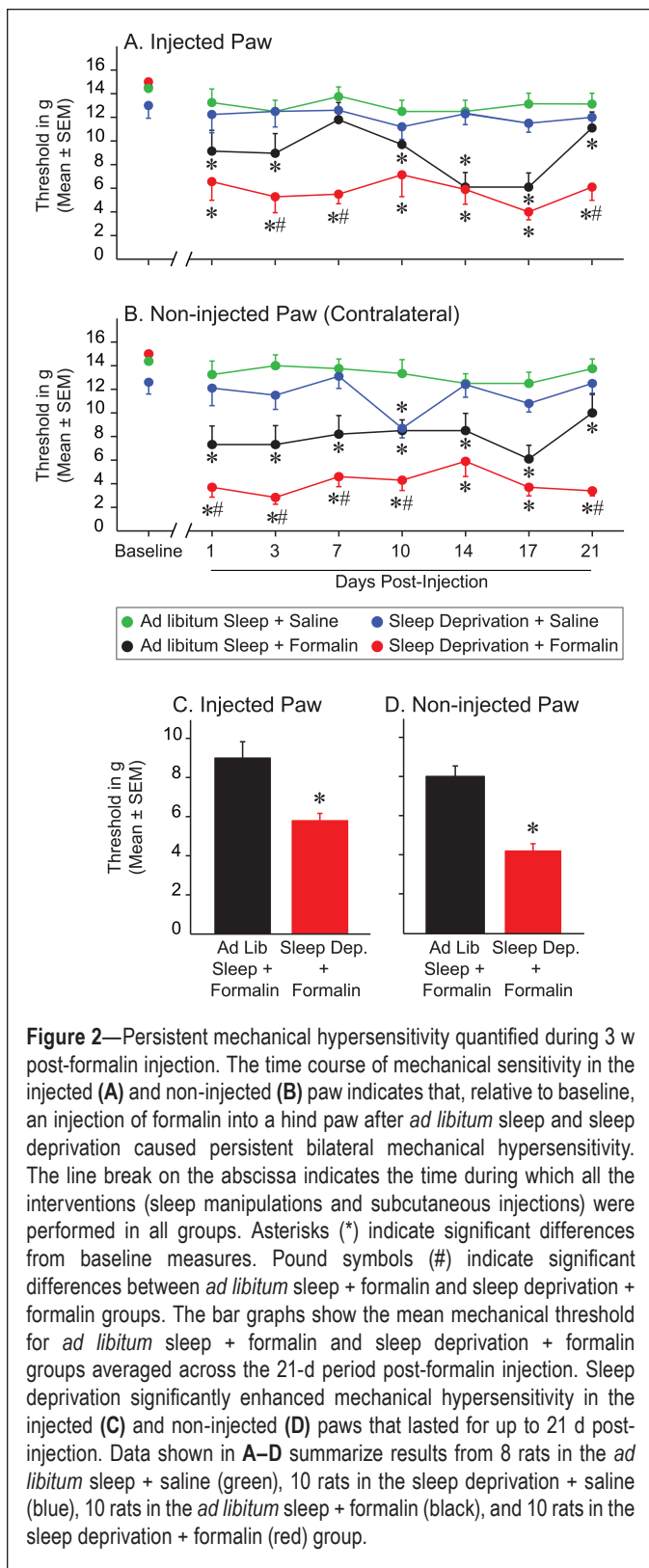


Figure 2—Persistent mechanical hypersensitivity quantified during 3 w post-formalin injection. The time course of mechanical sensitivity in the injected (A) and non-injected (B) paw indicates that, relative to baseline, an injection of formalin into a hind paw after *ad libitum* sleep and sleep deprivation caused persistent bilateral mechanical hypersensitivity. The bar graphs (Figures 2C and 2D) plot the mean threshold for mechanical stimulation averaged across day 1 to 21 and show that sleep deprivation significantly enhanced mechanical hypersensitivity in the injected (C) and non-injected (D) paws that lasted for up to 21 d post-injection. Data shown in A–D summarize results from 8 rats in the *ad libitum* sleep + saline group, 10 rats in the sleep deprivation + saline group, 10 rats in the *ad libitum* sleep + formalin group, and 10 rats in the sleep deprivation + formalin group.

lasted up to 60 min post-formalin injection. Thereafter, no signs of spontaneous pain or distress were observed; rats behaved normally including sleeping, feeding, and grooming. Consistent with previous reports of adverse consequences after formalin injection,^{42,47} mild edema in the injected paw developed in all rats; the edema resolved within the first 5 days

post-injection. None of the rats had signs of infection in the injection site, and all rats showed normal increasing weight trends during the experimental protocol.

Persistent Mechanical Hypersensitivity Caused by Formalin Injection was Enhanced by Previous Sleep Deprivation

Figure 2 illustrates the time course of the mechanical threshold quantified bilaterally during 3 w post-injection. Two-way ANOVA revealed a significant main group effect in the injected ($F = 16.77$; $df = 3, 238$; $P < 0.0001$) and contralateral ($F = 37.72$; $df = 3, 238$; $P < 0.0001$) limb. Formalin injection caused bilateral mechanical hypersensitivity that persisted during the 21-d pain testing period. Figure 2A shows that relative to the *ad libitum* sleep + formalin group, sleep deprivation (+ formalin) caused a significant decrease in the mechanical threshold (increased mechanical hypersensitivity) in the injected paw on days 3, 7, and 21 post-injection. Figure 2B shows that relative to the *ad libitum* sleep + formalin group, sleep deprivation caused a significant decrease in the mechanical threshold (increased mechanical hypersensitivity) in the non-injected paw on days 1, 3, 7, 10, and 21 post-injection. The bar graphs (Figures 2C and 2D) plot the mean threshold for mechanical stimulation averaged across day 1 to 21 and show that sleep deprivation significantly ($t = 3.267$; $df = 12$; $P = 0.034$, injected paw and $t = 5.792$; $df = 12$; $P < 0.0001$, non-injected paw) worsened mechanical hypersensitivity. In the sleep deprivation + saline group, there was no significant effect on mechanical sensitivity that persisted over the course of the testing period. The synergy index for the injected and non-injected paw was 1.196 and 1.396, respectively, and thus revealed that mechanical sensitivity in the sleep deprivation + formalin group was greater than the expected from the addition of measures from the sleep deprivation + saline and *ad libitum* sleep + formalin group. Figure 3 shows the time course of %MPE (thermal nociception) in the injected (Figure 3A) and non-injected (Figure 3B) paw. Relative to *ad libitum* sleep + saline, formalin injection (*ad libitum* sleep + formalin) caused a significant increase in %MPE during days 1 and 3 post-injection. There was no persistent effect on thermal nociception due to injection or sleep deprivation.

Allowing a Recovery Sleep Period Before Formalin Injection Diminishes Post-Injection Pain Levels and Duration

Figure 4 depicts the time course of bilateral mechanical thresholds and %MPE during the 21-day testing period in a group of rats that underwent sleep deprivation and were allowed a 24-h recovery period prior to receiving an injection of formalin into a hind paw. Two-way ANOVA indicated a significant effect of condition ($F = 32.96$; $df = 7,144$; $P < 0.0001$), time effect ($F = 13.48$; $df = 7,144$; $P < 0.0001$), and condition by time interaction ($F = 8.89$; $df = 7,144$; $P < 0.0001$). *Post hoc* tests revealed that, relative to baseline, formalin caused a significant decrease in mechanical hypersensitivity in the injected paw that persisted until day 10 post-injection (Figure 4A). There were no persistent changes in the threshold for mechanical stimulation in the paw contralateral to the injection side (Figure 4A), nor bilateral changes in the responses to thermal stimulation (Figure 4B).

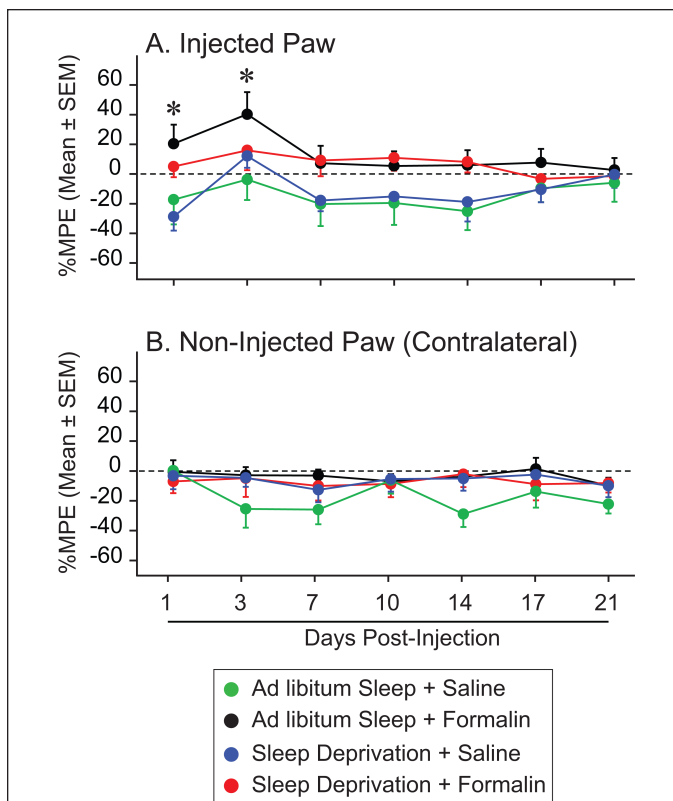


Figure 3—Time course of thermal nociception in the injected (A) and non-injected (B) paw during 3 w post-formalin injection. Relative to *ad libitum* sleep + saline, formalin injection caused a significant increase in the percentage of maximum possible effect (%MPE) during days 1 and 3 post-injection. There was no persistent effect on thermal nociception due to injection or sleep deprivation beyond 3 d post-injection. The dotted line at 0 %MPE represents baseline (pre-experiment) levels of thermal nociception. The number of rats (n) per group is the same as in Figure 2.

Figure 5A shows the time course of ipsilateral (i.e., injected paw) mechanical hypersensitivity in sleep deprivation + formalin and sleep deprivation/recovery + formalin groups, expressed as the mean percent change from baseline levels. Two-way ANOVA revealed a significant effect of condition ($F = 37.11$; $df = 1,144$; $P < 0.0001$), time effect ($F = 16.85$; $df = 7,144$; $P < 0.0001$), and condition by time interaction ($F = 7.26$; $df = 7,144$; $P < 0.0001$). Rats allowed a 24-h interval (recovery sleep) before receiving a formalin injection had a shorter time to recovery than those in the sleep deprivation + formalin group (i.e., not allowed a recovery sleep interval prior to formalin injection); levels of mechanical hypersensitivity in the injected paw were significantly lower on days 14 ($P = 0.001$), 17 ($P < 0.0001$) and 21 ($P < 0.0001$) post-injection. Regression analysis indicates that in the sleep deprivation/recovery + formalin group there was a significant ($F = 44.12$; $df = 1,5$; $P = 0.0012$) linear reduction in mechanical hypersensitivity during the post-injection period. Post-injection time accounted for 90% ($R^2 = 0.8982$) of the variance in mechanical sensitivity. The slope of the linear regression function for mechanical hypersensitivity in the sleep deprivation + formalin group was not significantly ($F = 0.31$; $df = 1,5$; $P = 0.6$) different from zero.

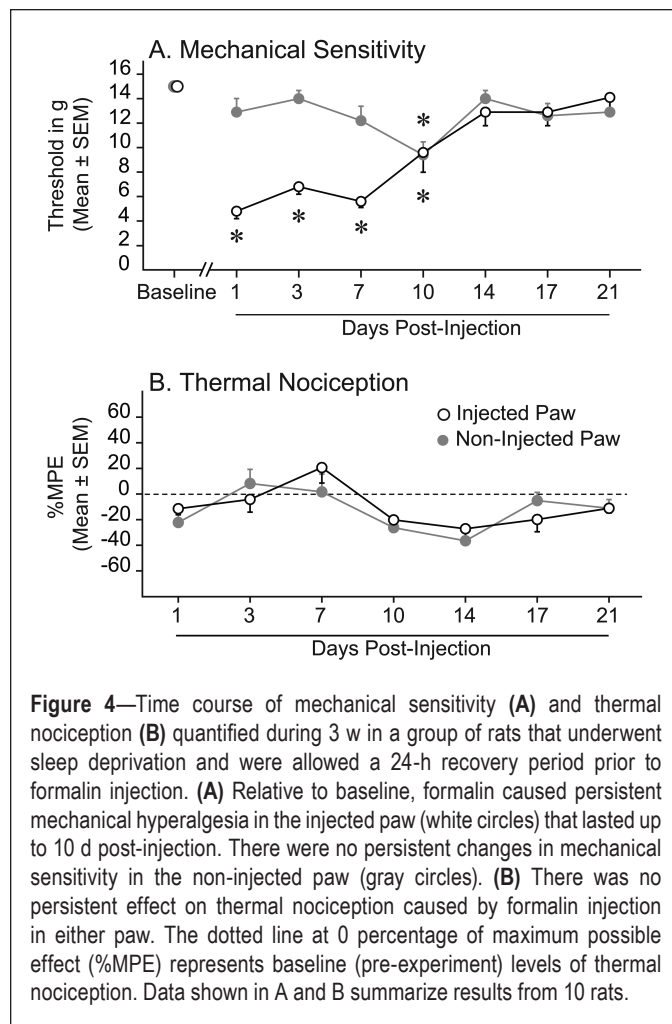


Figure 4—Time course of mechanical sensitivity (A) and thermal nociception (B) quantified during 3 w in a group of rats that underwent sleep deprivation and were allowed a 24-h recovery period prior to formalin injection. (A) Relative to baseline, formalin caused persistent mechanical hyperalgesia in the injected paw (white circles) that lasted up to 10 d post-injection. There were no persistent changes in mechanical sensitivity in the non-injected paw (gray circles). (B) There was no persistent effect on thermal nociception caused by formalin injection in either paw. The dotted line at 0 percentage of maximum possible effect (%MPE) represents baseline (pre-experiment) levels of thermal nociception. Data shown in A and B summarize results from 10 rats.

A statistical comparison between the regression function in the sleep deprivation + formalin group and that one in the sleep deprivation/recovery + formalin group revealed that both slopes were significantly ($F = 30.72$; $df = 1,10$; $P = 0.00025$) different.

A one-way ANOVA revealed a significant ($F = 4.08$; $df = 2,18$; $P = 0.035$) effect of sleep deprivation in mechanical hypersensitivity post-formalin injection (Figure 5B). Relative to *ad libitum* sleep + formalin, the mean mechanical threshold averaged across days 1 to 21 post-injection was significantly ($t = 3.379$; $df = 12$; $P = 0.0027$) reduced by sleep deprivation. There was no significant ($t = 0.312$; $df = 12$; $P = 0.38$) difference in mechanical sensitivity between the *ad libitum* sleep + formalin and sleep deprivation/recovery + formalin groups. Last, a sleep recovery period prior to formalin prevented the increase in mechanical hypersensitivity ($t = 2.491$; $df = 12$; $P = 0.0142$) caused by sleep deprivation (sleep deprivation + formalin versus sleep deprivation/recovery + formalin).

DISCUSSION

This study showed that a subcutaneous injection of formalin causes long-lasting mechanical hypersensitivity (i.e., mechanical allodynia, defined as pain evoked by a normally innocuous tactile stimulus) in the injected limb as well as in sites distant to the injection site. The data demonstrate that acute total sleep deprivation preceding a noxious inflammatory insult

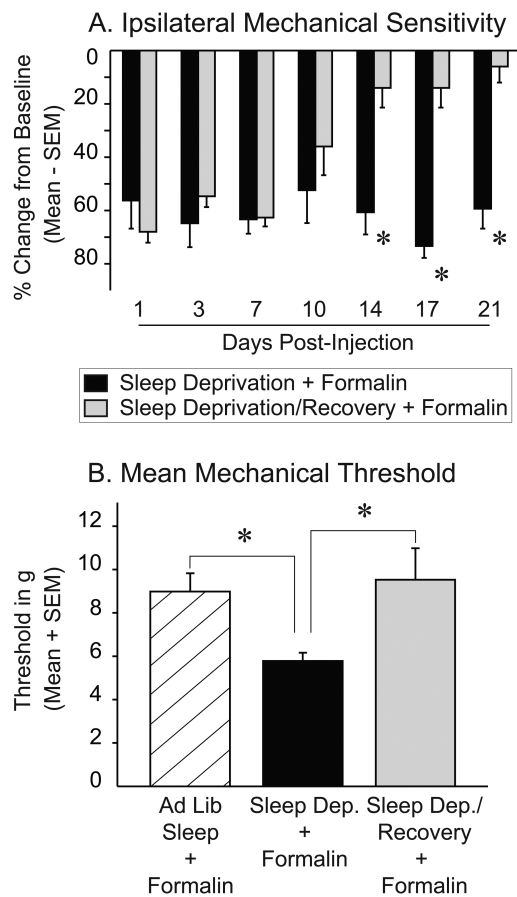


Figure 5—Duration and mean threshold of mechanical hypersensitivity in rats that underwent sleep deprivation and were allowed a 24-h recovery period prior to formalin injection. **(A)** Time course of mechanical hypersensitivity in the injected paw expressed as mean percent change from baseline levels. Relative to sleep deprivation + formalin (black bars), the duration of mechanical hyperalgesia was significantly shorter in the group that was allowed a recovery period between sleep deprivation and a formalin injection (gray bars). **(B)** Mean mechanical sensitivity averaged across days 1 to 21 post-formalin injection. Sleep deprivation significantly enhanced mechanical hyperalgesia caused by a formalin injection. A recovery period after sleep deprivation diminished nociception by preventing the mean decrease in the mechanical threshold caused by formalin injection.

significantly worsens mechanical hypersensitivity caused by formalin; the effect of a single episode of sleep deprivation caused a long-lasting effect. There was a synergistic effect on post-inflammatory mechanical hypersensitivity when sleep deprivation preceded the injection of formalin. Last, the results also showed that a recovery period prior to a noxious insult diminished mechanical hypersensitivity, which manifested in the injected limb only. The following paragraphs discuss the importance of sleep in chronic pain, the animal model, translational relevance and limitations.

Sleep loss is a growing public health concern.¹ Accumulating evidence indicates that sleep loss is associated with a wide range of health issues including chronic cardiovascular¹⁰ and metabolic disease,¹² obesity,¹³ cancer,⁶⁰ mental health disorders,¹⁴ as well as chronic pain.¹⁸ Chronic pain is

another major health care problem worldwide,^{61,62} with a significant economic burden and negative effect on the quality of life. Several lines of evidence indicate that sleep and pain have bidirectional interactions. Data from preclinical^{19,20,22,63} and human^{17,27,30,31,33,39,64,65} studies show that poor sleep quality, experimental sleep restriction and sleep deprivation enhances pain. Conversely, a long list of evidence supports the view that pain disrupts sleep.^{21,23,24,34–36,66–68} The current study shows that acute sleep deprivation worsens nociception caused by a formalin injection (Figure 2), lending additional support to the interpretation that sleep loss increases pain. Furthermore, human data suggest that sleep loss is a risk factor for developing chronic pain.^{18,32,40,41} Figure 2 illustrates the long-lasting negative impact of sleep deprivation on inflammatory-induced nociception subsequent to formalin injection. While the mechanical threshold in both *ad libitum* sleep + formalin and sleep deprivation + formalin group was significantly lower than their respective baseline levels, measures of mechanical sensitivity in the *ad libitum* sleep + formalin group on day 21 showed a trend to recovery (Figure 2). Taken together, these data suggest that acute sleep loss preceding an inflammatory insult may prolong the recovery period and potentially contribute to the development of chronic pain. The concept that a single episode of sleep deprivation can contribute to persistent allodynia is in line with extensive evidence showing that even short-term sleep deprivation can alter molecular, cellular, and network mechanisms that translate into long-term behavioral changes.^{69–72} Contrary to the detrimental effects of sleep deprivation, a recovery period from sleep deprivation allowed prior to formalin injection had a beneficial effect on nociceptive levels and duration. In the group allowed a sleep recovery period, mechanical hypersensitivity developed in the injected paw only (Figure 4A), had significantly shorter duration (10 versus 21 days, Figure 5A) and lower intensity than in the sleep deprivation + formalin group (Figure 5B). Notably, human data also show that a behavioral intervention used to increase sleep time in sleepy but otherwise healthy adults can successfully reduce pain sensitivity.²⁸ The translational relevance of the current study is supported by evidence that persistent post-surgical pain affects 10% to 50% of patients.⁷³ Chronic sleep problems before surgery are a strong predictor of postoperative pain.⁷⁴ The current study shows that acute sleep loss can negatively impact the intensity and duration of pain after an inflammatory insult. Hence, the results reported here underscore the importance of preoperative sleep management in the care of surgical patients. These data encourage future studies extending these findings to a model of surgical pain, investigating the mechanisms underlying the effects of sleep deprivation and recovery sleep on pain, and testing pre-emptive sleep interventions aimed at improving pain outcomes.

This study used a rodent model of somatic persistent inflammatory pain^{42,46–48,50} to examine the complex bidirectional interactions between sleep and chronic pain. As described in the Results section, a subcutaneous injection of formalin causes a number of nociceptive behaviors that last approximately 1 h, and are classically divided into an early phase and a late phase. Importantly, the presence of the early phase is key for development of the late phase. For example, preemptive

pharmacologic interventions, administration of volatile anesthetics, or brain stimulation to cause analgesia performed before the early phase attenuate or eliminate the late phase.^{47,75} Thus, the preceding evidence of confounding actions of inhaled anesthetics precluded the administration of brief general anesthesia for the injection of formalin in the current study. In the formalin model, the late phase as well as the subsequent prolonged allodynia and hyperalgesia mimic the symptoms of neuropathic pain in humans.^{42,46,47} Fu et al.⁴² reported that nociceptive responses to mechanical and thermal stimulation remained altered for 3 to 4 w after formalin injection. In the current study, formalin (*ad libitum* sleep + formalin) caused bilateral hypersensitivity evoked by mechanical stimulation that persisted during the 3-w testing period (Figure 2). However, there were no long-lasting changes in thermal nociception (Figure 3, day 1 to 21). Relative to the study by Fu et al.,⁴² there are two methodological differences that may account for the absence of persistent thermal hyperalgesia in the injected paw reported here. First, Fu et al.⁴² used a modified hot plate with the temperature set at 43.8°C, just 0.2°C above the measured threshold for noxious thermal stimulation. In contrast, the thermal stimulus in the Hargreaves' method rises up to 100°C when switched from idle to active intensity. Thus, the stimulus delivered with the Hargreaves' method is likely at the top of the intensity-response curve. The second difference is that each study used different behavioral measures (number of nociceptive behaviors⁴² versus latency to paw withdrawal) to assess thermal nociception. The disparity in the results reported here and those in the study by Fu and colleagues⁴² suggests that a different test may be needed for identifying changes in thermal hyperalgesia with the experimental protocol used in this study. The widespread mechanical (cutaneous) hypersensitivity, without long-lasting changes in thermal hyperalgesia in the formalin model fits well with the clinical presentation of many syndromes characterized by chronic centralized pain,⁷⁶ in which mechanical hyperalgesia is a more consistent finding than thermal hyperalgesia.⁷⁷

The prolonged duration of the allodynic response, relative to the relatively short duration (< 5 d) of peripheral inflammatory manifestations (skin flare, blisters, and paw edema), suggests a central mechanism. This notion is also supported by the bilateral nature of mechanical hypersensitivity illustrated in Figure 2, as well as by evidence showing that central changes in neural excitability underlie the late phase after formalin injection.^{46,47,49,50,78,79} Increased activation of primary sensory neurons caused by intense noxious peripheral stimulation can trigger a long-lasting increased excitability and synaptic efficacy of dorsal horn neurons, a phenomenon termed central sensitization.^{80,81} The net result of this activity-dependent plasticity is an increased gain of central nociceptive pathways, causing regional pain hypersensitivity that outlasts the duration of the initial stimulus (Figure 2). Furthermore, electrophysiological studies revealed that induction of long-term potentiation in spinal nociceptive neurons induces a long-lasting increase in the excitability of thalamo-cortical networks.⁸² These data support the interpretation that sustained nociceptor activation can induce changes in the excitability of pain pathways at both spinal and supraspinal levels. Consistent with a role for supraspinal nuclei in the pain

phenotype, several studies indicate that descending modulation of spinal mechanisms contribute to maintain central sensitization induced by peripheral formalin injection.^{48,78,83–85} Data from studies in humans suggest that augmentation of peripheral sensory input by abnormal activation of pain-processing brain areas is also a common underlying mechanism in several conditions with centralized pain.^{76,86,87}

The finding that acute sleep deprivation enhanced post-formalin nociception (Figure 2) suggests that sleep loss prior to an inflammatory insult can contribute to chronic pain by increasing the propensity to develop central sensitization. Several studies are congruent with the conclusion that sleep deprivation increases cortical and thalamic excitability.^{88–94} Microdialysis data show state-specific changes in neurotransmitter levels within the spinal cord,^{95–97} and sleep deprivation causes mechanical⁹⁸ and thermal⁹⁹ hypersensitivity by altering the neurochemical milieu within spinal sensory regions.^{98,99} Taken together, the data reviewed above support the hypothesis that sleep deprivation can facilitate and maintain central sensitization by altering descending (cortico-spinal) and ascending mechanisms that process or modulate pain.

Another relevant result from this study was that post-formalin levels, extension and duration of mechanical allodynia were significantly reduced in the group of rats allowed a sleep recovery period after sleep deprivation (Figures 4A, 5A, and 5B). Sleep deprivation increases neural excitability,^{88–94} and recovery sleep is expected to reduce the increase in excitability brought about by sleep deprivation. However, the magnitude of changes in post-formalin nociception (Figures 4A, 5A, and 5B) is consistent with the hypothesis that state-specific mechanisms may also limit the development of long-lasting diffuse allodynia in the formalin model. The brain regions and mechanisms that mediate sleep-induced changes in pain regulation remain to be elucidated. The ventrolateral column of the periaqueductal gray contributes to regulate states of sleep and wakefulness,^{100–105} and controls spinal nociceptive mechanisms via projections to the rostral ventromedial medulla.^{106–109} Future research will examine the periaqueductal gray – rostral ventromedial medulla – spinal cord pathway as a potential neural substrate underlying the effects of sleep loss and recovery on pain.

Limitations and Conclusions

This study was limited by the use of methods that assessed only the nociceptive component of pain. The present results encourage future studies using non-reflexive methods for evaluating sleep,¹¹⁰ cognitive and affective responses to sleep challenges, pain, and their interactions. Comparisons between different pain models, extending the testing period until full recovery will also be critical for testing the effect of sleep on post-insult pain duration. Another limitation is that the current experiments did not examine sex-related differences^{111,112} in the interactions between sleep and post-insult pain. Stress responses to sleep deprivation vary depending on the method used to maintain wakefulness.^{113,114} Future studies can address these limitations by including female rats, and determining to what extent stress plays a role in pain in the current experimental paradigm.

The results support the conclusion that sleep loss preceding an inflammatory insult worsens subsequent pain levels and can increase the susceptibility for that pain to persist. This study also suggests that enhanced sleep may be beneficial by reducing the intensity and duration of pain that can develop and persist after a noxious insult. These findings form the foundation for future studies to clarify the mechanisms by which sleep loss and recovery alter pain. Last, this study provides additional evidence in support of a link between insufficient sleep and pathology.

ABBREVIATIONS

ANOVA, analysis of variance
 %MPE, percentage of maximum possible effect
 NREM, nonrapid eye movement
 PWL, paw withdrawal latency
 REM, rapid eye movement
 SEM, standard error of the mean

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