Two Modalities of Orthopedic Pain Produce Unique Sleep Phenotypes

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INTRODUCTION: Sleep and pain have a well-established negatively reinforcing relationship; but this relationship is poorly understood in orthopedic models. Chronic pain can degrade sleep which, in turn, exacerbates pain experience. This relationship likely exists in many orthopedic diseases as chronic pain is often a prominent component for patients to seek medical care. There is a growing body of literature confirming patient sleep is disrupted in low back pain and joint pain conditions [1,2]. These data are predominantly collected by clinical questionnaires unable to capture sufficient detail to understand the role of sleep in development and maintenance of pain. Thus, managing both disrupted sleep and chronic pain in orthopedic patients is challenging and improved understanding of the relationship between sleep and pain in orthopedic models is needed to improve patient care.

Sleep disturbances in orthopedic patients is highly variable. Some of this variability may originate from complex pain modalities, specifically neuropathic or inflammatory pain. Neuropathic and inflammatory pain affect different nerve pathways with cognitive experiences manifesting in unique physical and psychological experiences. Inflammatory pain activates intended nociceptive pathways which productively communicate pain to enact protective measures. Neuropathic pain derives from damaged neural tissue which can recruit unintended nociceptive and sensory nerve pathways resulting in unpredictable locations of pain sensations that can be spontaneous or evoked. The objective of this work is to utilize preclinical models of these two pain modalities to investigate the unique ways sleep may be disrupted. We hypothesize that the sleep disruptions associated with chronic pain will present phenotypic differences between inflammatory and neuropathic modalities.

METHODS: Adult C57b/6 mice (n=30, female) were pair-housed under standard 12:12 hour light-dark cycles with ad libitum food and water. All animals were anesthetized (2-3% isoflurane) and prepared for aseptic surgery. To model neuropathic pain, animals then received either a T10 contusion (50 kdyne, IH-400 Infinite Horizon Impactor) spinal cord injury (SCI, n=7), a T10 laminectomy sham (LAMX, n=5), or no procedure (NAÏVE, n=6). To model inflammatory knee pain, animals received either an intra-articular knee injection of monoiodoacetate (MIA, n=6, 0.2 mg/10ul) or phosphate buffered saline (SALINE, n=5, 10ul). SCI and LAMX animals received 48 hours of post-operative meloxicam (2 mg/kg, twice daily), Baytril (2.5 mg/kg, daily), and supplemental hydration (0.5 ml sterile saline daily). Bladders were expressed twice daily as needed until animals voided independently.

Animals were acclimated to experimenters and equipment prior to behavioral studies. Baseline 3-stage sleep (wake, REM sleep, and non-REM sleep) and hind paw mechanical pain sensitivity (50% paw withdrawal threshold) were recorded before surgery and weekly for 6 weeks post-procedure to model chronic timelines. Sleep was noninvasively measured in animal homecages during the 12-hour dark cycle using novel electric field (EF) sensors developed by our group and previously validated against gold standard electroencephalogram (EEG) + electromyograph (EMG). EF sensors can passively translate animal movement into a voltage trace waveform from outside an animal homecage. Resulting voltage trace waveforms were scored for 3-stage sleep to calculate the following measures: percent time asleep, micro-arousal index (brief arousals/hour), sleep fragmentation index (# sleeping bouts/hour), average duration of sleep bouts, average duration of REM sleep bouts, percent time spent in REM sleep, and REM sleep latency. After 6 weeks, animals were euthanized then knee joints were dissected, processed for paraffin embedding, sectioned, and stained with toluidine blue (MIA, n=4, Saline n=3). Knee inflammation was measured via medial joint capsule width [3]. Neuropathic pain was confirmed in these SCI animals in another study through terminal electrophysiological experiments which measured spontaneous afferent firing activity in dorsal root ganglia [4]. Group means were analyzed using multifactorial ANOVAs with Tukey's HSD post-hoc test and correlations made using general linear regression models.

RESULTS: At 6 weeks post-procedure, both inflammatory (MIA, $p<0.001$) and neuropathic (SCI, $p<0.001$) pain models developed mechanical hypersensitivity relative to control groups. The neuropathic pain model at 6 weeks showed higher measures for sleep fragmentation ($p<0.001$), shorter average sleep events ($p<0.001$), and a higher micro-arousal index ($p<0.001$) compared to control groups. Medial joint capsule width was significantly larger in the inflammatory pain model relative to control ($p<0.01$). Mechanical hypersensitivity correlated with increased sleep fragmentation in both models (MIA: $p<0.05$, $R^2=0.15$ and SCI: $p<0.01$, $R^2=0.20$, **Figure 1A and 1B**). Furthermore, SCI group decreased sleep bout duration ($p<0.001$, $R^2=0.40$), REM sleep duration ($p<0.001$, $R^2=0.32$, **Figure 2**), and REM sleep latency ($p<0.001$, $R^2=0.35$) correlated with mechanical hypersensitivity. MIA animal percent time asleep tended to decreased with development of mechanical hypersensitivity ($p<0.05$, $R^2=0.12$).

DISCUSSION: These findings suggest inflammatory pain (MIA group) and neuropathic pain (SCI group) may associate with different sleep disruptions, with more severe sleep changes developing after SCI. With a thoracic level contusion, no central sleep control mechanisms were directly damaged, but SCI animals still developed fragmented sleep. Sleep changes in MIA animals were more subtle, but observed correlative trends suggest potential phenotypes with conserved sleep bout composition but altered percentage of time spent asleep might develop at longer timepoints. Importantly, mechanical hypersensitivity between MIA and SCI animals was not different, suggesting a similar evoked mechanical pain severity experience in both groups. This suggests non-evoked or non-inflammatory components of neuropathic pain may explain SCI some of the different in sleep changes between the models.

SIGNIFICANCE: Orthopedic pain has complex effects on the body and chronic pain even more so; separating the effects that different phenotypes of pain can have on sleep can improve patient outcomes by focusing treatment dependent on the type of chronic pain experienced.

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IMAGES:

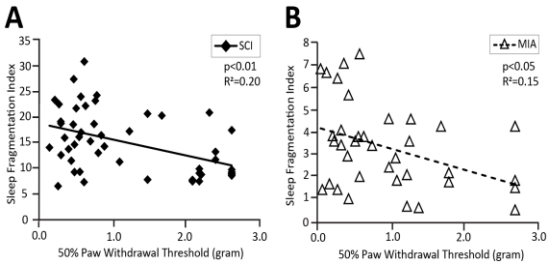

Figure 1: Sleep Becomes Fragmented with Mechanical Hypersensitivity. A) SCI animals. B) MIA animals.

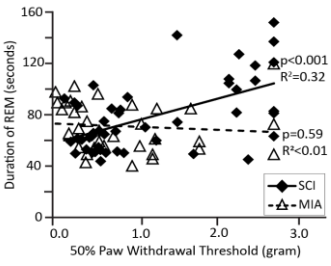

Figure 2: REM Duration Decreases with Mechanical Hypersensitivity After SCI but Not MIA.