

Hippocampal Hypersensitivity Associates with Joint Inflammation, Sleep Changes, and Mechanical Sensitivity

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INTRODUCTION: Approximately 25% of adults in the US grapple with knee pain¹, a number steadily climbing in tandem with the growing burden of debilitating chronic pain, which significantly affects their physical and emotional well-being. This concern also imposes a substantial financial strain on healthcare, surpassing \$250 billion—individuals enduring chronic pain frequently report associated sleep disturbances, compounding their pain experience. In addition, chronic pain and poor sleep can individually bring about changes in the central nervous system (CNS) functions, exacerbating concurrent mental health conditions. Unfortunately, this trio of factors is inadequately understood due to limited research that includes evaluation of all three: pain, sleep, and CNS metrics. Better understanding the interplay between pain, sleep, and CNS changes will improve clinical care of orthopedic patients with chronic pain and development of new pain management therapies.

While various CNS changes arise in response to ongoing pain and inadequate sleep, one particular region, the hippocampus, exhibits heightened sensitivity. The hippocampus, a delicate brain structure primarily responsible for memory and learning processes, can be adversely affected by sleep deprivation and chronic pain. This impairment extends to memory function and the hippocampus's capacity to adapt to stimuli, known as neuroplasticity. Neuroplasticity comes in two primary forms: long-term potentiation (LTP), which bolsters synaptic connections, and long-term depression, which weakens them. Although the connection between chronic pain and hippocampal LTP has been thoroughly investigated using neuropathic pain models, its understanding in other pain models, like joint pain, remains limited. These alternative models may exhibit distinct sleep, pain, and hippocampal activity interplays. Consequently, this research aims to utilize a preclinical model of inflammatory knee pain to comprehensively examine the experience of pain, sleep quality, and hippocampal LTP.

METHODS: To model inflammatory knee pain, adult c57b/6 mice were prepared for aseptic surgery, anesthetized (2-3% isoflurane), and received a 10 μ L intra-articular injection of either moniodoacetate (0.2mg/10 μ L MIA, n=5) or saline control (n=4). Hind paw mechanical sensitivity and three-stage sleep patterns (wake, non-rapid-eye-movement (REM) sleep, and REM sleep) were measured after 6 weeks as previously described². Sleep was evaluated through innovative, noninvasive electric field motion sensors conceived and validated by our group³.

After 6 weeks, terminal electrophysiology experiments evaluated left and right hippocampus LTP. Animals were deeply anesthetized with isoflurane prior to euthanasia. Brains were dissected, chilled in Ca²⁺ free artificial cerebrospinal fluid (ACSF) bubbled with 95% O₂/5% CO₂, mounted, coronally sectioned (350 μ m, Bregma -1.94 mm), then stabilized for 120 minutes in ACSF at 35°C. Sections were immobilized on an 8x8 microelectrode array (MEA, MED-A64MD1, Alpha Med Scientific) and imaged to confirm placement. Visual confirmation of evoked field excitatory postsynaptic potentials (fEPSP) dictated the best test stimulation (-40 μ A, 10s duration) site to assess hippocampal LTP. Baseline responses were recorded for 30 minutes prior to being tetanized (10-30 μ A bursts, 100 pulses for 1 second, 100 Hz, 50% amplitude). fEPSP responses were observed for 60 minutes to determine early LTP responses and fEPSP slope was calculated for each waveform and normalized to baseline. At euthanasia, knees were dissected and processed for paraffin embedding to histologically evaluate joint damage. Knees were sectioned (10 μ m), mounted, stained with toluidine blue, and scored for synovial inflammation (medial joint capsule width) according to the Osteoarthritis Research Society International recommendations⁴. Mean differences were analyzed using students t-tests and correlations were calculated using a general linear regression model.

RESULTS SECTION: Six weeks post-injection, MIA-treated animals exhibited heightened mechanical sensitivity ($p < 0.001$) and a decreased percentage of time spent in REM sleep ($p < 0.009$) compared to the sham control group. MIA-treated animals displayed hyperexcited fEPSP slopes immediately after tetanus stimulation ($p < 0.023$) relative to the saline controls. While there were no discernible group differences during early LTP, some MIA animals (3/5) and a portion of the saline animals (1/2) maintained plasticity.

Mechanical hypersensitivity correlated with post-tetanus hyperexcitability ($p < 0.08$, $R^2 = 0.49$, **Figure 1A**) and percent time spent in REM sleep ($p < 0.001$, $R^2 = 0.92$, **Figure 1B**). Wider synoviums (an indicator of joint inflammation) correlated with post-tetanus hyperexcitability ($p < 0.11$, $R^2 = 0.43$, **Figure 1C**) and percent time spent in REM sleep ($p < 0.059$, $R^2 = 0.54$, **Figure 1D**). Lastly, mechanical hypersensitivity also strongly correlated with wider synoviums ($p < 0.038$, $R^2 = 0.61$). This correlation highlighted that as mechanical sensitivity increased, there was a corresponding rise in the extent of joint inflammation.

DISCUSSION: These results unveil a direct relationship between mechanical sensitivity, sleep disruption, and post-tetanus hyperexcitability. Specifically, heightened mechanical sensitivity correlated with measures of premature arousals (reduced REM), increased inflammation, and increased neural hyperexcitability. Inflammatory knee pain might be activating somatic pathways potentially incited evoked pain responses during active REM sleep and causing premature arousal. Notably, REM sleep plays a crucial role in neural well-being, thus any impairment might aggregate and compromise CNS activity, such as the observed hippocampal hyperexcitability.

SIGNIFICANCE: Together, these findings are the first to explore the complex interconnectedness of sleep, pain, and CNS plasticity within a joint pain model. Further work will unravel this complex relationship with the potential to yield enhanced alternative pain treatments, such as chronotherapies, particularly relevant to orthopedic patients.

REFERENCES: ¹Nguyen +, 2011, PMID: 22147711;
²Chaplan +, 1994, PMID: 7990513; ³Kloefkorn +, 2020 PMID: 32619585; ⁴Gerwin +, 2010, PMID: 20864021.

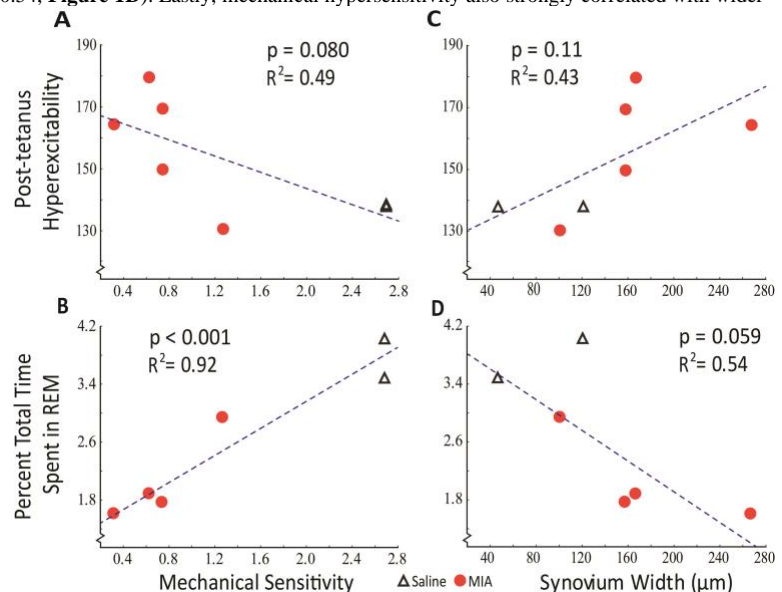


Figure 1: Correlations Between Chronic Pain, Sleep, and Hippocampus Activity. A&B) Mechanical hypersensitivity correlates with hippocampal hyperexcitability and reduced REM sleep percentage. C&D) Wider synoviums (indicating increased inflammation) correlate with hippocampal hyperexcitability and reduced REM sleep percentage. All points represent animal means.