Temporally Characterizing Sleep Features in a Preclinical Low Back Pain Model

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INTRODUCTION: There is a strong bidirectional relationship between low back pain (LBP) and sleep disruption, with over 50% of LBP patients reporting sleep disturbances [1]. Clinically, inadequate quality sleep and enhanced pain can have profound negative impacts on quality of life if unaddressed. Though this relationship has been observed in clinical cohorts, additional studies are needed to elucidate the directional and causal mechanisms linking sleep and pain. Recent studies have identified potential mediators to the sleep-pain relationship, including anxiety, stress, and fatigue, all of which create barriers to effective pain management [2]. Our lab conducted an in-depth longitudinal study aimed at assessing how sleep features are altered with low back pain-like progression in a preclinical model. We **hypothesized** that sleep quality would degrade post significant pain-like onset and would correspond with increased anxiety-like behaviors. To our knowledge, this is the first study to noninvasively characterize sleep quality in a preclinical LBP model. This study provides a foundation for understanding a potential pathway by which improved sleep can mitigate LBP symptoms.

METHODS: All procedures were approved by the Arizona State University and University of Nebraska-Lincoln Institutional Animal Care and Use Committees. The data shown encompasses two separate studies. Both studies utilized male (M) and female (F) adult Sprague-Dawley rats, with injury consisting of an L5-L6 annular puncture and scrape, further described in Lillyman et al [3]. **Study 1** consisted of animals evenly split into sham and injured groups measuring pain-like behavior (n=20/group/sex), and sleep (n<12/group/sex) biweekly until week 15, and anxiety (n=20/group/sex) at weeks 0 and 14. Pain-like behavior was assessed using pressure algometry. Anxiety-like behavior was evaluated using an open field assessment. To **noninvasively** characterize sleep in **both studies**, electric field (EF) sensors were attached to the exterior of the rodents' home cage and data was collected over the 12-hour light cycle [4]. EF sensor data was scored in 10-second epochs with scoring criterion adhering to previously published methods [5]. Metrics extracted include sleep fragmentation index (**SFI, #sleep bouts/hour**), where higher indices indicate less consolidated sleep bouts, and REM bout durations, a stage often preserved in response to sleep deprivation. **Study 2** consisted of animals evenly split into sham and injured groups measuring pain-like behavior (n=12/group/sex) at weeks 6, 12, and 18. Sleep (n=8/group/sex) and anxiety (n=12/group/sex) were assessed at week 18. Pain-like behavior was assessed using grip strength. Anxiety-like behavior was assessed using a 5-minute video recorded during open exploration of an elevated plus maze (EPM). Time spent and number of entries into open arms, closed arms, and the center of the apparatus was quantified using ANY-maze software. Sleep was assessed as outlined above. At the end of **study 1** and **2**, animals were humanely euthanized, and brain tissue was collected from all animals that underwent sleep assessment. Immunohistochemistry (IHC) is underway to detect neuroinflammatory differences between sham and injured groups.

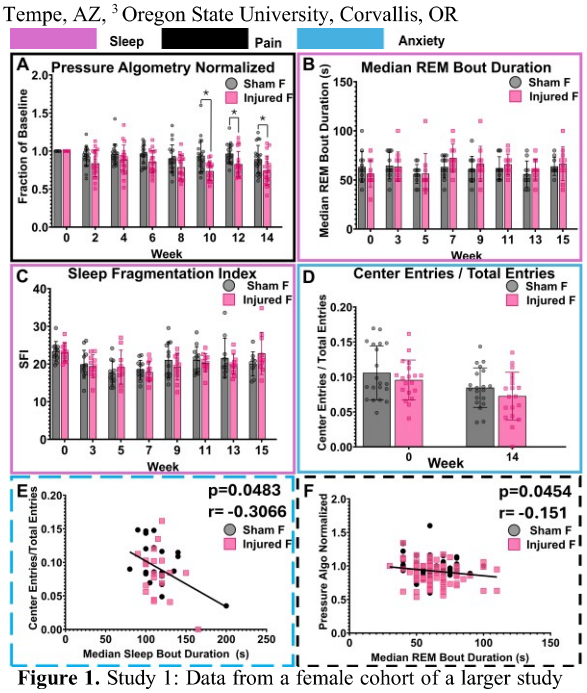

Figure 1. Study 1: Data from a female cohort of a larger study examining relationships between sleep, pain and anxiety through week 15.

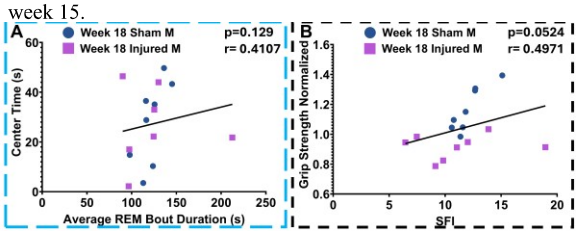

Figure 2. Study 2: Data from a male cohort of a larger study examining the relationships between sleep, pain, and anxiety at week 18.

RESULTS SECTION: In **study 1**, injured females showed a significant increase in pain-like behavior at weeks 10, 12, and 14 compared to sham animals (**Fig 1A,** 2-way ANOVA with Tukey's post-hoc). Injured males also exhibited a significant increase in pain-like behavior compared to sham males (data not shown). No differences were observed with sleep fragmentation index or median REM bout duration before or directly following significant pain-like onset between sham and injured females **(Fig 1B&C**, Multiple Mann-Whiney test**)** or males (data not shown) in **study 1**. Sham and injured female cohorts did not exhibit differences in entries to the center region in the open field assay -a proxy for anxiety- at weeks 0 or 14 (**Fig 1D**, 2-way ANOVA with Tukey's post-hoc). However, a significant correlation was found between anxiety and median sleep bout duration in **study 1** females (**Fig 1E,** p=0.0486; r=-0.3066, Spearman). Increased pain-like behavior (decreased pressure algometry) also significantly correlated with an increased REM bout duration in **study 1 females** (**Fig 1F**, p=0.0454; r=-0.151, Spearman). Correlations were not significant between sleep-pain assays in male animals (not shown). These data suggest that, in females, pain-like behavior and anxiety correlate with altered sleep behavior. In **Study 2**, preliminary analysis on male animals revealed a significant increase in pain-like behavior (grip strength) at weeks 12 and 18 compared to sham animals (data not shown). Male week 18 sleep data showed no significant differences across all metrics between sham and injured animals (data not shown). However, when week 18 male pain-like behavior (grip strength) was correlated with SFI, a trending relationship was found (p=0.0524; r=0.4971, **Fig 2B**, Spearman), suggesting more pain may be correlated to less sleep fragmentation. Correlations between average REM bout durations and anxiety-like behavior (EPM) revealed a less robust trend in males when compared to **study 1** females (p=0.129; r=0.4107, **Fig 2A**, Spearman). Analysis is currently underway for brain IHC to determine how brain changes may interact with these findings. Statistical Analysis was performed in GraphPadPrism10 (*p<0.05).

DISCUSSION: Consistent with our hypothesis, the results from **study 1** indicate that sleep changes do not temporally precede significant pain-like onset in a LBP model. Despite this, when looking at how sleep features correspond with pain, we observed a significant correlation between increased REM bout duration and enhanced pain-like behavior in the female cohort. These results indicate that, at least in the early stages of pain-like progression, REM sleep is acutely promoted. Though neither sleep nor anxiety independently exhibit significant differences, anxiety and sleep also significantly correlated in the female cohort, suggesting that more anxious animals have longer sleep bout durations. Preliminary data from **study 2** shows trending correlations between male pain-like behavior and sleep, a relationship not exhibited in **study 1,** suggesting that the impact between sleep and pain is a time-dependent process that may appear earlier in female cohorts. As evidenced by **study 2**, sleep-pain correlations are likely to carry over to week 18. Future work will continue to characterize the relationship between sleep, pain, and anxiety in **study 2** to more clearly identify differences at a later timepoint.

SIGNIFICANCE/CLINICAL RELEVANCE: Over half of the chronic LBP population presents with sleep issues. This study seeks to temporally investigate how sleep quality influences LBP development and vice versa, with the intent to provide more comprehensive and efficacious health interventions.

REFERENCES: [1]. Alsaadi et al, 2011. [2]. Whibley et al, 2019. [3] Lillyman et al, 2022. [4] Kloefkorn et al, 2021. [5] Kloefkorn et al, 2022.

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