Electric Field Sensors Enable Noninvasive Three-State-Sleep Scoring in Rats

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INTRODUCTION: Increasing evidence from clinical and laboratory-based studies suggests that sleep is an important modulator of pain [1-3]. Sleep disruption has been reported in 88% of chronic pain patients, indicating sleep is a potential predictor of chronic pain onset [4,5]. Likewise, sleep deprivation is also responsible for eliciting chronic pain symptoms such as hyperalgesia and musculoskeletal pain in healthy individuals, implying the relationship between sleep and pain is bidirectional [6,7]. We hypothesize this relationship likely exists in animal models of chronic pain, specifically chronic low back pain (CLBP), and can be expanded upon with a sleep quantification system. Currently, there are preexisting methods to assess sleep such as polysomnography (PSG), wearable technologies, and at-home electroencephalography (EEG). However, these methods pose challenges such as invasive procedures and complex data analysis with respect to PSG and poor reproducibility regarding at-home EEG and wearable technology [8,9]. There is a need to characterize sleep noninvasively and reliably in rat models of chronic pain to understand the relationship between pain and sleep**.** To address this, electric field (EF) sensor data can be utilized to quantify three-stage sleep wake cycles while remaining noninvasive, inexpensive, and enabling rodents to remain in their home cage with their cage mate [10]. To this end, we developed an analysis system, previously validated in mice [10], that measures stages of sleep in rats to increase clinical relevance of our CLBP model and expand upon the ties between pain and sleep.

METHODS: EF sensors (Plessey Semiconductors, PS25251, 1 cm^2, +/- 5V) were connected to a data acquisition system powered by a computer running LABVIEW (National Instruments) software to measure alterations in the local electric field and acquire time-matched video recordings. These sensors can convert animal movement into a voltage trace waveform detect alterations in respiration characteristic of rapid-eye-movement (REM) and non-REM (NREM) sleep stages when affixed to the exterior of rat home cages. The cage interior was temporarily modified via a grounded plexiglass divider to ensure sensor data is targeting a single animal without losing interaction with their cage mate, sans touch. Two sensors were shielded and placed 1/3rd of the way up the exterior half of the cage on the front end and far left side with the targeted animal (**Fig. 1A**). The cage was electrically shielded by surrounding the perimeter in grounded fine metal mesh fabric (VeilShield) to reduce noise with an accompanying fan to reduce humidity. EF sensor data and accompanying videos were acquired overnight (12-16 hours). The data was then manually scored in Spike2 (Cambridge Electronic Design) in 10-second epochs from voltage trace waveforms and exported to MATLAB to calculate sleep metrics, as coded by the Kloefkorn Lab [10, 11], such as: total and percentage time spent asleep, total and percentage time spent in NREM and REM sleep, average sleep bout duration, microarousal index, sleep fragmentation index, average REM sleep duration, REM sleep latency, number of awakenings, and number of arousals per hour of sleep. For validation, the accompanying video was scored manually with Elan (Max Planck Institute for Psycholinguistics) software and continuous state transitions were converted into 10-second epochs for direct comparison with EF sensor scoring. Data was collected on two animals and compared to previous PSG-validated EF sensor sleep data in mice as collected by the Kloefkorn Lab [10].

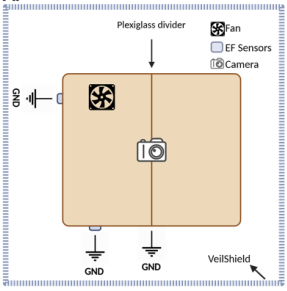
RESULTS: EF sensor and video scoring were compared epoch-to-epoch yielding a 12.2% aggregate error that breaks into the following respective state error: 2.6% Wake, 15.6% NREM, and 50% REM in this preliminary analysis. This error is primarily concentrated in transition states between stages, which is common among other scoring systems such as PSG [12]. Sleep measures between video and EF sensors were relatively similar (**Fig. 1B**). However, the video method skewed higher in sleep bout duration, decreased percentage time spent in REM sleep, REM sleep duration, and increased REM sleep latency which can be attributed to the difficulty in visually discerning REM sleep characteristics and missed wake events. EF sensors can detect wake and REM events earlier and more reliably. EF sensor voltage trace waveforms were visually similar between mouse data and rat data (**Fig. 2**) lending credence to direct translation between mouse and rat applications.

DISCUSSION: We were able to capture three-state sleep in rats, specifically discerning between REM and Non-REM sleep by real-time video validation of these sleep events. The results suggest that EF sensors are a promising new approach to noninvasively quantify sleep in both mice and rats and mitigate accuracy and invasiveness challenges inherent in other sleep analysis methods.

SIGNIFICANCE: This system is a robust tool to assess the three-stage-sleep wake cycle. In conjunction with other pain measures, associative sleep changes can now be incorporated into rat models of chronic pain to improve clinical translation.

REFERENCES: [1] Aili et al, 2015. [2] Tomim et al, 2016. [3] Bigatti et al, 2008. [4] Morin et al, 2006. [5] Smith et al, 2004. [6] Tiede et al, 2010.[7] Kundermann et al, 2004.[8] Bastianini et al, 2017.[9] Pollak et al, 2001.[10] Kloefkorn et al, 2020.[11] Kloefkorn et al, 2022.[12] Rosenberg & Hout, 2013.

A.



B.

Scoring Method	# Wake Events	Microarousal Index (MAI)	Sleep Fragmentation Index (SFI)	# Sleep Events	Sleep Bout Duration (sec)
EF Sensors	9	7.9	7.4	10	350
VIDEO	7	6.7	6.1	8	445

Scoring Method	% time spent in Sleep (%)	# REM Events	AVG Duration REM Events (sec)	% total time spent in REM (%)	REM Latency (sec)
EF Sensors	64.6	3	103.3	6.3	133.3
VIDEO	68	2	50	2.8	230

Figure 1. EF Sensor Setup and Video Validation. (A) Schematic of sleep collection system. (B) Preliminary data from one recorded animal regarding the differences between EF sensor collection and acquired video. (A) generated in BioRender

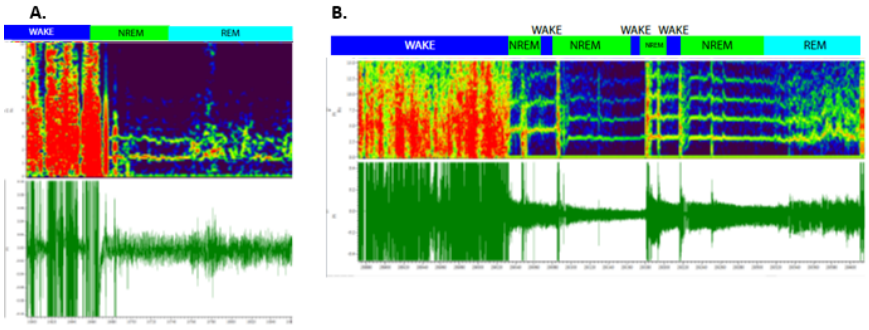


Figure 2. Spectrogram of EF Voltage Trace Between Rat (A) and Mouse (B). The characteristics between waveforms across the species of mouse and rat are visually similar, suggesting potential translatability of sleep-based applications. The arousal state is scored on top, indicating which of the three stages were scored based on the accompanying spectrogram in the middle. High powered frequencies are indicated in red while blue denotes low power frequencies. The bottom graph (in green) indicates the EF sensor voltage trace waveforms.