Sleep spindles as a diagnostic and therapeutic target for chronic pain

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Abstract

Pain is known to disrupt sleep patterns, and disturbances in sleep can further worsen pain symptoms. Sleep spindles occur during slow wave sleep and have established effects on sensory and affective processing in mammals. A number of chronic neuropsychiatric conditions, meanwhile, are known to alter sleep spindle density. The effect of persistent pain on sleep spindle waves, however, remains unknown, and studies of sleep spindles are challenging due to long period of monitoring and data analysis. Utilizing automated sleep spindle detection algorithms built on deep learning, we can monitor the effect of pain states on sleep spindle activity. In this study, we show that in a chronic pain model in rodents, there is a significant decrease in sleep spindle activity compared to controls. Meanwhile, methods to restore sleep spindles are associated with decreased pain symptoms. These results suggest that sleep spindle density correlates with chronic pain and may be both a potential biomarker for chronic pain and a target for neuromodulation therapy.

Introduction

Chronic pain affects one in four adults worldwide,[1] and diagnosis currently relies on self-reported verbal pain assessments. Reliable and useful assessment of pain is crucial to its diagnosis and effective management. Therefore, the development of objective biomarkers can revolutionize our ability to understand and treat pain. Biomarkers that demonstrate disease mechanisms are particularly useful, as they are more likely to be reproducible across distinct populations and may guide therapies.

Poor quality sleep has been shown to be a risk factor for chronic pain,[2–6] with numerous studies implicating sleep in pain-associated depression and anxiety as well.[7,8] A causal link between chronic pain and sleep deficits, however, is not well established. Nevertheless, there are reports that suggest interventions during sleep may have potential benefits in treating pain.[9,10] Thus, objective measurements of sleep quality present a promising diagnostic and therapeutic target for pain.

Sleep spindles are bursts of high-frequency (9–16 Hz) oscillations of neural activity that occur during stage 2 nonrapid eye movement (NREM) sleep. While spindle waves are generated by the thalamus, they are relayed through thalamocortical oscillations to the cortex. Spindles have been well studied in mammalian models.[11] It has been shown that spindles have functions

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in both memory consolidation and sensory processing.[12,13] Deficits in spindle activity during sleep have also been linked to numerous pathologies including epilepsy and other neuropsychiatric illness.[14,15] Recent studies have shown that spindles are involved in the attenuation of signals from the external environment, thus supporting a role for spindle waves in processing nociceptive inputs.[16]

The challenge of studying sleep spindles is the labor-intensive process of spindle identification and characterization. However, with recent advances in automated detection of sleep spindles using neural network algorithms,[17,18] it has become more feasible to study sleep spindle activity as a potential biomarker for certain health states. In this study, we apply a recently developed deep learning method, known as SpindleNet, to characterize sleep spindle activity in chronic pain-treated rats.[17] Our results indicate that sleep spindle density is decreased in a model of chronic pain. Furthermore, we show that pink noise, an established method to increase spindle density, can effectively decrease pain sensitivity.

Materials and methods

Rats

Experiments were conducted on male Sprague-Dawley rats obtained from Taconic Farms, Albany, NY. All procedures in this study were approved by the New York University School of Medicine (NYU SoM) Institutional Animal Care and Use Committee (IACUC) as consistent with the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals to ensure minimal animal use and discomfort. Animals were given on average 14 days to acclimate to the facility prior to experimentation.

EEG surgery

Rats were put under anesthesia (isoflurane, 1.5%–2%) and placed in a stereotactic frame before undergoing implantation. A midline incision of 4 cm was made and several small screw-based electrodes were anchored on superficial holes drilled on the skull pertaining to prefrontal cortex (Bregma +3.5 mm, midline) and bilateral hind limb S1 (Bregma −2 mm, 2 mm lateral) regions. Screws were soldered to female Mill-Max connectors, and ground and reference screws were anchored above the cerebellum. Dental cement was used to secure equipment with bone screws. Rats were allowed to recover for at least one week after surgery.

Sleep measurements

Sleep studies were performed over 4–5 h in a quiet, isolated room in ambient temperature during the typical day cycle. The timing of recording was consistent throughout the course of study. Each rat was acclimated to sleep testing conditions in their home cage. Intan RHD2000 boards were connected to the rat at the start of the experiment and two video cameras recorded the rat for the duration of the experiment. Each rat received one week of acclimation, two days of baseline recordings, and experimental conditions were split between control rats and rats after Complete Freund's Adjuvant (CFA) injection.[19] After baseline recordings, rats received CFA injection in the hind paw, and their sleep was measured one, three, and seven days after injection.

Analysis

After isolating sleep using manual labeling of video, electroencephalogram (EEG) time series acquired at 1 kHz were band pass filtered and down sampled to 200 Hz and then fed into a deep neural network (SpindleNet) to automatically detect spindle episodes. Spindle density was calculated as a measure of the number of spindles detected per minute of sleep recorded and normalized by average baseline recording data to calculate relative spindle density over time and compare across rats.

Allodynia testing

Mechanical allodynia testing was performed using the Dixon up-down method with von-Frey filaments.[20,21] Rats were placed on a mesh table, allowed to acclimate, and the hind limb paw was stimulated with filaments of logarithmically increasing stiffness. Withdrawal thresholds were recorded and 50% mechanical nociceptive threshold was computed, as described previously.[22,23]

Pink noise stimulation

The stimuli were bursts of pink 1/f noise of 50 ms duration, with a 5-ms rising and falling time, respectively. Pink instead of white noise was used because it sounds softer and is therefore more comfortable to hear. Pink noise stimulation was performed using a computer speaker at fixed volume located near the rat in the controlled sleep environment. The noise file was generated by Audacity's built-in pink noise generator. Stimulation frequency was normalized from a Gaussian distribution to a mean of 10 s between noises with a standard deviation of 3.33 s. Noise experiments were conducted at the end of a regular sleep recording session.

CFA injection

To induce chronic inflammatory pain, 0.1 ml of CFA (mycobacterium tuberculosis, Sigma-Aldrich) was suspended in an oil-saline (1:1) emulsion and injected

subcutaneously into the plantar portion of the hind paw. Control rats received equal volume of saline injection.

Spared nerve injury

Rats underwent a spared nerve injury (SNI) procedure, as described previously.[22,24] During the procedure, rats were anesthetized (isoflurane 1.5%–2%). An inferomedial incision was made from the left knee, and the biceps femoris was dissected to expose the sciatic nerve bundle with its three terminal branches: sural, common peroneal, and tibial nerves. The tibial and peroneal nerves were severed and tied off with nonabsorbent 5–0 silk sutures at the proximal point of the trifurcation and then cut distal to each knot to prevent reattachments. The sural nerve was spared. The muscle layer was then sutured closed with 4–0 absorbable sutures and the skin was closed with 3–0 silk sutures. Rats were allowed to recover for at least one week after surgery.

Sleep staging

Video and accelerometer measurements of the rat's movement were used to classify sleep versus wake states. Among sleep periods, NREM sleep was primarily determined by the high delta/theta EEG power ratio and the presence of slow waves and sleep spindles.

Automatic spindle detection

We used a previously established method, known as SpindleNet, to detect sleep spindles from a single-channel EEG.[17] SpindleNet is a deep learning-based method for online detection of sleep spindles. SpindleNet consists of a convolution neural network (CNN) and recurrent neural network (RNN). It was operated and run on single-channel downsampled (200 Hz) sleep EEG signals. Compared to other state-of-the-art detection methods, SpindleNet can achieve low detection latency and high detection accuracy and specificity. In addition, SpindleNet can produce robust performance with various EEG sampling frequencies and signal-to-noise ratio (SNR). SpindleNet was run on a Linux computer with an embedded Nvidia GPU card.

Statistical analysis

Nociceptive thresholds and sleep spindle densities for rats were presented as mean $\pm$ standard error of the mean. Unpaired t-tests were performed to compare the nociceptive threshold and spindle densities of CFA-treated rats to saline controls, to compare sleep spindle density with and without pink noise stimulation and to compare sleep spindle frequency, power, and duration between artificial and natural spindles. An unpaired t-test was also performed to compare the mean

nociceptive thresholds of rats treated with SNI before and after 14 consecutive days of pink noise stimulation. A linear regression was performed to determine correlation between nociceptive thresholds and sleep spindle density with R^2 value in the plot. For all tests, a P value < 0.05 was considered statistically significant. All of the data were analyzed using GraphPad Prism version 7 software and MATLAB (MathWorks).

Results

Each rat slept for a minimum of 1 h in the sleep recording apparatus for every 3 h of experimentation (Figure 1 (a)). During this time, we recorded EEG signals. Sleep versus wake state was classified based on the video and accelerometer measurement of rat's movement (Figure 1 (b) and (c)), and such classification was used to search for sleep spindles given the EEG signal (Figure 1(d)). Overall, sleep duration did not differ significantly between experimental groups, recording days, or experiment sessions.

A deep neural network, SpindleNet, has been recently developed to automatically detect spindle episodes in real time (Figure 1(d) and (e)). The output of SpindleNet provided soft thresholds for the detection of spindles (Figure 3(e)), which were left consistent across all rats and sessions to allow correlations across time. SpindleNet rejected any spindles that did not meet the minimum duration necessary to be considered a spindle episode. Detected spindles that met our predetermined probability threshold and the minimum duration criterion were counted for the duration of the sleep session. We computed the relative sleep spindle density measures by the ratio of the number of isolated spindles (detected by SpindleNet) per minute of sleep (exclusively for NREM sleep).

Our first research question was to investigate the impact of chronic pain on sleep spindles. We used CFA to induce persistent inflammatory pain in the hind paws. We found that CFA-treated rats, compared with saline-treated rats (controls), showed a marked reduction in spindle density during sleep one day after the onset of pain (Figure 2(a)). We correlated these relative spindle densities with behavior by measuring the mechanical nociceptive threshold (Figure 2(b)). As rats recovered from CFA, spindle density returned to baseline or the pre-CFA levels. In contrast, control rats showed no symptoms of mechanical allodynia, nor did they show changes in spindle density over the time course of the experiment. We observed an inverse correlation between relative spindle activity and allodynia (Figure 2(c)), indicating a close relationship between sleep spindle density and persistent pain. These results also suggest that spindle density may indicate the severity of pain.
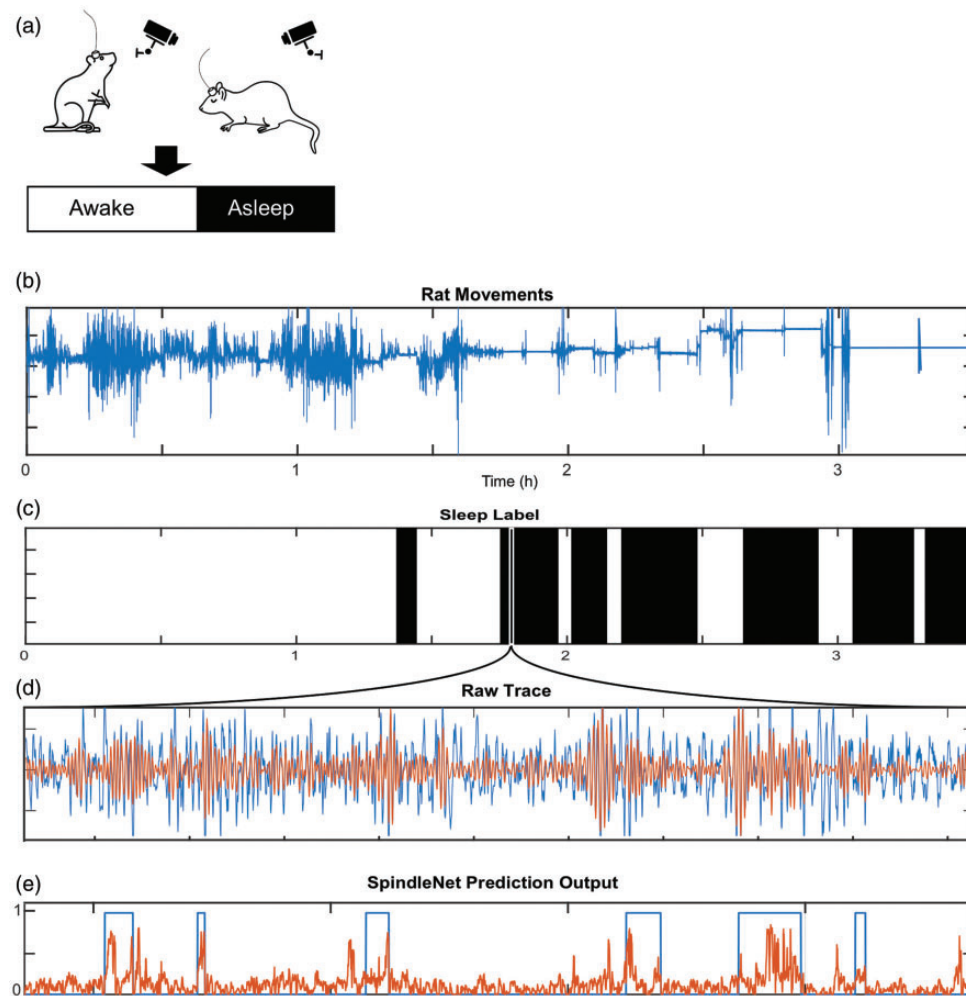
Figure 1. Automated sleep spindle density measurements. (a) This schematic represents the experimental setup with cameras recording rats movement in sleep and awake states. (b) Rat movements obtained from an accelerometer during sleep, indicating activity of the rat during a representative recording session. (c) Sleep scores manually obtained from monitoring video feed of the rat during experimentation. (d) Raw EEG signal at 1 kHz (blue) obtained from sleep superimposed with downsampled to 200 Hz signal (orange). (e) Raw output (orange) probabilities from the SpindleNet automated detection network threshold (blue) for recognizing spindles.

Our next research question was to investigate whether acoustic stimulation could affect sleep spindles and chronic pain symptoms. To dissect a possible causal relationship between pain symptoms and sleep deficits, we analyzed if restoration of sleep spindles could relieve pain. Pink noise, a type of low frequency acoustic stimulation, is a well-known method to enhance sleep spindles.[25–29] Thus, we introduced random pink noise based on a Gaussian random distribution with average interval between stimuli of 10 s. Rats subject to such noise showed an immediate intrasession increase in spindle density (Figure 3). Detected sleep spindles had an average frequency between 10 and 15 Hz with variable duration (Figure 3(d)). There was no statistically significant difference in the shape

of spindles in the context of pink noise and spindles in the absence of pink noise, with both sets of spindles exhibiting the same frequency, duration, and power distributions. We further examined the effect of pink noise on pain symptoms. In order to maximize the treatment effects of acoustic stimulation, we exposed rats to 14 consecutive days of acoustic stimulation. We used SNI to model stable chronic pain. SNI-treated rats were exposed to 14 consecutive days of pink noise stimulation during 8 h of sleep in a quiet room. We measured allodynia response before and after the pink noise treatment regimen and observed a statistically significant change in behavioral pain response as manifested by relief of mechanical allodynia (Figure 3 (e)). Together, these results indicate that rhythmic
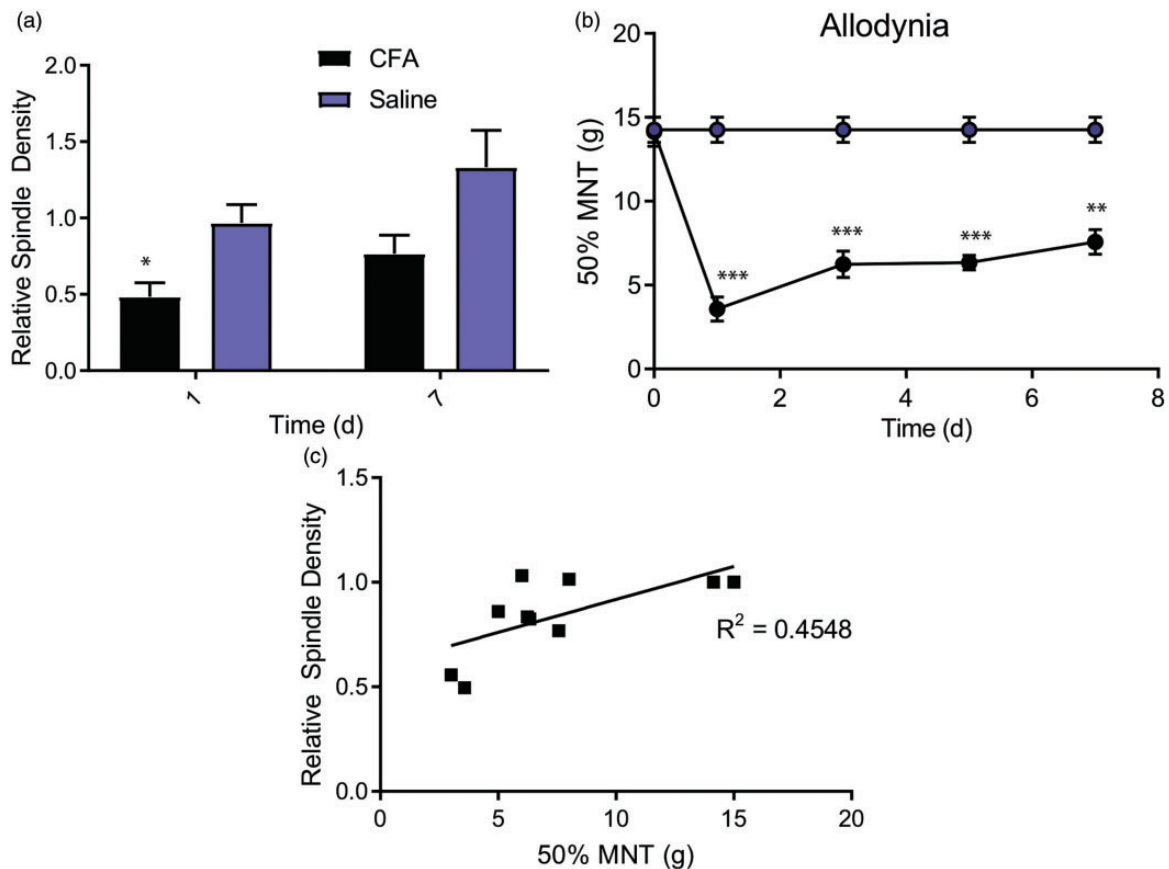
Figure 2. Sleep spindle density decreases in the chronic pain state. Individual rats have different baseline spindle densities, thus all were normalized to the average of their baseline recordings. (a) Relative spindle density for CFA-treated rats (n = 3) and saline controls (n = 4) on days 1 and 7 after injection. An unpaired t-test showed statistically significant decrease in average spindle density in the CFA-treated rats compared to controls one day post-injection (P = 0.0189). Differences in spindle density diminished over time as rodents recovered from inflammatory pain induced by the CFA model and since baseline recordings increased as seen on day 7 post-injection (unpaired t-test; P = 0.1902). (b) The mechanical nociceptive threshold (50% MNT) for CFA- and saline-treated rats. After injection with CFA, rodents experienced significant reductions in nociceptive threshold from baseline compared to saline; unpaired t-test on day 1 (P = 0.0002), day 3 (P = 0.0008), day 5 (P = 0.0004), and day 7 (P = 0.0016). (c) As CFA-treated rats recovered from persistent pain, as indicated by nociceptive threshold measurements, we observed a correlation between nociceptive threshold and spindle density in S1, using a linear regression analysis ($R^2 = 0.4548$). CFA: Complete Freund's Adjuvant; MNT: mechanical nociceptive threshold.

acoustic stimulation, likely by elevating spindle density, could reduce chronic pain symptoms.

Discussion

The appropriate assessment of pain, particularly chronic pain, is essential to understanding pain mechanisms and providing proper treatment. Current pain assessment in both humans and animal models relies on subjective verbal or behavioral reports. However, the development of objective neurophysiological measures can complement subjective behavioral measures to yield promising advances in the diagnosis and management of pain.

All sensory information received from the external environment, including nociception, passes through the thalamus before reaching the cortex. The thalamus, through its interaction with the cortex, has a long established role in attention and sensory gating mechanisms during sleep.[30,31] Sleep spindle waves occur during NREM sleep, and they have been shown to play a role in learning, memory consolidation, and sensory processing.[12,13,32] Meanwhile, deficits in spindle activity during sleep have also been linked to numerous pathologies including epilepsy, altered mentation, poor memory retention, and other neuropsychiatric illness.[14,15] Interestingly, recent studies have also demonstrated the role for sleep spindles in the attenuation of signals from the external environment, as a form of sensory gating.[16] This role in sensory gating suggests the involvement for spindle waves in processing nociceptive inputs as well.

A number of studies have demonstrated a relationship between spindle density and sleep stability, with the underlying mechanisms being perturbations in thalamocortical oscillation.[33,34] At the same time, an
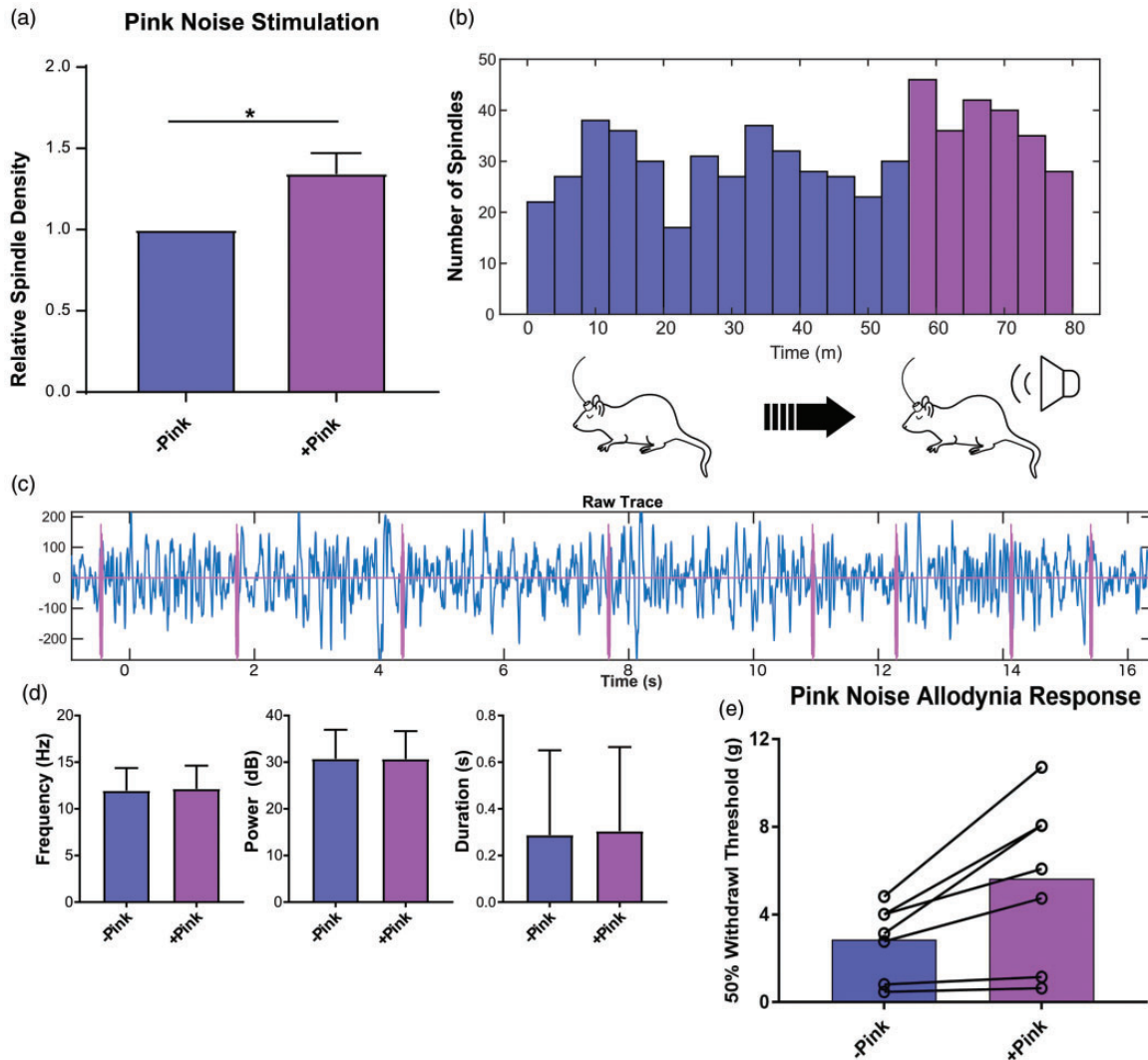
Figure 3. Pink noise stimulation reduces pain behaviors. (a) In the presence of pink noise, we found that for (n = 3) sessions across (n = 3) CFA-treated rats, there was a significant increase (P = 0.04, unpaired Student's t-tests) in spindle density. (b) A representative sleep session where blue indicates spindles during silence and pink denotes the presence of aforementioned pink noise stimulation, revealing spindle enhancement. (c) The raw trace of the EEG signal (blue) superimposed with pink noise waveform (pink) shows how spindles might be stimulated. Spindles detected were in previously accepted frequency ranges of 10–15 Hz. (d) There was no statistically significant difference in detected spindle frequency (P = 0.0508), power (P = 0.9357), or duration (P = 0.9357) between artificial and natural spindles after unpaired t-test analysis. (e) Effect of pink noise stimulation on nociceptive threshold in rats with spared nerve injury. After pink noise stimulation of (n = 7) rats for 14 consecutive days, we compared 50% withdrawal threshold with allodynia testing before and after treatment and found that there was a significant increase after treatment with an average increase of 2.77 g (P = 0.016; unpaired Student's t-tests).

increasing number of studies have also shown that chronic pain is associated with poor sleep quality, especially interruptions in slow wave, NREM sleep.[2,3,35,36] This is not surprising, given the role for thalamocortical dysrhythmia in chronic pain.[37–39] Since sleep spindle density has been implied in many functional roles in memory consolidation and sensory gating, it is important to accurately identify and characterize sleep spindles. Our SpindleNet can serve as an excellent spindle detector due to its robust performance.[10] Our study

found that depressed spindle waves are associated with chronic pain. Due to the role of spindles in sensory gating, cognition, and affect, through modulations of thalamocortical oscillation, it is quite plausible that deficits in spindle waves would contribute to the pathology or pathogenesis of chronic pain. Our finding that acoustic stimulation exposure can enhance spindle waves and over time decrease allodynia in rats with chronic pain provides further support for this hypothesis.

The use of EEG findings as a biomarker for neuro-psychiatric diseases has gained considerable interest over the last decade. Compared with other neuroimaging modalities such as the magnetic resonance imaging, EEG is cheaper, more portable, and easier to use. Modern source localization has further improved the spatial resolution for high-density EEG studies, advancing the specificity and sensitivity of EEG bio-markers.[40,41] Acute pain triggers characteristic evoked potentials (or event-related potentials, ERPs) on the EEG as well as elevated theta and gamma powers. More importantly, the power of theta and gamma oscillations are altered in chronic pain state, and changes in these EEG nociceptive response have been shown to predict the presence of pain and analgesic effects.[42–47] Furthermore, recent machine learning-driven feature extractions of EEG signals have been shown to distinguish pain patients and healthy controls.[48] These studies support the use of EEG as a potential tool to derive biomarkers or biosignatures for pain. In this study, we have demonstrated a unique use of spindle activity during sleep as an EEG finding for pain states. Sleep spindles can be measured easily at home or in the hospital and can be used to track the presence and progression of pain symptoms. Future work in humans is needed to explore the use of spindle density changes as a potential biomarker for chronic pain.

Our results also have therapeutic implications, as our strategy to enhance spindles is associated with decreased mechanical allodynia. Acoustic stimulation is noninvasive, and much research has been conducted in the use of acoustic stimuli to manipulate spindles, with multiple studies showing measurable behavioral impacts on memory and cognition in human subjects.[14,26,49,50] However, protocols for spindle manipulation vary, with some groups utilizing bursts of white or pink noise as in this study, and others even implementing closed-loop electrical or acoustic stimulation in real time.[51,52] It remains to be seen if random acoustic stimulation is as effective as closed-loop acoustic stimulation, suggesting further study is required in exploring optimal spindle manipulation paradigms, especially with regard to impact on behavior.

A limitation of our study is that we only recorded from male rats and did not directly measure the sex difference in the relationship between sleep and pain. Future studies shall comprehensively address the role of sex in this important relationship. In addition, future studies are needed to carefully measure EEG responses in a number of cortical regions, including but not limited to recordings in the S1, after a number of pain conditions, and the response of sleep spindles to pink noise stimulation in these pain models.

In summary, we have applied a deep neural network approach to automate the analysis of sleep spindles in rodents. Our study indicates that spindle density is decreased in the chronic pain state. Acoustic stimulation, meanwhile, can enhance spindles and relieve chronic pain symptoms. Therefore, our study suggests the potential for sleep spindles to function as possible targets for pain diagnosis and for therapeutic neuromodulation.

Author Contributions

ZSC and JW conceived and designed the experiments. BC, PK, LH, DV, and QZ performed the experiments. ZSC and JW interpreted the data and wrote the manuscript with other authors' inputs.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: ZSC, PK, and JW are inventors of a pending US patent application. The remaining authors have no conflict of interest.

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