HHS Public Access

Author manuscript

Sci Transl Med. Author manuscript; available in PMC 2025 April 17.

Published in final edited form as:

Sci Transl Med. 2024 April 17; 16(743): eadg3036. doi:10.1126/scitranslmed.adg3036.

Nociceptor spontaneous activity is responsible for fragmenting non–rapid eye movement sleep in mouse models of neuropathic pain

Chloe Alexandre[1,2,*], **Giulia Miracca**[3,4], **Victor Duarte Holanda**[1,2], **Ashley Sharma**[1,2], **Kamila Kourbanova**[1,2], **Ashley Ferreira**[3,4], **Maíra A. Bicca**[1,2], **Xiangsunze Zeng**[4], **Victoria A. Nassar**[1,2], **Seungkyu Lee**[4], **Satvinder Kaur**[3], **Sridevi V. Sarma**[5], **Pierre Sacré**[6], **Thomas E. Scammell**[3,†], **Clifford J. Woolf**[4,†], **Alban Latremoliere**[1,2,*,†]

[1]Department of Neurosurgery, Neurosurgery Pain Research institute, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA

[2]Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA

[3]Department of Neurology, Beth israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115, USA

[4]FM Kirby Neurobiology Center, Boston Children's Hospital and Department of Neurobiology, Harvard Medical School, Boston, MA 02115, USA

[5]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21205, USA

[6]Department of Electrical Engineering and Computer Science, School of Engineering, University of Liège, Liège, Belgium

Abstract

Spontaneous pain, a major complaint of patients with neuropathic pain, has eluded study because there is no reliable marker in either preclinical models or clinical studies. Here, we performed a comprehensive electroencephalogram/electromyogram analysis of sleep in several mouse models of chronic pain: neuropathic (spared nerve injury and chronic constriction injury), inflammatory (Freund's complete adjuvant and carrageenan, plantar incision) and chemical pain (capsaicin). We find that peripheral axonal injury drives fragmentation of sleep by increasing brief arousals from non–rapid eye movement sleep (NREMS) without changing total sleep amount. In contrast to neuropathic pain, inflammatory or chemical pain did not increase brief arousals. NREMS

[*]Corresponding author. alatrem1@jhmi.edu (A.L.); calexa41@jhmi.edu (C.A.).

Author contributions: C.A., T.E.S., C.J.W., and A.L. conceived the study and designed the experiments with help of coauthors as follows: EEG/EMG implantations were performed by C.A., G.M., A.F., and V.D.H. Sleep scoring was carried out by C.A., G.M., A.F., A.L., K.K., A.S., and V.D.H. Sleep analyses were performed by C.A. Spectral analyses were conducted by C.A., P.S., and S.V.S. Optogenetic stimulations were performed by V.N. and A.L. Co-monitoring of paw movements and EEG after SNi performed by X.Z. DRG neurons cultures and CGRP ELISA conducted by S.L. Sensory testing was performed by A.L. and G.M. Pain surgeries and intraplantar injections were carried out by A.L. PB^{CGRP} ablation by S.K. Brain perfusion, immunohistochemistry, and PB^{CGRP+} cell quantifications were performed by M.A.B. The manuscript was written by C.A., T.E.S., C.J.W., and A.L, with input from all coauthors.

[†]These authors contributed equally to this work.

Competing interests: C.A., T.E.S., C.J.W., and A.L. have pending US Patent application (no. 17/251, 755) submitted by Childrens Medical Center Corp and Beth Israel Deaconess Medical Center Inc. that covers "Biomarkers of neuropathic pain." The other authors declare that they have no competing interests.

fragmentation was reduced by the analgesics gabapentin and carbamazepine, and it resolved when pain sensitivity returned to normal in a transient neuropathic pain model (sciatic nerve crush). Genetic silencing of peripheral sensory neurons or ablation of $CGRP^+$ neurons in the parabrachial nucleus prevented sleep fragmentation, whereas pharmacological blockade of skin sensory fibers was ineffective, indicating that the neural activity driving the arousals originates ectopically in primary nociceptor neurons and is relayed through the lateral parabrachial nucleus. These findings identify NREMS fragmentation by brief arousals as an effective proxy to measure spontaneous neuropathic pain in mice.

INTRODUCTION

Preclinical research on neuropathic pain has relied heavily on mechanical and thermal stimulus-evoked pain-related behavior. Although it is pertinent to assess pain arising from normally innocuous stimuli (allodynia) and the exaggerated responses to noxious stimuli (hyperalgesia), it has limited translational value because the main symptom of patients with neuropathic pain is spontaneous pain (pain arising in the absence of any external stimulus) (1–3). Spontaneous pain is triggered by ectopic activation of nociceptive pain pathways (4–6). Spontaneous pain can be categorized into either persistent “burning” ongoing pain or paroxysmal “electric shock”–type sensations that typically last only for several seconds (1, 3, 7). Ongoing pain arises in multiple chronic pain etiologies (inflammatory, diabetic, or traumatic neuropathic pain) (3, 8–10), whereas paroxysmal pain attacks are more frequent after peripheral nerve injury (amputation, avulsion, or nerve crushes during invasive surgeries) (1, 8, 11).

Objectively quantifying the symptom of spontaneous pain in preclinical models has been a major challenge, which has hampered the mechanistic studies required to develop reliable diagnostic tools and effective treatments (3, 12). Monitoring behaviors like flinching, licking, or biting can detect spontaneous pain-related behaviors, but this is challenging because these events are relatively rare and sporadic (13–16) and can be repressed by other behavioral activities (17–20). The grimace scale can detect signs of ongoing, sustained pain, especially during inflammatory (21) and chemotherapy-induced neuropathic pain (22). The conditioned place preference test is an operant behavioral assay developed to detect relief of spontaneous tonic (ongoing) pain after nerve injury (23). This assay is helpful to detect potential analgesic signals, but it cannot quantify pain intensity or identify sporadic pain attacks, and it can be influenced by drugs with addictive properties.

Another strategy to detect sporadic spontaneous pain-associated events is to monitor behaviors or physiological parameters directly affected by pain (24). Changes in heart rate, heart rate variability, pupil dilatation, and sweating can detect pain. Unfortunately, these readout measures often require a sustained pain input and display relatively low specificity (12). Chronic pain is also strongly associated with symptoms of depression and anxiety (25, 26). These comorbidities are pertinent clinically, but they tend to develop relatively slowly (several weeks) after surgery, at least in mice (27).

People with nerve injury report poor sleep quality (28–30), but the exact nature of their sleep disturbances and the underlying neural mechanisms are now unknown. Because both

sleep and pain are heavily influenced by environmental and psychological factors that evolve over time, very few longitudinal polysomnographic (PSG) studies have been performed in patients with neuropathic pain (31). Short-term, postoperative PSG studies report less non–rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS), but exposure to general anesthetics and postoperative care could confound these studies (32, 33). Similarly, rodent sleep studies yield varying results, possibly because of differences in species, strains, surgical protocols, and anesthetics used or environmental housing conditions such as bedding types (table S1). In mice, peripheral nerve injury results in heightened sensory arousal during NREMS, with more cortico-autonomic arousals and increased NREMS fragility (34), which may contribute to NREMS fragmentation (35–40) but not in all cases (34). Changes in total NREMS or REMS amount does appear to depend on the type of nerve injury performed (see table S1) (35–45).

In short, the nature and origin of the neural signal responsible for neuropathic pain-related sleep disruptions, including sleep fragmentation, have not yet been characterized. In addition, which of these sleep disturbances are specific to neuropathic pain is also unknown.

Given that painful peripheral stimuli trigger an arousal from sleep within hundreds of milliseconds (17, 46) and that nerve injury causes spontaneous ectopic firing of injured nociceptors (4, 5, 47), which is associated with spontaneous pain (48–50), we investigated whether neuropathic pain causes pain-induced arousals from sleep in mice and the neural pathways involved.

RESULTS

Single-pulse optogenetic activation of nociceptors triggers brief arousals

We characterized the nature of the sleep awakenings caused by brief, acute nociceptor stimulation during NREMS, using an optogenetic approach. We crossed mice that express Cre recombinase under the Na(v)1.8 promoter, a sodium channel expressed in all medium- (Aδ) and small-diameter (C) sensory neurons, including nociceptors (51), or mice expressing Cre recombinase under the transient receptor potential vanilloid type 1 (TRPV1) promoter, which is restricted to nociceptors (17), with Cre-dependent channelrhodopsin (ChR2)–tdTomato mice (52). We instrumented the resulting animals for electroencephalogram/electromyogram (EEG/EMG) recordings and subjected them to a transdermal 10-ms laser pulse to the hindpaw to trigger a single action potential in a small number of sensory neurons (Fig. 1A) (17). In awake Na(v)1.8::ChR2 mice, transdermal photostimulation of nerve terminals triggered nociceptive withdrawal reflexes in a power- and duration-dependent manner (fig. S1A). The same stimulation applied during NREMS (at least 20 s of preceding NREMS confirmed by EEG/EMG monitoring) triggered an arousal (EEG desynchronization associated with an increase in EMG activity; Fig. 1A) with a latency of 86.9 ± 3.9 ms (Fig. 1B). The vast majority of optogenetic stimulations resulted in a brisk withdrawal reflex of the hindpaw and an arousal lasting on average 5.7 ± 0.6 s (Fig. 1C) before NREMS resumed. In TRPV1::ChR2 mice, transdermal photostimulation of the hindpaw during NREMS also elicited a quick arousal, with an average duration of 11.3 ± 1.2 s (fig. S1B). Arousals lasting less than 16 s in mice are called brief arousals (53, 54) or microarousals (55) and occur spontaneously during sleep. The EEG power spectral density

of brief arousals triggered by optogenetic stimulation of the hindpaw revealed a spectral profile similar to those occurring spontaneously during the same recording session (Fig. 1D and fig. S1C). Although 10-ms photostimulation caused a systematic brisk withdrawal reflex of the hindpaw, this stimulus might not be long or sufficient enough to induce measurable changes in the fronto-parietal EEG. We therefore applied a repeated train of optogenetic stimulations (10× in 10 s) during wake and found that it increased the relative EEG gamma (30 to 100 Hz) power compared with spontaneous wake segments of a similar duration (fig. S1D). Last, we found that repeated optogenetic stimulations (10-ms pulse every 15 s for 5 consecutive minutes) were required to completely prevent mice from reentering NREMS (fig. S1E). Shortly after the repeated stimulation train ended, animals quickly returned to sleep (fig. S1E). Together, these data indicate that a single, acute activation of nociceptors (even an intense stimulus) elicits only a brief arousal from NREMS rather than a full, conscious awakening, whereas persistent nociceptor input is required to prevent sleep, alter the EEG, and cause insomnia-like behaviors.

Peripheral nerve injury causes NREMS fragmentation by brief arousals

Next, we tested whether peripheral nerve injury, which results in brief bursts of spontaneous activity in injured sensory neurons (4, 5, 47, 50), triggers brief arousals from sleep. Mice instrumented for EEG/EMG recordings were subjected to the spared nerve injury (SNI) model, where two distal branches of the sciatic nerve are cut and ligated (Fig. 1E), causing permanent damage and persistent evoked pain hypersensitivity (fig. S1F) (56). In addition to the standard scoring of vigilance states (57), we manually identified and flagged all brief arousals (<16 s; Fig. 1, F to I, and fig. S1G; see Materials and Methods) from NREMS during the light and dark periods to determine their number, duration, and spectral profile. The distribution of brief arousals derived from these EEG/EMG activity patterns across 24 hours shows a characteristic circadian pattern with an increase during the dark period (Fig. 1G), when mice are most active and the pressure to sleep is lower. After SNI, the number of brief arousals increased during both the light (+24 ± 4%) and dark (+25 ± 9%) periods compared with baseline (Fig. 1G). In addition, the distribution of wake-from-NREMS episodes, as a function of their mean duration, showed an increase in very short wake (<16 s) episodes after SNI (Fig. 1H and fig. S1H) and can therefore serve as a reliable proxy measure for assessing sleep fragmentation after nerve injury.

Spontaneous brief arousals preferentially occur during the declining phase of an infraslow (~50 s) oscillation of the EEG sigma (10 to 15 Hz) power (34, 55, 58), a phase proposed to be a period of “sleep fragility” (58). Consistent with these studies, we observed an infraslow fluctuation of the EEG sigma power during NREMS, peaking at 0.017 Hz (Fig. 1, I and J) at baseline with no changes after SNI (Fig. 1, I and J). The onset of flagged brief arousals clustered around the trough of the 0.02-Hz oscillation, with a mean “preferred” phase of 140.7 ± 2.2° at baseline and 138.5 ± 2.6° after SNI (Fig. 1J), indicating that the phase-coupling to the infraslow fluctuation was not altered by the nerve injury. Brief arousals present a unique spectral profile, distinct from both NREMS or wakefulness (fig. S1, I and J), with an intermediate arousal degree between NREMS and full wakefulness. The EEG spectral profile of brief arousals before and after SNI was similar (Fig. 1K and

fig. S1K). These results indicate that nerve injury severely fragments NREMS sleep by increasing the occurrence of brief arousals during periods when sleep is fragile.

Because optogenetically induced pain arousals from NREMS were also associated with paw withdrawals, we coupled EEG/EMG recordings with high-definition video capture in a separate cohort of mice to monitor subtle behavioral changes occurring during brief arousals, including those targeted at the injured hindpaw. We found that, after nerve injury, mice had more brief arousals coupled with a brief lifting of the injured paw (but not licking; Fig. 1L). Overall, we estimate that about 50% of brief arousals caused by nerve injury are associated with a subtle paw flinch/lift. These subtle paw flinches were not associated with global changes in body position, general movement, or body core temperature (fig. S1, M to P).

The increase in the number of brief arousals after SNI led to fragmented NREMS, as indicated by shorter and more frequent NREMS episodes, without changing the total amount of NREMS or its overall circadian distribution (Fig. 2A and fig. S2A). The NREMS spectral profile, when not interrupted by brief arousals, was also unchanged after SNI (fig. S2B). Male and female mice subjected to SNI developed NREMS fragmentation by brief arousals to a similar degree (Fig. 2B and fig. S2C).

We then conducted a longitudinal study to investigate the duration of sleep disturbances after nerve injury and performed EEG/EMG recordings in SNI mice over the course of 7 weeks. The SNI-induced fragmentation of NREMS by brief arousals was present at 1 week and continued for at least 7 weeks after SNI (Fig. 2C and fig. S2D), with little interindividual variability (Fig. 2, D and E). In contrast, SNI did not change the duration or number of REMS episodes (Fig. 2, F and G), the distribution of wake-from-REMS episodes or the REMS spectral profile (fig. S2, E and F), indicating that SNI does not disrupt REMS architecture. Sham procedures, in which the sciatic nerve was exposed but not injured, did not affect the occurrence of brief arousals or any other sleep-wake parameters when tested over the same time course as nerve-injured mice (fig. S2, G to J).

Next, we performed a chronic constriction injury (CCI) of the sciatic nerve (59, 60) on mice implanted for sleep recordings (Fig. 3A). CCI triggers a local inflammation and partial nerve ischemia that damage most axons and cause neuropathic pain symptoms in rodents. CCI also fragmented NREMS, with more frequent and briefer NREMS episodes during the light period, but, during the dark period, NREMS fragmentation was less consistent (Fig. 3A and fig. S3A). NREMS fragmentation was caused by an increase in very short wake episodes interrupting NREMS (Fig. 3B). This effect was maximal 5 weeks after CCI and started to attenuate after 11 weeks (Fig. 3, C and D). During the dark period, CCI animals spent more time in NREMS, at the expense of wake, compared with baseline (fig. S3, A to C). CCI did not alter REMS architecture at any of the time points tested (fig. S3, A to C).

Last, to test whether the NREMS fragmentation caused by nerve injury recovers with a return of normal sensory sensitivity, we used a model of transient neuropathic pain produced by crushing the sciatic nerve (SNCrush; Fig. 3E). After this procedure, mice displayed a transient phase of pain-like hypersensitivity to mechanical and cold (4°C) stimuli 2

to 4 weeks after injury, which returned to normal values at about 5 to 6 weeks after the injury. SNCrush transiently increased the number of brief arousals and shortened the average duration of NREMS episodes (similar to SNI and CCI), and this resolved by 5 weeks after injury, when the SNCrush mice recovered normal sensory responses from the hindpaw (Fig. 3, F to H, and fig. S3, D to F). Together, these results indicate that three different mouse models of traumatic peripheral nerve injury develop NREMS fragmentation by brief arousals and that sleep fragmentation coincides with abnormal sensory responses and resolves when pain-related behaviors subside.

Inflammatory and chemical pain do not fragment NREMS

Next, we tested whether other models of pain hypersensitivity also develop NREMS fragmentation by increasing brief arousals. We assessed inflammatory pain caused by intraplantar injection of Freund's complete adjuvant (FCA; Fig. 4A) or carrageenan (Fig. 4B), as well as by capsaicin (Fig. 4C), and postoperative pain after skin incision of the plantar surface of the hindpaw (Fig. 4E). There was no change in the average duration of NREMS episodes and no increase in brief arousals in any of these pain models (Fig. 4, A to D, and fig. S4, A to F), although animals displayed evoked pain hypersensitivity across several modalities (Fig. 4, A to D, and fig. S4, A to F). These data reveal that NREMS fragmentation by brief arousals is independent of evoked pain hypersensitivity and is specific to post-injury neuropathic pain.

Ectopic activation of injured nociceptors is responsible for NREMS fragmentation by brief arousals

To identify the neurons responsible for triggering the NREMS fragmentation after nerve injury, we crossed mice with Cre recombinase–dependent expression of the light chain of the tetanus toxin (tet-tox) (61) with Na(v)1.8-Cre (Fig. 5A). Expression of the light chain of tet-tox blocks synaptic vesicle exocytosis and thereby synaptic input to the spinal cord, which we confirmed by measuring blockade of neuropeptide calcitonin gene–related peptide (CGRP) release from dorsal root ganglion (DRG) neurons from Na(v)1.8::tet-tox mice or wild-type (WT) littermates. Both capsaicin (0.5 μM) and KCl (50 mM) triggered release of CGRP from cultured WT sensory neurons; however, CGRP release was nearly abolished in Na(v)1.8::tet-tox neurons (Fig. 5A). Behaviorally, Na(v)1.8::tet-tox mice were viable and displayed a blunted response to noxious stimuli (Fig. 5B), whereas responses to innocuous stimuli were normal (fig. S5, A to C) (62). At baseline, sleep-wake architecture was similar in Na(v)1.8::tet-tox mice and their WT littermates (Fig. 5C and fig. S5D). After SNI, littermate mice developed NREMS fragmentation with an increase in brief arousals and a decrease in NREMS episode average duration compared with baseline, whereas Na(v)1.8::tet-tox SNI mice were protected from the NREMS fragmentation (Fig. 5, D and E). These data indicate that sleep fragmentation after nerve injury is initiated by injured peripheral Na(v)1.8-expressing nociceptors. Next, we crossed mice that express the diphtheria toxin in a Cre-dependent manner with TRPV1-Cre animals, producing TRPV1::DTA mice where TRPV1 lineage cells are ablated (63), and found that they too did not develop NREMS fragmentation after SNI (Fig. 5F and fig. S5E).

Both Na(v)1.8::tet-tox and TRPV1::DTA mice developed mechanical allodynia to the same extent as their littermates after nerve injury (fig. S5F) (63), indicating that stimulus-evoked pain hypersensitivity after SNI is not the likely cause of NREMS fragmentation. To test this formally, we pharmacologically blocked the spared nociceptor skin nerve terminals of SNI mice (intact sural and saphenous innervation) and recorded their sleep (Fig. 5G). We used a cocktail of 2% lidocaine/0.5% QX-314 (64, 65) to produce an extended (9 hours) nociceptor-selective blockade in addition to the shorter duration (2 hours) total nerve blockade produced by lidocaine. Intraplantar injection of 2% lidocaine/0.5% QX-314 did not restore the average duration of NREMS episodes and even slightly increased the number of brief arousals during full sensory blockade (lidocaine effect covering the first 2 hours), whereas the extended blockade (9 hours) did not change these parameters compared to vehicle-injected mice (Fig. 5G). We conclude that the neural activity responsible for NREMS fragmentation after nerve injury is generated by small-diameter nociceptor sensory neurons [populations expressing Na(v)1.8 and of TRPV1-lineage] and that this does not require peripheral stimuli (Lido-QX314 results), indicating, instead, that it is a consequence of spontaneous ectopic activity in injured peripheral sensory neurons. Because a return of normal sleep continuity coincided with peripheral nerve regeneration and sensory recovery after sciatic nerve crush (SNCrush results), we suggest that injured nociceptors are responsible for fragmenting NREMS by brief arousals after axonal injury in a peripheral nerve.

Using NREMS fragmentation by brief arousals to detect possible analgesic efficacy

To examine whether the sleep fragmentation associated with spontaneous neuropathic pain is modulated by analgesics, we intraperitoneally administered gabapentin, a first-line medication for neuropathic pain (66, 67), and found that it dose-dependently decreased the number of brief arousals during NREMS and increased NREMS episode average duration in SNI mice (Fig. 6, A and B). At 30 mg/kg, gabapentin did not reduce mechanical allodynia in awake mice (fig. S6A) and did not cause major sedation, as indicated by unaltered onset of NREMS and normal sleep amount after drug administration (Fig. 6C). However, at this dose, NREMS episodes were longer than in non-injured state (see Fig. 6B) during the first 2 hours, suggesting a possible transient sedative/somnolence side effect. In support of this, EEG power in the gamma band (30 to 100 Hz) was decreased during both wakefulness and NREMS for 4 hours (fig. S6, C to D) and for 2 hours during REMS (fig. S6E). Carbamazepine, a sodium channel blocker used to block paroxysmal pain in trigeminal neuralgia (68), also reduced the number of brief arousals and increased NREMS continuity (Fig. 6D) without sedation (Fig. 6E) when administered at 5 mg/kg subcutaneously (sc), a dose that does not relieve stimulus-evoked pain. In contrast, morphine [1 mg/kg intraperitoneally (ip)] did not normalize NREMS episode duration or the number of brief arousals in SNI mice (Fig. 6F). The reduction in brief arousals triggered by neuropathic pain could therefore be a marker of analgesic efficacy specifically for the spontaneous pain element of this condition.

CGRP-expressing neurons of PB mediate nerve injury–induced brief arousals

To identify the pathway through which spontaneous activity in nociceptors after nerve injury drives cortical arousal, we genetically targeted the lateral parabrachial nucleus (PB), which

is both a major relay for ascending nociceptive signals (69) and one where chemogenetic or optogenetic activation wakes sleeping mice (70). Within this nucleus, CGRP-expressing neurons are activated by nociceptive inputs (71), and we selectively ablated these PB neurons by bilaterally microinjecting an AAV coding for Cre-dependent expression of diphtheria toxin (AAV-Flex-DTA) into the lateral PB of CGRP-CreER mice that express Cre under the CGRP promoter (Fig. 7A and fig. S7A). To induce recombination, mice received tamoxifen for 5 consecutive days, starting just before adeno-associated virus (AAV) microinjections. We confirmed the nearly total deletion of CGRP neurons by counting the CGRP-positive neurons within the lateral PB (Fig. 7A). After SNI, the PB^{CGRP}-DTA mice showed no increase in brief arousals, whereas CreER-negative mice displayed the typical NREMS fragmentation from brief arousals (Fig. 7, B to D), revealing that the CGRP-expressing neurons of the lateral PB are necessary for driving the fragmentation of sleep produced by peripheral nerve injury.

DISCUSSION

Peripheral nerve injury fragments NREMS by triggering brief arousals, without reducing the total amount of sleep. These brief arousals are driven by an input from injured primary sensory neurons in a peripheral nerve that are then relayed through the CGRP-containing neurons of the lateral PB, a critical relay of nociceptive information processing (69, 70). These arousing signals do not require external peripheral input from the injury site and are likely triggered by brief, spontaneous activity arising ectopically in injured nociceptors. Such activity causes brief arousals when asleep, and we propose that the number of brief arousals during NREMS is an objective surrogate of spontaneous neuropathic pain in vivo that can be used to measure the efficacy of analgesic medications.

Two main types of spontaneous pain have been characterized: ongoing and paroxysmal pain (3). Their qualitative description, temporal profile, and prevalence depending on chronic pain etiologies (72, 73) imply that different mechanisms could be involved for each. Our results suggest that, after nerve injury, NREMS fragmentation by brief arousals is caused by paroxysmal pain attacks, whereas other assays might be able to preferentially monitor ongoing pain. Paroxysmal pain is extremely prevalent after severe nerve injury and is almost always reported in patients with a neuroma (1), and it is much rarer in chronic inflammatory pain settings. In our experiments, only nerve injury/neuropathic models increased the number of brief arousals, and these arousals resolved after nerve regeneration and target reinnervation and in response to carbamazepine, a drug that is clinically effective for paroxysmal neuropathic pain attacks (47, 74, 75). Second, using an optogenetic approach to activate nociceptors, we found that a single acute photostimulation caused a brief arousal, whereas repeated activation (similar to ongoing pain sensation) is required to prevent sleep. It is possible then that paroxysmal pain causes brief arousals and that tonic, ongoing pain leads to an insomnia-like situation. Ongoing pain might also be contributing to changes in other assays like the grimace scale and conditioned place preference. After SNI, there is an increase in grimace scale score that occurs in the first weeks after surgery, but not at later, more chronic time points (22) when robust NREMS fragmentation by brief arousals is still present. Similarly, the establishment of conditioned place aversion requires a sustained or relatively high frequency painful stimulation, whereas sparse nociceptive inputs

are insufficient (76). As a result, tests of conditioned place preference are more likely to capture relief from ongoing or frequent pain rather than detect the sporadic spontaneous pain attacks that are responsible for sleep fragmentation.

We estimate that peripheral nerve injury triggers a pain-associated brief arousal every 6 to 7 min of NREMS, which is in line with the temporal spontaneous activation profile of individual sensory neurons observed in imaging studies (50). These brief arousals are not associated with behaviors like licking of the hindpaw or escape behaviors, suggesting that they are more sensitive than the more complex pain-associated motor behaviors evoked in awake mice and that the short cortical activation time that they trigger is insufficient to recruit conscious/cognitive structures.

Our results suggest that episodes of paroxysmal pain after nerve injury are the main driving mechanism for NREMS fragmentation, and, therefore, measuring the rate of brief arousals within NREMS could be a helpful marker of this type of pain. Further, our results imply that a profiling of the exact nature of sleep disturbances in chronic pain states might represent a powerful diagnostic readout for different spontaneous pain symptoms, which would complement quantitative sensory testing of evoked pain (3).

The fragmentation of NREMS by pain-induced brief arousals could contribute to several other comorbidities in patients with neuropathic pain. In humans, experimental sleep fragmentation (by acoustic startle every 2 min) without sleep loss is associated with complaints of next day sleepiness, a negative mood, decreased mental flexibility, and difficulty sustaining attention (77), which are also major complaints from patients with neuropathic pain (30). Furthermore, experimental sleep fragmentation is sufficient to dampen hedonic capacity in healthy individuals (78), another major comorbidity present with neuropathic pain. NREMS disruption from spontaneous nociceptor activation may contribute then to several major comorbidities of chronic neuropathic pain.

We found that the neural activity responsible for triggering brief arousals arises spontaneously in Na(v) 1.8-positive and TRPV1-lineage sensory neurons after injury and does not require input evoked in intact afferents by peripheral stimuli. Because a return of normal sleep continuity coincided with peripheral nerve regeneration and sensory recovery after sciatic nerve crush, we suggest that injured nociceptors are responsible for fragmenting NREMS by brief arousals after axonal injury in a peripheral nerve. There are three anatomical sites where ectopic action potential can be generated: the site of the nerve injury (79), in DRG cell bodies (50), or presynaptic central terminals within the spinal cord. Peripheral nerve injury causes a sprouting of sympathetic fibers within the DRG, which is associated with a spontaneous firing of clusters of sensory neurons and pain-related behaviors in awake animals (50). In the same study, spontaneous firing also occurred in some injured neurons independent of sympathetic sprouting. This DRG neuron activation profile is in line with the triggering of brief arousals by a small number of axons when recruited by transdermal optogenetic stimulation (17). Another possible source of the ectopic activation of injured sensory neurons is the site of injury, where axotomized axons form a neuroma (79), which is often a source of abnormal axonal firing. Injured fibers can locally overexpress sodium channels; increase production of several factors that

increase excitability, such as tetrahydrobiopterin (60, 80, 81); and attract immune cells, including macrophages, T cells, and natural killer cells, which can promote excitability (82–84). Determining the possible involvement of any or all these factors in the generation of spontaneous neuropathic pain can now be assessed by monitoring the rate of brief arousals during NREMS.

Last, our experiments identify CGRP-expressing neurons in the lateral PB (PB^{CGRP}) as a critical relay through which nerve injury drives brief arousal from NREMS. The PB is a major target for spinal nociceptive–specific projection neurons from lamina I (69), and it is activated by a wide range of nociceptive stimuli (85, 86). Activation of excitatory neurons within the lateral PB of naïve mice is sufficient to cause a place aversion (87) and their inhibition reduces pain-related responses (87). PB^{CGRP} neurons are involved in several components of pain behavior in awake mice, including escaping from a noxious stimulus and forming an aversive memory (the driving mechanism for conditioned place aversion) (88). PB^{CGRP}-ablated mice show deficits in reacting to a noxious heat source and in developing conditioned place aversion, and the activation of these neurons promotes aversion (88). PB^{CGRP} neurons are activated by parabrachial dynorphin-positive neurons that are directly innervated by nociceptive spinal neurons (89). PB^{CGRP} neurons can drive cortical arousals from sleep through their projections to the basal forebrain and lateral hypothalamus (70) contributing to brief arousals during sleep.

Our data suggest that the anatomical pathways responsible for NREMS fragmentation after nerve injury are the same nociceptive pathways that trigger spontaneous pain in awake animals. Whether generating a conscious pain sensation requires neural circuits only activated when awake remains unknown. The spectral analyses indicate that a brief input from injured nociceptors causes arousal, but the return to sleep is quick (less than 16 s) and may occur before any triggering of conscious pain perception. For example, in people, pain-induced arousals lasting less than 1 min are not remembered the next day (46). Similarly, in one study of patients with trigeminal neuralgia, paroxysmal attacks were described by bed partners as producing agitated sleep rather than full awakenings remembered by the patients (90). However, even if these nociceptive episodes might not be experienced as conscious pain, they seem to increase the incidence of pain-related nightmares in patients with chronic pain (91).

Our study has limitations. Brief arousals occur spontaneously during NREMS, and our spectral power analyses of EEG recordings did not identify a specific signature for brief arousals caused by nociceptive stimulation. We demonstrated the increase in brief arousals caused by nerve injury originates in sensory neurons, but we cannot determine which individual brief arousal is caused by pain. Future studies aiming at identifying such possible features within the EEG to label specifically pain-induced brief arousals are needed to move toward validation of a reliable marker for spontaneous pain. We found that both Na(v)1.8::tet-tox and TRPV1::DTA mice are protected against NREMS fragmentation after nerve injury, but the exact nature of the sensory fibers involved and whether the same fibers contribute to NREMS fragmentation over time are not yet known. Last, no PSG studies have been carried out on patients with traumatic neuropathic pain to test for an increase or

abnormally high number of brief arousals and how this correlates with their sleep and pain complaints.

In conclusion, our findings causally link a specific sleep disturbance with spontaneous ectopic activity in nociceptors after nerve injury, an input that also generates spontaneous pain. Sleep disturbances, moreover, increase pain sensitivity, reduce opioid analgesic potency, and delay recovery (57, 92–95). Our results suggest that those changes in injured nociceptors that drive spontaneous pain are major drivers of sleep disruption rather than increases in sensitivity to peripheral stimuli in intact afferents. Therefore, measuring brief arousals during NREMS after nerve injury could be a useful marker of the paroxysmal spontaneous activity in nociceptors that triggers spontaneous pain and a valuable method for evaluating the efficacy of therapeutic interventions for managing paroxysmal neuropathic pain to improve the quality of life in patients.

MATERIALS AND METHODS

Study design

The major goal of this study was to characterize the sleep disturbances caused by peripheral nerve injury in mice and determine the neural pathways involved. All mice were implanted for EEG/EMG recordings, and they were monitored for their sleep and pain behaviors before and after induction of a painful condition. Sleep-wake behaviors in various models of neuropathic pain were analyzed at different time points, up to 11 weeks after nerve injury. We also analyzed sleep-wake behaviors in models of inflammatory and chemical pain to test the specificity of the phenotype to neuropathic pain. For gain-of-function studies, we used mice with Cre recombinase–dependent expression of channelrhodopsin crossed with Na(v)1.8-Cre or TRPV1-Cre animals. For loss-of-function approaches, we used mice with Cre-dependent expression of tet-tox or diphtheria toxin [Na(v)1.8-Cre, TRPV1-Cre, and CGRP-creER]. Last, we used pharmacological approaches to perform skin nerve silencing or systemic administration of clinically relevant drugs.

For experiments involving transgenic mice, randomization was achieved at weaning time: Mice were separated on the basis of their sex and placed in their new home cage, and only cages with a mixed representation of transgenic mice and their littermates were used for behavioral experiments. All experiments used at least two independent litters and were duplicated. Behavioral assays and genotyping were performed by different experimenters. For experiments involving regular C57BL/6J mice, animals were housed for at least 1 week for acclimation in their new home environment before enrolling them in experiments. Mice were then randomized into specific experimental groups. For pharmacological experiments using sleep readouts, mice were first injected with vehicle and then with increasing doses of a compound. There was at least 1 week washout between injections. For experiments using pain readouts, mice were treated only once with vehicle or drug. The person performing surgeries was blind to the genotype of mice or their experiment allocation. The persons performing pain or sleep analyses were blind to the treatment or experimental group. For all EEG/EMG recordings, a preliminary automatic scoring was visually inspected and corrected as needed by trained and blinded experimenters. In addition to the standard scoring of vigilance states (wake, NREMS, and REMS), we manually tagged brief arousals from

NREMS. Animals with inferior or poor EMG signals were excluded from the brief arousal analysis (indicated in table S2). Sample size was chosen on the basis of our previous experiments using similar assays and the literature. The exact numbers of mice used in each study are indicated in the respective figure legends, and all statistical tests are detailed in table S2. All procedures were approved in advance by the Institutional Animal Care and Use Committees of Johns Hopkins School of Medicine, Beth Israel Deaconess Medical Center, Boston Children's Hospital, and Harvard Medical School and were performed in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*.

Statistics

Statistical analysis was performed using Prism version 10.2.0 for Windows, GraphPad Software (La Jolla, CA, USA). Data are expressed as means ± SEM unless otherwise stated. Data were checked for normality using the Shapiro-Wilk method to decide for parametric statistics. Paired or unpaired student's *t* tests were used to determine significance between two groups as appropriate. Comparison between more than two groups, i.e., for nerve injury time-course (SNI, SNCrush, and Sham), drug (gabapentin) conditions, or multiple time bins, were analyzed by one- or two-way analysis of variance (ANOVA) with repeated measured when appropriate. In the case of significance, ANOVA analysis was followed by the appropriate multiple-comparisons tests. $P \leq 0.05$ was considered significant. When normality was violated, nonparametric post hoc tests (Mann-Whitney tests for unpaired and Wilcoxon tests for paired) were used. Circular statistics were done using the CircStat for Matlab toolbox. For complete statistical analyses, refer to table S2.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

We thank C. Saper and R. Srinivasa for thoughtful discussions and comments on this study. We are grateful to S. Dymecki for providing fl-STOP-fl-Tet-tox mice. A.L. wishes to thank Martine Latremoliere for life-long support.

Funding:

This work was supported, in part, by the NIH grant R01NS112266 and Blaustein Pain Research Fund Grants to A.L.; NIH grant DE022912 to C.J.W. and T.E.S.; NIH grants NS105076-01 and AT011447 and IDDRC of Boston Children's Hospital grant U54 HD090255 to C.J.W.; NIH grant R01NS112175 to S.K.; NIH grant R01AT011844 to S.V.S., and Blaustein Pain Research Fund Grants to C.A.

Data and materials availability:

All data associated with this study are present in the paper or the Supplementary Materials. Fl-STOP-fl-tet-tox mice were obtained from S. Dymecki (Harvard University) and CGRP-CreER mice obtained from P.-T. Chuang (University of California, San Francisco) under material transfer agreements.

REFERENCES AND NOTES

1. Attal N, Fermanian C, Fermanian J, Lanteri-Minet M, Alchaar H, Bouhassira D, Neuropathic pain: Are there distinct subtypes depending on the aetiology or anatomical lesion? Pain 138, 343–353 (2008). [PubMed: 18289791]
2. Backonja MM, Stacey B, Neuropathic pain symptoms relative to overall pain rating. J. Pain 5, 491–497 (2004). [PubMed: 15556827]
3. Baron R, Binder A, Wasner G, Neuropathic pain: Diagnosis, pathophysiological mechanisms, and treatment. Lancet Neurol. 9, 807–819 (2010). [PubMed: 20650402]
4. Devor M, Ectopic discharge in Abeta afferents as a source of neuropathic pain. Exp. Brain Res 196, 115–128 (2009). [PubMed: 19242687]
5. Djouhri L, Koutsikou S, Fang X, McMullan S, Lawson SN, Spontaneous pain, both neuropathic and inflammatory, is related to frequency of spontaneous firing in intact C-fiber nociceptors. J. Neurosci 26, 1281–1292 (2006). [PubMed: 16436616]
6. Tanelian DL, Maclver MB, Analgesic concentrations of lidocaine suppress tonic A-delta and C fiber discharges produced by acute injury. Anesthesiology 74, 934–936 (1991). [PubMed: 2021210]
7. Finnerup NB, Attal N, in The Oxford Handbook of the Neurobiology of Pain, Wood JN, Ed. (Oxford University Press, 2020), pp. 659–678.
8. Thakur M, Dickenson AH, Baron R, Osteoarthritis pain: Nociceptive or neuropathic? Nat. Rev. Rheumatol 10, 374–380 (2014). [PubMed: 24686507]
9. Walsh DA, McWilliams DF, Mechanisms, impact and management of pain in rheumatoid arthritis. Nat. Rev. Rheumatol 10, 581–592 (2014). [PubMed: 24861185]
10. Hunter DJ, McDougall JJ, Keefe FJ, The symptoms of osteoarthritis and the genesis of pain. Rheum. Dis. Clin. North Am 34, 623–643 (2008). [PubMed: 18687276]
11. Buch NS, Qerama E, Brix Finnerup N, Nikolajsen L, Neuromas and postamputation pain. Pain 161, 147–155 (2020). [PubMed: 31568042]
12. Tracey I, Woolf CJ, Andrews NA, Composite pain biomarker signatures for objective assessment and effective treatment. Neuron 101, 783–800 (2019). [PubMed: 30844399]
13. Deseure K, Adriaensen H, Nonevoked facial pain in rats following infraorbital nerve injury: A parametric analysis. Physiol. Behav 81, 595–604 (2004). [PubMed: 15178152]
14. Deseure K, Hans G, Behavioral study of non-evoked orofacial pain following different types of infraorbital nerve injury in rats. Physiol. Behav 138, 292–296 (2015). [PubMed: 25455862]
15. Deseure K, Hans GH, Differential drug effects on spontaneous and evoked pain behavior in a model of trigeminal neuropathic pain. J. Pain Res 10, 279–286 (2017). [PubMed: 28184169]
16. Vos BP, Strassman AM, Maciewicz RJ, Behavioral evidence of trigeminal neuropathic pain following chronic constriction injury to the rat's infraorbital nerve. J. Neurosci 14, 2708–2723 (1994). [PubMed: 8182437]
17. Browne LE, Latremoliere A, Lehnert BP, Grantham A, Ward C, Alexandre C, Costigan M, Michoud F, Roberson DP, Ginty DD, Woolf CJ, Time-resolved fast mammalian behavior reveals the complexity of protective pain responses. Cell Rep. 20, 89–98 (2017). [PubMed: 28683326]
18. Henrich MC, Frahm KS, Coghill RC, Andersen OK, Spinal nociception is facilitated during cognitive distraction. Neuroscience 491, 134–145 (2022). [PubMed: 35381321]
19. Legrain V, Guerit JM, Bruyer R, Plaghki L, Attentional modulation of the nociceptive processing into the human brain: Selective spatial attention, probability of stimulus occurrence, and target detection effects on laser evoked potentials. Pain 99, 21–39 (2002). [PubMed: 12237181]
20. Mogil JS, Animal models of pain: Progress and challenges. Nat. Rev. Neurosci 10, 283–294 (2009). [PubMed: 19259101]
21. Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AM, Ferrari MD, Craig KD, Mogil JS, Coding of facial expressions of pain in the laboratory mouse. Nat. Methods 7, 447–449 (2010). [PubMed: 20453868]

22. Mogil JS, Pang DSJ, Silva Dutra GG, Chambers CT, The development and use of facial grimace scales for pain measurement in animals. Neurosci. Biobehav. Rev 116, 480–493 (2020). [PubMed: 32682741]
23. King T, Vera-Portocarrero L, Gutierrez T, Vanderah TW, Dussor G, Lai J, Fields HL, Porreca F, Unmasking the tonic-aversive state in neuropathic pain. Nat. Neurosci 12, 1364–1366 (2009). [PubMed: 19783992]
24. Rice ASC, Finnerup NB, Kemp HI, Currie GL, Baron R, Sensory profiling in animal models of neuropathic pain: A call for back-translation. Pain 159, 819–824 (2018). [PubMed: 29300280]
25. Norman GJ, Karelina K, Zhang N, Walton JC, Morris JS, Devries AC, Stress and IL-1beta contribute to the development of depressive-like behavior following peripheral nerve injury. Mol. Psychiatry 15, 404–414 (2010). [PubMed: 19773812]
26. Sieberg CB, Taras C, Gomaa A, Nickerson C, Wong C, Ward C, Baskozos G, Bennett DLH, Ramirez JD, Themistocleous AC, Rice ASC, Shillo PR, Tesfaye S, Edwards RR, Andrews NA, Berde C, Costigan M, Neuropathic pain drives anxiety behavior in mice, results consistent with anxiety levels in diabetic neuropathy patients. Pain Rep. 3, e651 (2018). [PubMed: 29922743]
27. Zhou W, Jin Y, Meng Q, Zhu X, Bai T, Tian Y, Mao Y, Wang L, Xie W, Zhong H, Zhang N, Luo MH, Tao W, Wang H, Li J, Li J, Qiu BS, Zhou JN, Li X, Xu H, Wang K, Zhang X, Liu Y, Richter-Levin G, Xu L, Zhang Z, A neural circuit for comorbid depressive symptoms in chronic pain. Nat. Neurosci 22, 1649–1658 (2019). [PubMed: 31451801]
28. Miettinen T, Mantyselka P, Hagelberg N, Mustola S, Kalso E, Lotsch J, Machine learning suggests sleep as a core factor in chronic pain. Pain 162, 109–123 (2021). [PubMed: 32694382]
29. Sun Y, Laksono I, Selvanathan J, Saripella A, Nagappa M, Pham C, Englesakis M, Peng P, Morin CM, Chung F, Prevalence of sleep disturbances in patients with chronic non-cancer pain: A systematic review and meta-analysis. Sleep Med. Rev 57, 101467 (2021). [PubMed: 33827029]
30. Turk DC, Dworkin RH, Revicki D, Harding G, Burke LB, Cella D, Cleeland CS, Cowan P, Farrar JT, Hertz S, Max MB, Rappaport BA, Identifying important outcome domains for chronic pain clinical trials: An IMMPACT survey of people with pain. Pain 137, 276–285 (2008). [PubMed: 17937976]
31. Bjurstrom MF, Irwin MR, Polysomnographic characteristics in nonmalignant chronic pain populations: A review of controlled studies. Sleep Med. Rev 26, 74–86 (2016). [PubMed: 26140866]
32. Chouchou F, Khoury S, Chauny JM, Denis R, Lavigne GJ, Postoperative sleep disruptions: A potential catalyst of acute pain? Sleep Med. Rev 18, 273–282 (2014). [PubMed: 24074687]
33. Kehlet H, Jensen TS, Woolf CJ, Persistent postsurgical pain: Risk factors and prevention. Lancet 367, 1618–1625 (2006). [PubMed: 16698416]
34. Cardis R, Lecci S, Fernandez LM, Osorio-Forero A, Chung PCS, Fulda S, Decosterd I, Luthi A, Cortico-autonomic local arousals and heightened somatosensory arousability during NREMS of mice in neuropathic pain. eLife 10, e65835 (2021). [PubMed: 34227936]
35. Ito H, Navratilova E, Vagnerova B, Watanabe M, Kopruszinski C, Moreira de Souza LH, Yue X, Ikegami D, Moutal A, Patwardhan A, Khanna R, Yamazaki M, Guerrero M, Rosen H, Roberts E, Neugebauer V, Dodick DW, Porreca F, Chronic pain recruits hypothalamic dynorphin/kappa opioid receptor signalling to promote wakefulness and vigilance. Brain 146, 1186–1199 (2023). [PubMed: 35485490]
36. Liu YY, Yin D, Chen L, Qu WM, Chen CR, Laudon M, Cheng NN, Urade Y, Huang ZL, Piromelatine exerts antinociceptive effect via melatonin, opioid, and 5HT1A receptors and hypnotic effect via melatonin receptors in a mouse model of neuropathic pain. Psychopharmacology 231 , 3973–3985 (2014). [PubMed: 24700387]
37. Narita M, Niikura K, Nanjo-Niikura K, Narita M, Furuya M, Yamashita A, Saeki M, Matsushima Y, Imai S, Shimizu T, Asato M, Kuzumaki N, Okutsu D, Miyoshi K, Suzuki M, Tsukiyama Y, Konno M, Yomiya K, Matoba M, Suzuki T, Sleep disturbances in a neuropathic pain-like condition in the mouse are associated with altered GABAergic transmission in the cingulate cortex. Pain 152, 1358–1372 (2011). [PubMed: 21396773]

38. Wu YE, Li YD, Luo YJ, Wang TX, Wang HJ, Chen SN, Qu WM, Huang ZL, Gelsemine alleviates both neuropathic pain and sleep disturbance in partial sciatic nerve ligation mice. Acta Pharmacol. Sin 36, 1308–1317 (2015). [PubMed: 26388157]
39. Zhou H, Li M, Zhao R, Sun L, Yang G, A sleep-active basalocortical pathway crucial for generation and maintenance of chronic pain. Nat. Neurosci 26, 458–469 (2023). [PubMed: 36690899]
40. Ho A, Lee SJ, Drew VJ, Jung J, Kang J, Cheong C, Kim T, Sleep disturbance correlated with severity of neuropathic pain in sciatic nerve crush injury model. J. Sleep Res e14137 (2024). [PubMed: 38199868]
41. Andersen ML, Tufik S, Sleep patterns over 21-day period in rats with chronic constriction of sciatic nerve. Brain Res. 984, 84–92 (2003). [PubMed: 12932842]
42. Kontinen VK, Ahnaou A, Drinkenburg WH, Meert TF, Sleep and EEG patterns in the chronic constriction injury model of neuropathic pain. Physiol. Behav 78, 241–246 (2003). [PubMed: 12576121]
43. Leys LJ, Chu KL, Xu J, Pai M, Yang HS, Robb HM, Jarvis MF, Radek RJ, McGaraughty S, Disturbances in slow-wave sleep are induced by models of bilateral inflammation, neuropathic, and postoperative pain, but not osteoarthritic pain in rats. Pain 154, 1092–1102 (2013). [PubMed: 23664655]
44. Monassi CR, Bandler R, Keay KA, A subpopulation of rats show social and sleep-waking changes typical of chronic neuropathic pain following peripheral nerve injury. Eur. J. Neurosci 17, 1907–1920 (2003). [PubMed: 12752790]
45. Tokunaga S, Takeda Y, Shinomiya K, Yamamoto W, Utsu Y, Toide K, Kamei C, Changes of sleep patterns in rats with chronic constriction injury under aversive conditions. Biol. Pharm. Bull 30, 2088–2090 (2007). [PubMed: 17978481]
46. Bastuji H, Perchet C, Legrain V, Montes C, Garcia-Larrea L, Laser evoked responses to painful stimulation persist during sleep and predict subsequent arousals. Pain 137, 589–599 (2008). [PubMed: 18063478]
47. Tanelian DL, Brose WG, Neuropathic pain can be relieved by drugs that are use-dependent sodium channel blockers: Lidocaine, carbamazepine, and mexiletine. Anesthesiology 74, 949–951 (1991). [PubMed: 1850581]
48. Haroutounian S, Nikolajsen L, Bendtsen TF, Finnerup NB, Kristensen AD, Hasselstrom JB, Jensen TS, Primary afferent input critical for maintaining spontaneou pain in peripheral neuropathy. Pain 155, 1272–1279 (2014). [PubMed: 24704366]
49. Truini A, Padua L, Biasiotta A, Caliandro P, Pazzaglia C, Galeotti F, Inghilleri M, Cruccu G, Differential involvement of A-delta and A-beta fibres in neuropathic pain related to carpal tunnel syndrome. Pain 145, 105–109 (2009). [PubMed: 19535205]
50. Zheng Q, Xie W, Luckemeyer DD, Lay M, Wang XW, Dong X, Limjunyawong N, Ye Y, Zhou FQ, Strong JA, Zhang JM, Dong X, Synchronized cluster firing, a distinct form of sensory neuron activation, drives spontaneous pain. Neuron 110, 209–220.e6 (2022). [PubMed: 34752775]
51. Agarwal N, Offermanns S, Kuner R, Conditional gene deletion in primary nociceptive neurons of trigeminal ganglia and dorsal root ganglia. Genesis 38, 122–129 (2004). [PubMed: 15048809]
52. Madisen L, Mao T, Koch H, Zhuo JM, Berenyi A, Fujisawa S, Hsu YW, Garcia III AJ, Gu X, Zanella S, Kidney J, Gu H, Mao Y, Hooks BM, Boyden ES, Buzsaki G, Ramirez JM, Jones AR, Svoboda K, Han X, Turner EE, Zeng H, A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing. Nat. Neurosci 15, 793–802 (2012). [PubMed: 22446880]
53. Franken P, Dijk DJ, Tobler I, Borbely AA, Sleep deprivation in rats: Effects on EEG power spectra, vigilance states, and cortical temperature. Am. J. Physiol 261, R198–R208 (1991). [PubMed: 1858947]
54. Tobler I, Deboer T, Fischer M, Sleep and sleep regulation in normal and prion protein-deficient mice. J. Neurosci 17, 1869–1879 (1997). [PubMed: 9030645]
55. Antila H, Kwak I, Choi A, Pisciotti A, Covarrubias I, Baik J, Eisch A, Beier K, Thomas S, Weber F, Chung S, A noradrenergic-hypothalamic neural substrate for stress-induced sleep disturbances. Proc. Natl. Acad. Sci. U.S.A 119, e2123528119 (2022). [PubMed: 36331996]

56. Decosterd I, Woolf CJ, Spared nerve injury: An animal model of persistent peripheral neuropathic pain. Pain 87, 149–158 (2000). [PubMed: 10924808]
57. Alexandre C, Latremoliere A, Ferreira A, Miracca G, Yamamoto M, Scammell TE, Woolf CJ, Decreased alertness due to sleep loss increases pain sensitivity in mice. Nat. Med 23, 768–774 (2017). [PubMed: 28481358]
58. Lecci S, Fernandez LM, Weber FD, Cardis R, Chatton JY, Born J, Luthi A, Coordinated infraslow neural and cardiac oscillations mark fragility and offline periods in mammalian sleep. Sci. Adv 3, e1602026 (2017). [PubMed: 28246641]
59. Bennett GJ, Xie YK, A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 33, 87–107 (1988). [PubMed: 2837713]
60. Latremoliere A, Latini A, Andrews N, Cronin SJ, Fujita M, Gorska K, Hovius R, Romero C, Chuaiphichai S, Painter M, Miracca G, Babaniyi O, Remor AP, Duong K, Riva P, Barrett LB, Ferreiros N, Naylor A, Penninger JM, Tegeder I, Zhong J, Blagg J, Channon KM, Johnsson K, Costigan M, Woolf CJ, Reduction of neuropathic and inflammatory pain through inhibition of the tetrahydrobiopterin pathway. Neuron 86, 1393–1406 (2015). [PubMed: 26087165]
61. Ray RS, Corcoran AE, Brust RD, Kim JC, Richerson GB, Nattie E, Dymecki SM, Impaired respiratory and body temperature control upon acute serotonergic neuron inhibition. Science 333, 637–642 (2011). [PubMed: 21798952]
62. Abrahamsen B, Zhao J, Asante CO, Cendan CM, Marsh S, Martinez-Barbera JP, Nassar MA, Dickenson AH, Wood JN, The cell and molecular basis of mechanical, cold, and inflammatory pain. Science 321,702–705 (2008). [PubMed: 18669863]
63. Mishra SK, Tisel SM, Orestes P, Bhangoo SK, Hoon MA, TRPV1-lineage neurons are required for thermal sensation. EMBO J. 30, 582–593 (2011). [PubMed: 21139565]
64. Binshtok AM, Bean BP, Woolf CJ, Inhibition of nociceptors by TRPV1-mediated entry of impermeant sodium channel blockers. Nature 449, 607–610 (2007). [PubMed: 17914397]
65. Roberson DP, Binshtok AM, Blasl F, Bean BP, Woolf CJ, Targeting of sodium channel blockers into nociceptors to produce long-duration analgesia: A systematic study and review. Br. J. Pharmacol 164, 48–58 (2011). [PubMed: 21457220]
66. Dworkin RH, Backonja M, Rowbotham MC, Allen RR, Argoff CR, Bennett GJ, Bushnell MC, Farrar JT, Galer BS, Haythornthwaite JA, Hewitt DJ, Loeser JD, Max MB, Saltarelli M, Schmader KE, Stein C, Thompson D, Turk DC, Wallace MS, Watkins LR, Weinstein SM, Advances in neuropathic pain: Diagnosis, mechanisms, and treatment recommendations. Arch. Neurol 60, 1524–1534 (2003). [PubMed: 14623723]
67. Dworkin RH, Jensen MP, Gammaitoni AR, Olaleye DO, Galer BS, Symptom profiles differ in patients with neuropathic versus non-neuropathic pain. J. Pain 8, 118–126 (2007). [PubMed: 16949878]
68. Backonja MM, Use of anticonvulsants for treatment of neuropathic pain. Neurology 59, S14–S17 (2002). [PubMed: 12221151]
69. Gauriau C, Bernard JF, Pain pathways and parabrachial circuits in the rat. Exp. Physiol 87, 251–258 (2002). [PubMed: 11856971]
70. Kaur S, Wang JL, Ferrari L, Thankachan S, Kroeger D, Venner A, Lazarus M, Wellman A, Arrigoni E, Fuller PM, Saper CB, A genetically defined circuit for arousal from sleep during hypercapnia. Neuron 96, 1153–1167.e5 (2017). [PubMed: 29103805]
71. Campos CA, Bowen AJ, Roman CW, Palmiter RD, Encoding of danger by parabrachial CGRP neurons. Nature 555, 617–622 (2018). [PubMed: 29562230]
72. Pan F, Jones G, Clinical perspective on pain and pain phenotypes in osteoarthritis. Curr. Rheumatol. Rep 20, 79 (2018). [PubMed: 30382417]
73. Conaghan PG, Cook AD, Hamilton JA, Tak PP, Therapeutic options for targeting inflammatory osteoarthritis pain. Nat. Rev. Rheumatol 15, 355–363 (2019). [PubMed: 31068673]
74. Burchiel KJ, Carbamazepine inhibits spontaneous activity in experimental neuromas. Exp. Neurol 102, 249–253 (1988). [PubMed: 3181365]
75. Wiffen PJ, Derry S, Moore RA, Kalso EA, Topiramate for neuropathic pain and fibromyalgia in adults. Cochrane Database Syst. Rev 2013, CD005451 (2013).

76. Zhou H, Zhang Q, Martinez E, Dale J, Hu S, Zhang E, Liu K, Huang D, Yang G, Chen Z, Wang J, Ketamine reduces aversion in rodent pain models by suppressing hyperactivity of the anterior cingulate cortex. Nat. Commun 9, 3751 (2018). [PubMed: 30218052]
77. Martin SE, Engleman HM, Deary IJ, Douglas NJ, The effect of sleep fragmentation on daytime function. Am. J. Respir. Crit. Care Med 153, 1328–1332 (1996). [PubMed: 8616562]
78. Reid MJ, Climent-Sanz C, Finan PH, The sleep-reward-pain pathway model: An integrative review. Curr. Sleep Med. Rep 8, 97–104 (2022).
79. Devor M, Wall PD, Type of sensory nerve fibre sprouting to form a neuroma. Nature 262, 705–708 (1976). [PubMed: 958442]
80. Tegeder I, Costigan M, Griffin RS, Abele A, Belfer I, Schmidt H, Ehnert C, Nejim J, Marian C, Scholz J, Wu T, Allchorne A, Diatchenko L, Binshtok AM, Goldman D, Adolph J, Sama S, Atlas SJ, Carlezon WA, Parsegian A, Lotsch J, Fillingim RB, Maixner W, Geisslinger G, Max MB, Woolf CJ, GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence. Nat. Med 12, 1269–1277 (2006). [PubMed: 17057711]
81. Cronin SJF, Rao S, Tejada MA, Turnes BL, Licht-Mayer S, Omura T, Brenneis C, Jacobs E, Barrett L, Latremoliere A, Andrews N, Channon KM, Latini A, Arvanites AC, Davidow LS, Costigan M, Rubin LL, Penninger JM, Woolf CJ, Phenotypic drug screen uncovers the metabolic GCH1/BH4 pathway as key regulator of EGFR/ KRAS-mediated neuropathic pain and lung cancer. Sci. Transl. Med 14, eabj1531 (2022). [PubMed: 36044597]
82. Costigan M, Moss A, Latremoliere A, Johnston C, Verma-Gandhu M, Herbert TA, Barrett L, Brenner GJ, Vardeh D, Woolf CJ, Fitzgerald M, T-cell infiltration and signaling in the adult dorsal spinal cord is a major contributor to neuropathic pain-like hypersensitivity. J. Neurosci 29, 14415–14422 (2009). [PubMed: 19923276]
83. Davies AJ, Kim HW, Gonzalez-Cano R, Choi J, Back SK, Roh SE, Johnson E, Gabriac M, Kim MS, Lee J, Lee JE, Kim YS, Bae YC, Kim SJ, Lee KM, Na HS, Riva P, Latremoliere A, Rinaldi S, Ugolini S, Costigan M, Oh SB, Natural killer cells degenerate intact sensory afferents following nerve injury. Cell 176, 716–728.e18 (2019). [PubMed: 30712871]
84. Vicuna L, Strochlic DE, Latremoliere A, Bali KK, Simonetti M, Husainie D, Prokosch S, Riva P, Griffin RS, Njoo C, Gehrig S, Mall MA, Arnold B, Devor M, Woolf CJ, Liberles SD, Costigan M, Kuner R, The serine protease inhibitor SerpinA3N attenuates neuropathic pain by inhibiting T cell-derived leukocyte elastase. Nat. Med 21, 518–523 (2015). [PubMed: 25915831]
85. Kayser V, Viguier F, Ioannidi M, Bernard JF, Latremoliere A, Michot B, Vela JM, Buschmann H, Hamon M, Bourgoin S, Differential anti-neuropathic pain effects of tetrodotoxin in sciatic nerve- versus infraorbital nerve-ligated rats – Behavioral, pharmacological and immunohistochemical investigations. Neuropharmacology 58, 474–487 (2010). [PubMed: 19747496]
86. Buritova J, Besson JM, Bernard JF, Involvement of the spinoparabrachial pathway in inflammatory nociceptive processes: A c-Fos protein study in the awake rat. J. Comp. Neurol 397, 10–28 (1998). [PubMed: 9671276]
87. Sun L, Liu R, Guo F, Wen MQ, Ma XL, Li KY, Sun H, Xu CL, Li YY, Wu MY, Zhu ZG, Li XJ, Yu YQ, Chen Z, Li XY, Duan S, Parabrachial nucleus circuit governs neuropathic pain-like behavior. Nat. Commun 11 , 5974 (2020). [PubMed: 33239627]
88. Han S, Soleiman MT, Soden ME, Zweifel LS, Palmiter RD, Elucidating an affective pain circuit that creates a threat memory. Cell 162, 363–374 (2015). [PubMed: 26186190]
89. Choi S, Hachisuka J, Brett MA, Magee AR, Omori Y, Iqbal NU, Zhang D, DeLisle MM, Wolfson RL, Bai L, Santiago C, Gong S, Goulding M, Heintz N, Koerber HR, Ross SE, Ginty DD, Parallel ascending spinal pathways for affective touch and pain. Nature 587, 258–263 (2020). [PubMed: 33116307]
90. Devor M, Wood I, Sharav Y, Zakrzewska JM, Trigeminal neuralgia during sleep. Pain Pract. 8, 263–268 (2008). [PubMed: 18503619]
91. Mathes J, Schuffelen J, Dickmann B, Gieselmann A, Pietrowsky R, Pain and nightmares—A diary study of patients with chronic pain. Dreaming 32, 183–193 (2022).
92. Alexandre C, Latremoliere A, Finan PH, in The Oxford Handbook of the Neurobiology of Pain, Wood JN, Ed. (Oxford University Press, 2020), pp. 557–608.

93. Vanini G., Sleep deprivation and recovery sleep prior to a noxious inflammatory insult influence characteristics and duration of pain. Sleep 39, 133–142 (2016). [PubMed: 26237772]

94. Wang PK, Cao J, Wang H, Liang L, Zhang J, Lutz BM, Shieh KR, Bekker A, Tao YX, Short-term sleep disturbance-induced stress does not affect basal pain perception, but does delay postsurgical pain recovery. J. Pain 16, 1186–1199 (2015). [PubMed: 26342649]

95. Kourbanova K, Alexandre C, Latremoliere A, Effect of sleep loss on pain-New conceptual and mechanistic avenues. Front. Neurosci 16, 1009902 (2022). [PubMed: 36605555]

96. Franken P, Malafosse A, Tafti M, Genetic variation in EEG activity during sleep in inbred mice. Am. J. Physiol 275, R1127–R1137 (1998). [PubMed: 9756543]

97. Prerau MJ, Brown RE, Bianchi MT, Ellenbogen JM, Purdon PL, Sleep neurophysiological dynamics through the lens of multitaper spectral analysis. Physiology 32, 60–92 (2017). [PubMed: 27927806]

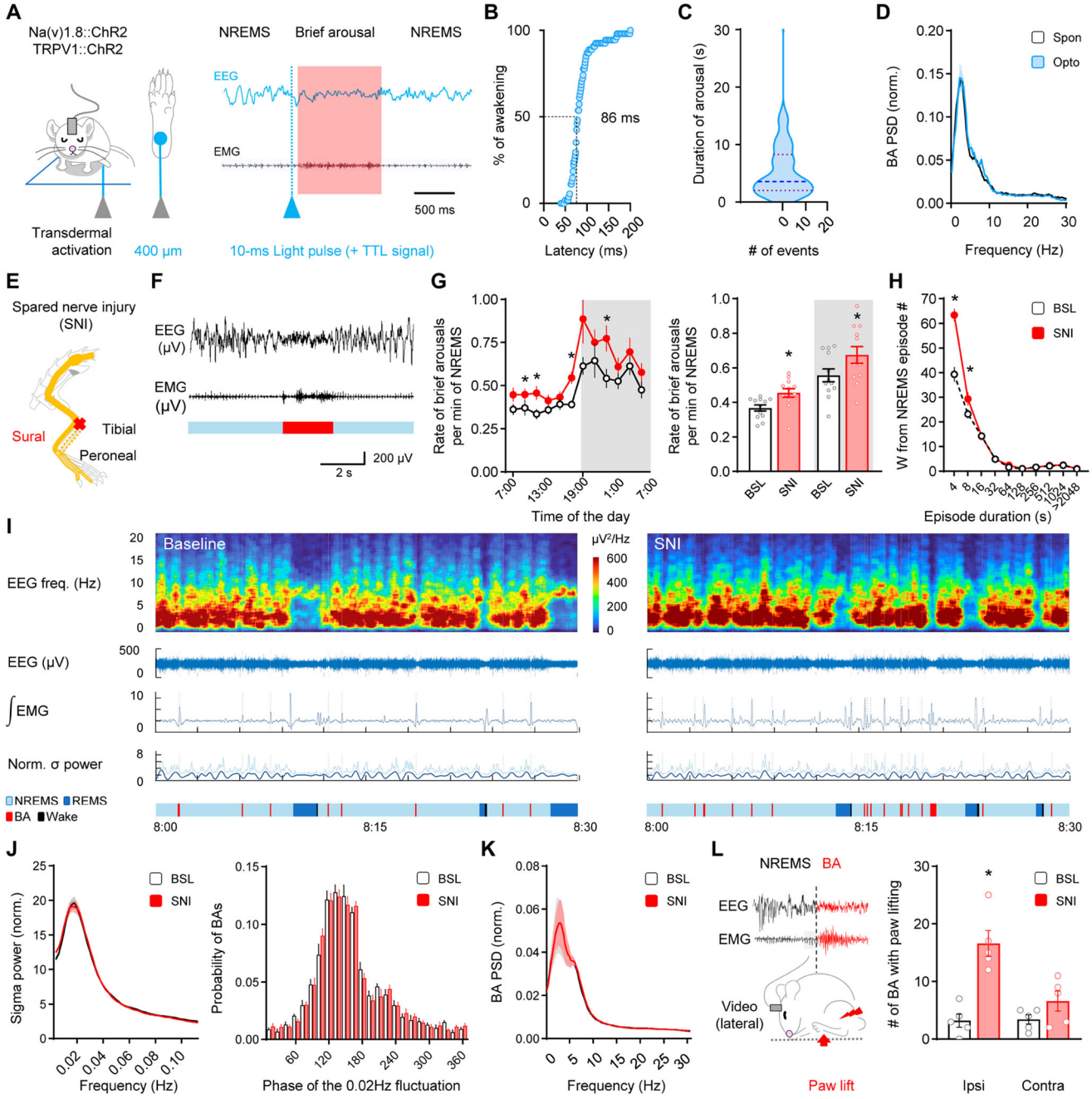

Fig. 1. Acute activation of nociceptors causes brief arousals during NREMS.

(**A**) Left: Schematic representation of the experimental protocol to trigger transdermal activation of nociceptive fibers during NREMS using a 400-µm-diameter optical fiber. Right: Representative EEG and EMG traces showing an arousal triggered by nociceptive optogenetic activation in a Na(v)1.8::ChR2 mouse. The dashed line represents the 10-ms photostimulation, and the red box highlights the arousal. TTL, transistor-transistor logic. (**B**) Latency of arousal triggered by optogenetic activation of Na(v)1.8-positive fibers in Na(v)1.8::ChR2 mice (n = 54 trials in four mice). (**C**) Duration of arousals triggered by

nociceptive optogenetic activation in Na(v)1.8::ChR2 mice. Blue dashed line indicates the median; purple dashed lines represent 25th and 75th quartiles. (**D**) EEG power spectral density during spontaneous (n = 35) and optogenetically induced (n = 38) arousals in Na(v)1.8::ChR2 mice. (**E**) Schematic representation of the SNI model. (**F**) Representative EEG and EMG traces showing a brief arousal (BA). The duration of a BA was determined by the duration of the EMG activation. In blue, NREMS; in red, BA. (**G**) Left: Hourly rate of brief arousals (number per minute of NREMS) across 24-hour period; and right: computed over the light and dark periods before (BSL) and after SNI in C57BL/6J wild-type (WT) mice. (**H**) Distribution of wake (W) episodes originating from NREMS as a function of their duration, determined by traditional scoring (4-s epoch), before (BSL) and after SNI. (**I**) Representative EEG and EMG recordings (30 min) under (left) baseline conditions and after (right) SNI procedure. Shown (top to bottom) are EEG spectrogram, raw EEG waveform, filtered and integrated EMG trace, filtered and normalized EEG sigma (10 to 15 Hz) power, and color-coded vigilance states. Brief arousals are in red, NREMS in light blue, REMS in dark blue, and wake in black. (**J**) Left: EEG power spectral density of the sigma power (10 to 15 Hz) during NREMS and (right) histograms of the phase angle values of the 0.02 Hz-fluctuation at brief arousal onset before (BSL) and after SNI. (**K**) Normalized EEG power spectral density during brief arousals before (BSL) and after SNI. Shading areas represent SEM. (**L**) Left: Schematic drawing of a paw lift occurring during brief arousals recorded by video combined with EEG/EMG recordings, and (right) quantification of paw lifts during brief arousals before (BSL) and after SNI. For all panels, data are presented as means ± SEM [n = 4 Na(v)1.8::ChR2 mice; n = 12 WT SNI mice for sleep analyses and n = 5 WT mice for EEG + paw video analyses]. Circles overlaid on the bar in histograms represent data from each individual animal. *P < 0.05 in comparison with baseline (BSL). For analysis of variance (ANOVA) values and post hoc test; please refer to table S2.

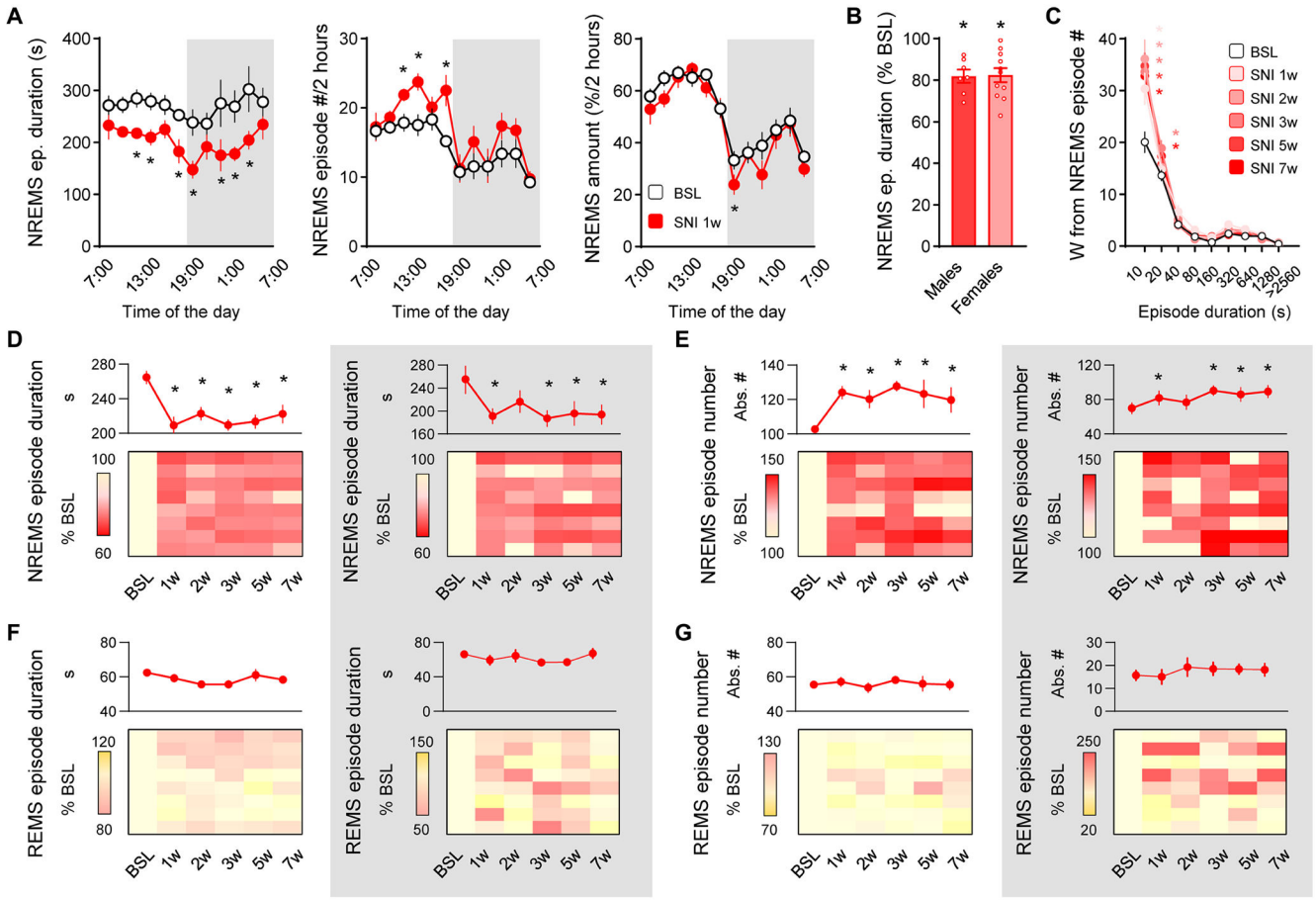

Fig. 2. Peripheral nerve injury fragments NREMS in mice.

(**A**) Left: NREMS episode mean duration; center: number; and right: amount, per 2-hour bins before (BSL) and 1 week after SNI. (**B**) NREMS episode duration in % of BSL after SNI in male and female WT mice ($n = 7$ males and 11 females). (**C**) Distribution of wake episodes originating from NREMS during the light period as a function of duration, determined by traditional scoring (10-s epoch), before (BSL) and after SNI. (**D** and **E**) NREMS episode average duration (D) and number (E) during the light and dark periods at BSL and at different time points after SNI. Each row of the heat maps shows data from one mouse. Average absolute values represented on top and individual data shown as heatmap below (in % of own BSL). (**F** and **G**) REMS episode average duration (F) and number (G) during the light and dark periods at BSL and at various times after SNI. Average absolute values represented on top, and individual data shown as heatmap below (in % of own BSL). Data are presented as means ± SEM ($n = 8$ C57BL/6J WT mice for time-course analyses, $n = 7$ males and $n = 11$ females for sex analysis). Circles overlaid on the bar in histograms represent data from each individual animal. $*P < 0.05$ in comparison with baseline. For ANOVA values and post hoc tests, refer to table S2. w, weeks.

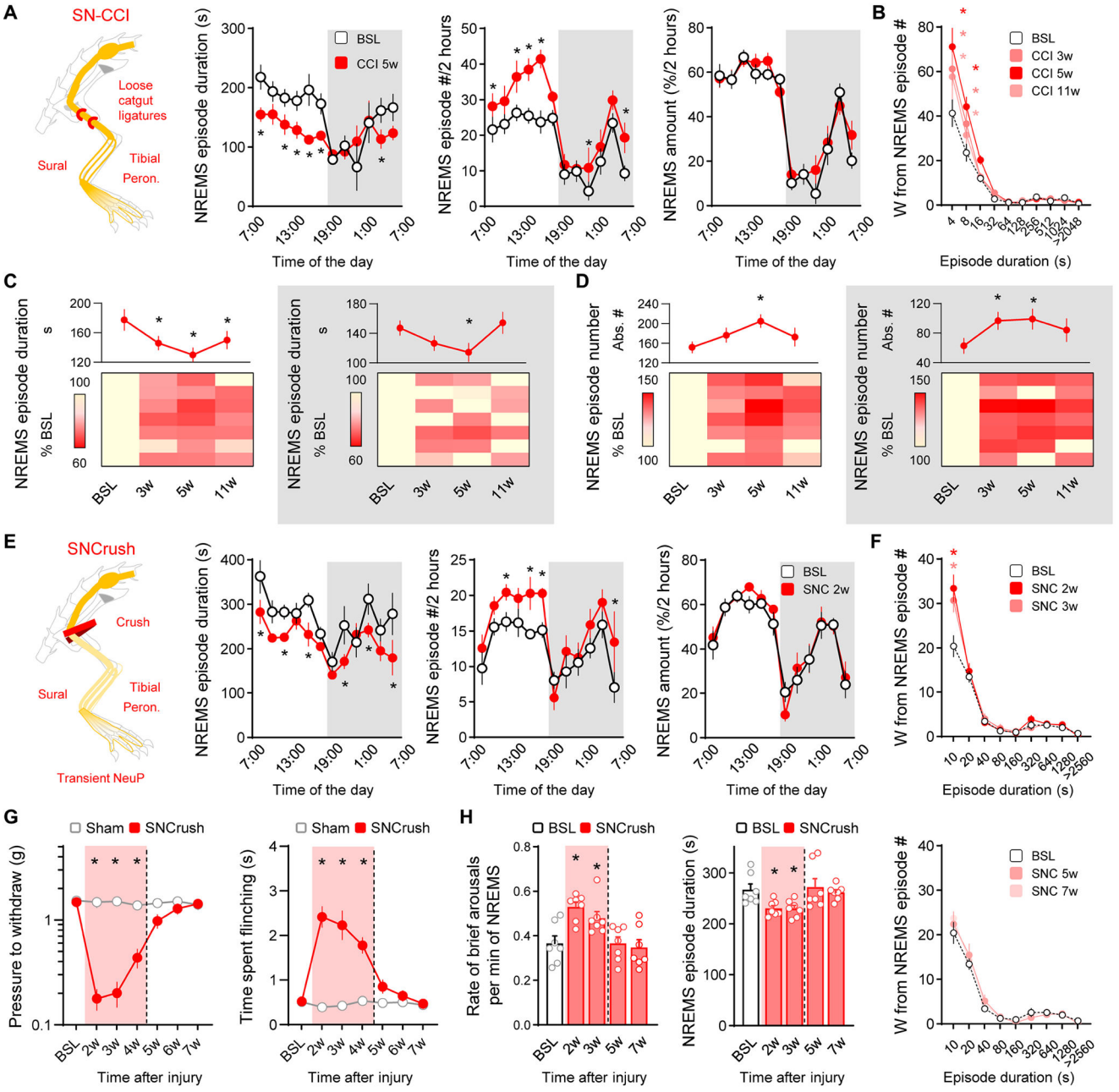

Fig. 3. CCI and sciatic nerve crush fragment NREMS.

(**A**) Far left: Mice were instrumented for EEG/EMG monitoring and assessed for baseline sleep and pain behaviors, two chromic catgut ligatures were then loosely tied around the common sciatic nerve without blocking epineural vascularization (CCI) and assessed for 11 weeks. Left: NREMS episode mean duration; center: number; and right: amount, per 2-hour bins before (BSL) and 5 weeks after CCI. (**B**) Distribution of wake episodes originating from NREMS during the light period as a function of duration, determined by traditional scoring (5-s epoch), before (BSL) and after CCI. (**C** and **D**) NREMS episode average duration (C) and number (D) during the light and dark periods at BSL and at different time

points after CCI. Each heatmap row shows data from one mouse (n = 7 mice). Averaged absolute values represented on top and individual data shown as heatmap below (in % of own BSL). (**E**) Far left: Mice were instrumented for EEG/EMG monitoring and assessed for baseline sleep and pain behaviors, then the common sciatic nerve was crushed (SNCrush), and animals were assessed for 7 weeks. Left: NREMS episode mean duration; center: number; and right: amount, per 2-hour bins before (BSL) and 2 weeks after SNCrush. (**F**) Distribution of wake episodes (W) originating from NREMS during the light period as a function of duration, determined by traditional scoring (10-s window), before (BSL) and (top) 2 and 3 weeks or (bottom) 5 and 7 weeks after SNCrush. (**G**) Left: Punctate mechanical allodynia thresholds assessed by von Frey filaments; and right: cold hyperalgesia assessed by acetone paw test after SNCrush. (**H**) Left: Brief arousal rate. Right: NREMS episode average duration before (BSL) and at different time points after SNCrush. Time points where pain hypersensitivity is present are highlighted in red. Data presented as means ± SEM (all mice are C57BL/6J WT; n = 7 CCI, n = 7 SNCrush for sleep analyses, and n = 10 mice for SNCrush pain behaviors). Circles overlaid on the bar in histograms represent data from each individual animal. *P < 0.05 in comparison with baseline. For ANOVA values and post hoc test, refer to table S2.

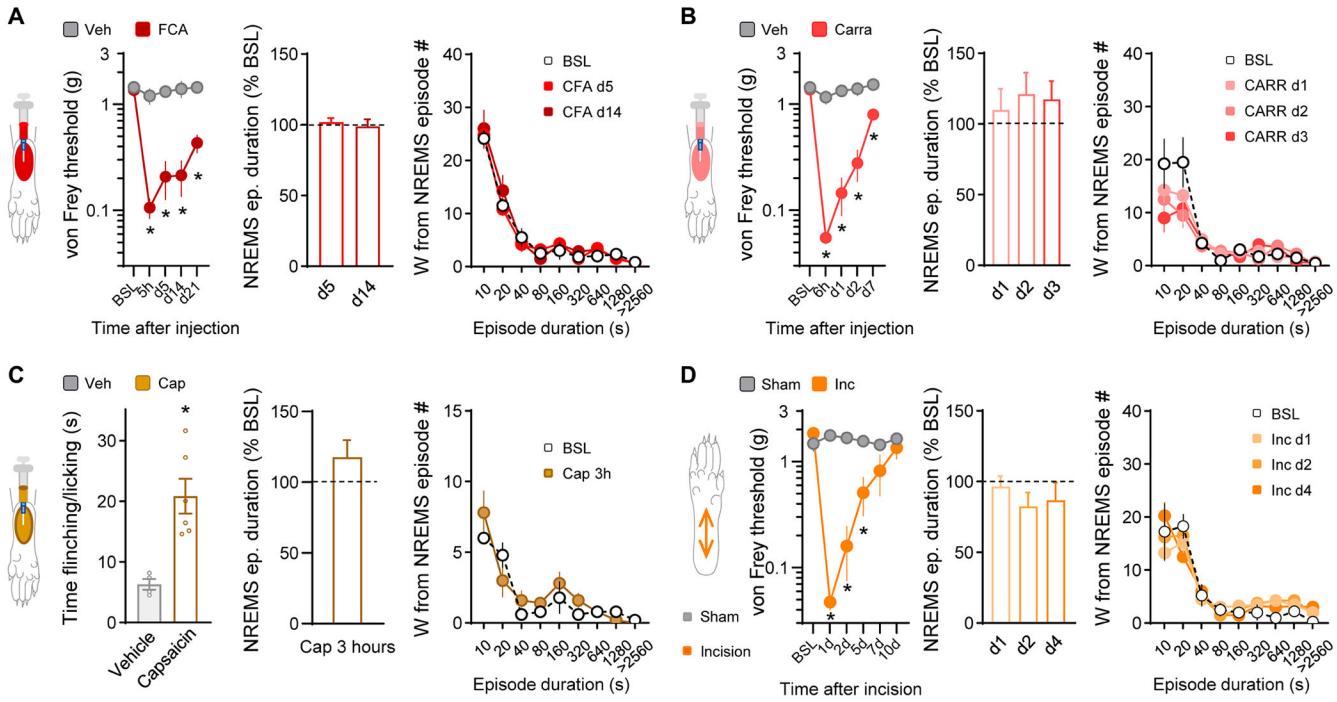

Fig. 4. Inflammatory and neurogenic pain does not fragment NREMS.

(**A** to **D**) Left: Mechanical pain hypersensitivity; center: NREMS episode average duration; and right: distribution of wake episodes originating from NREMS as a function of duration during the light period (FCA, CARR, and INC) or for 3 hours (after cap injection) in mice after: (A) intraplantar FCA injection (20 μg in 10 μl); (B) intraplantar carrageenan (CARR) injection (1% in 10 μl); (C) intraplantar injection of capsaicin (1 μg in 10 μl); (D) plantar skin incision (INC) of hindpaw. Data presented as means ± SEM (all mice are C57BL/6J WT, $n = 6$ FCA, $n = 6$ CARR, $n = 5$ capsaicin, and $n = 4$ plantar incision). *$P < 0.05$ in comparison with baseline (BSL) for inflammatory models. *$P < 0.05$ in comparison with sham animals for the incisional model. For complete statistical analyses (ANOVA values and post hoc test), refer to table S2. d, days.

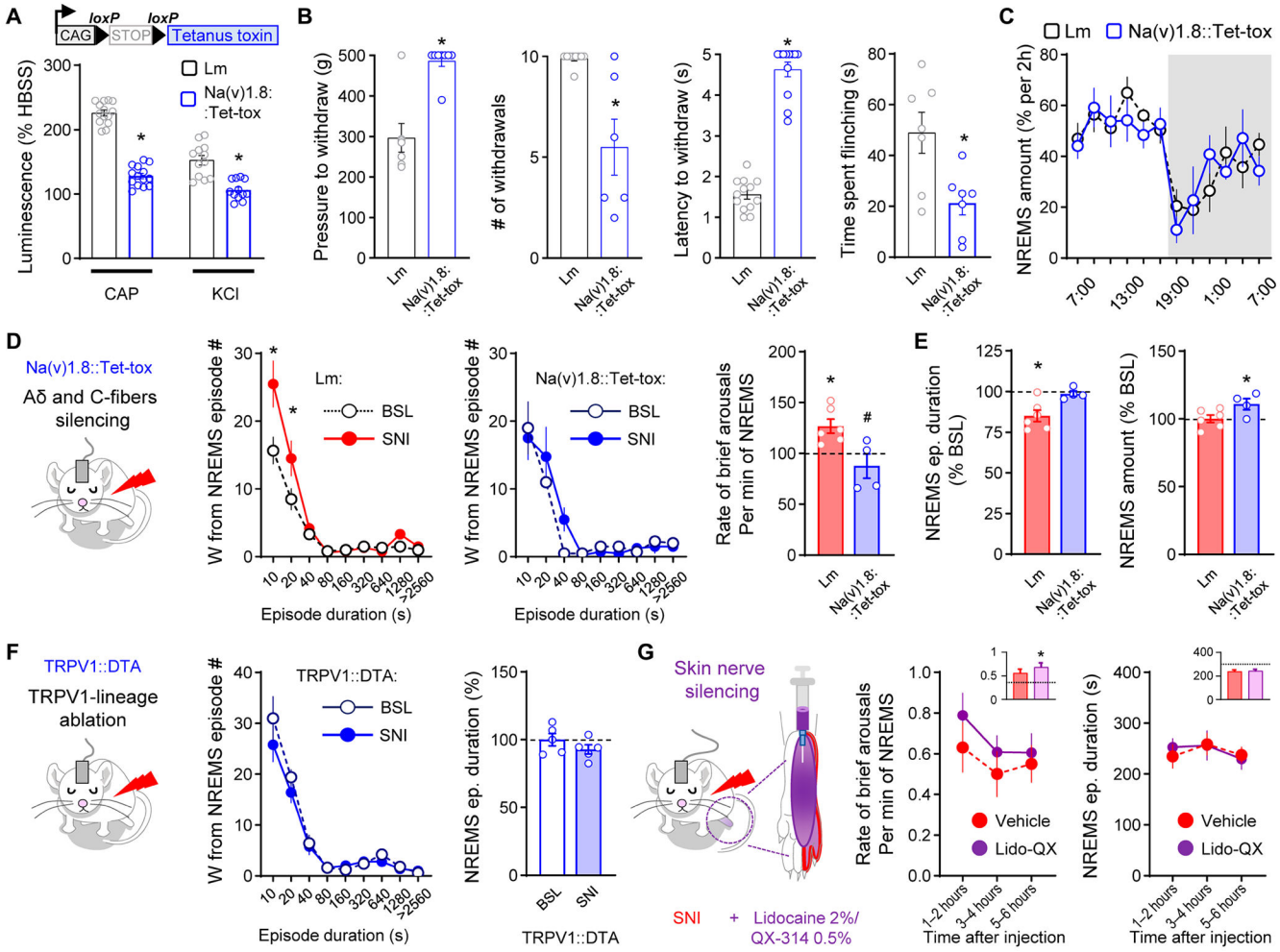

Fig. 5. The neural activity responsible for NREMS fragmentation by brief arousals originates ectopically in sensory neurons.

(**A**) Top: Genetic construct to produce Cre recombinase-dependent expression of tet-tox and (bottom) CGRP protein amount quantified by ELISA from supernatant of cultured sensory neurons from Na(v)1.8::tet-tox mice (Tg) and littermates (Lm) exposed to capsaicin or KCl (n = 4 mice per group). HBSS, Hanks' balanced salt solution. (**B**) Left to right: Behavioral responses of Na(v)1.8::tet-tox mice and littermates to calibrated forceps (n = 7 Lm and n = 8 Tg), pinprick (n = 9 Lm and n = 6 Tg), laser heat (n = 13 Lm and n = 12 Tg), and intraplantar injection of capsaicin (1 μg in 20 μl; n = 7 Lm and n = 7 Tg), respectively. (**C**) NREMS amount per 2-hour bins over 24 hours in naïve (baseline conditions) Na(v)1.8::tet-tox and littermate mice (n = 6 Lm and n = 4 Tg). (**D**) Far left: Na(v)1.8::tet-tox mice (silenced Aδ and C-fibers) were subjected to SNI surgery. Left, center: Distribution of wake episodes originating from NREMS during the light period as a function of duration before (BSL) and after SNI in Na(v)1.8::tet-tox mice and littermates. Right: Brief arousal rate (per min of NREMS) expressed in % of baseline (dashed line) after SNI in Na(v)1.8::tet-tox mice and littermates (n = 6 Lm, n = 4 Tg). (**E**) Left: NREMS episode duration; and right: amount in % of baseline (dashed line) after SNI in Na(v)1.8::tet-tox mice and littermates (n = 6 Lm and n = 4 Tg). (**F**) Left: TRPV1-Cre mice were bred with fl-STOP-fl-DTA mice to

produce animals with TRPV1-lineage ablation, which underwent SNI. Center: Distribution of wake episodes originating from NREMS during the light period as a function of duration before (BSL) and after SNI in TRPV1::DTA mice; and right: NREMS episode duration in % of baseline (dashed line) after SNI in TRPV1::DTA mice ($n = 5$). (**G**) Intraplantar injection of 2% lidocaine and 0.5% QX-314 (10 μl was performed to block nerve terminals in the skin of SNI mice instrumented for EEG/EMG monitoring, and vehicle control. Left: Brief arousal rate and NREMS episode average duration in SNI mice after injection of 2% lidocaine/0.5% QX-314 or vehicle. Insets: Results for 6 hour after injection ($n = 5$ veh and 5 lido-QX). Dashed line represents the NREMS episode duration before injury (BSL). Data are presented as means ± SEM. Circles overlaid on the bar in histograms represent data from each individual animal. *$P < 0.05$ in comparison with baseline. #$P < 0.05$ in comparison with littermates. For complete statistical analyses (ANOVA values and post hoc test), refer to table S2.

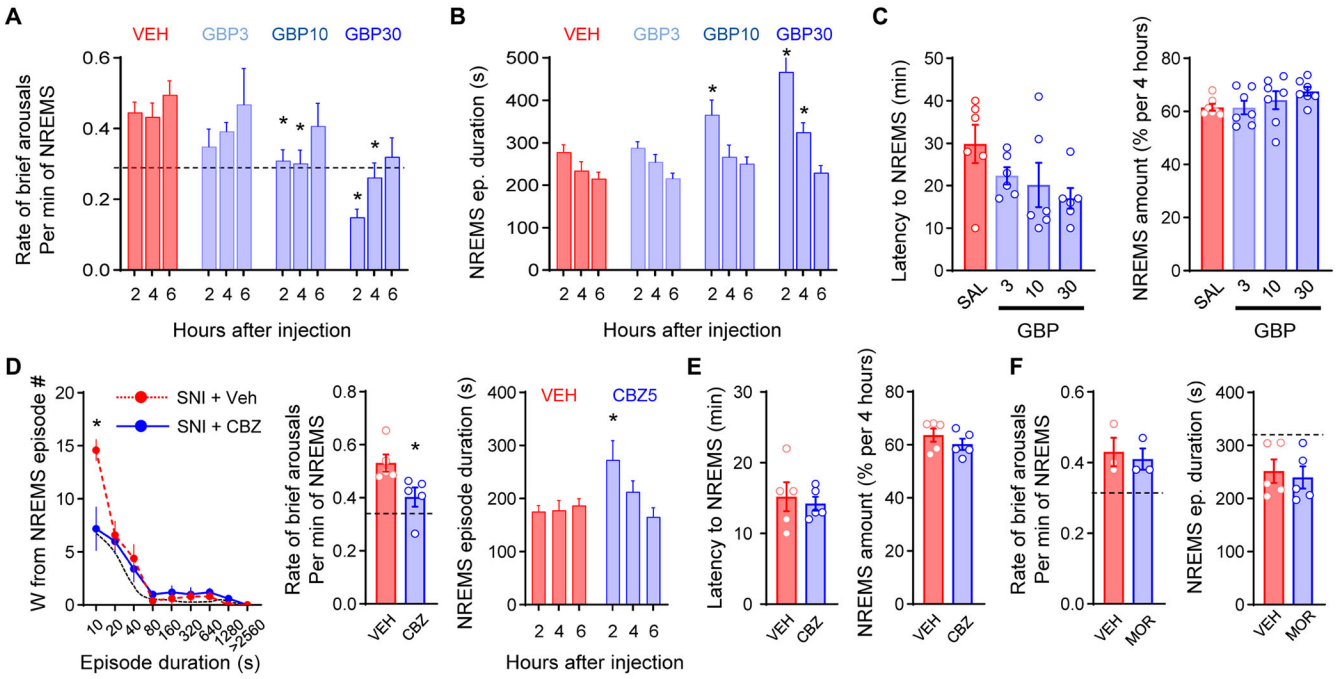

Fig. 6. Pharmacologic and genetic restoration of sleep continuity in SNI mice without causing sedation.

(**A** and **B**) (A) Brief arousal rate and (B) NREMS episode duration in 2-hour bins in SNI mice treated with vehicle or gabapentin (3 to 30 mg/kg ip). Dashed line represents the brief arousal rate before injury (BSL). (**C**) Left: NREMS latency; and right: NREMS amount averaged over 4 hours after injection in SNI mice of vehicle or gabapentin (3 to 30 mg/kg ip). (**D**) Left: Distribution of wake episodes originating from NREMS as a function of duration; center: rate of brief arousals during the first 4 hours; and right: NREMS episode average duration in 2-hour bins after injection of carbamazepine (5 mg/kg sc) in SNI mice. Dashed line represents the brief arousal rate at baseline. (**E**) Left: NREMS onset latency; and right: total NREMS amount during the first 4 hours after injection in SNI mice treated with vehicle or carbamazepine (5 mg/kg sc). (**F**) Left: Brief arousal rate; and right: NREMS episode duration for 2 hours after injection of morphine (1 mg/kg ip) in SNI mice. Data presented as means ± SEM (all mice are C57BL/6J WT; $n = 7$ for gabapentin, $n = 5$ for carbamazepine, and $n = 5$ for morphine). All pharmacological injections were performed at 12:00 p.m. Circles overlaid on the bar in histograms represent data from each individual animal. $*P < 0.05$ in comparison with vehicle. For complete statistical analyses (ANOVA values and post hoc test), refer to table S2.

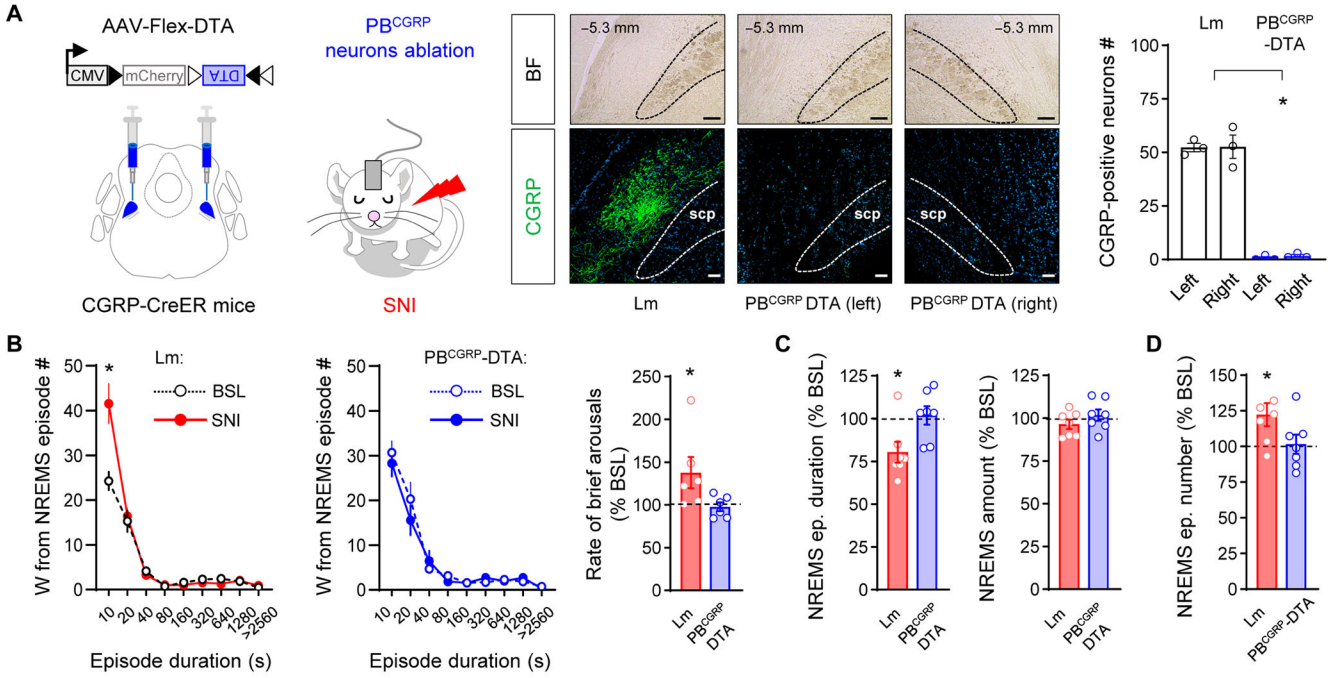

Fig. 7. Ablation of CGRP-positive neurons in the lateral PB prevents NREMS fragmentation after nerve injury.

(**A**) Left: Genetic construct of the AAV-Flex-DTA vector used for bilateral microinjections into the PB of CGRP-CreER mice or WT littermates; center: histological validation of CGRP-positive neuron deletion; and right: quantification of CGRP-positive neurons within the lateral PB. Scale bar, 50 μm (n = 3 mice per group). BF, bright field; Scp, superior cerebellar peduncle. (**B**) Left, center: Distribution of wake episodes originating from NREMS during the light period as function of duration; and right: rate of brief arousal 2 weeks after SNI as a percentage from baseline in WT littermates and PB^{CGRP}-DTA. (**C**) Left: NREMS episode average duration; and right: amount expressed in % of baseline in PB^{CGRP}-DTA and Lm mice 2 weeks after SNI. (**D**) NREMS episode number expressed as % of baseline in PB^{CGRP}-DTA and control mice 2 weeks after SNI. Data presented as means ± SEM (n = 7 PB^{CGRP}-DTA and n = 7 control Lm). Circles overlaid on the bar in histograms represent data from each individual animal. *P < 0.05 in comparison with vehicle. For complete statistical analyses (ANOVA values and post hoc test), refer to table S2.