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Descending Facilitation Maintains Long-term Spontaneous Neuropathic Pain

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Abstract

Neuropathic pain is frequently characterized by spontaneous pain (i.e. pain at rest) and in some cases, cold and touch-induced allodynia. Mechanisms underlying the chronicity of neuropathic pain are not well understood. Rats received spinal nerve ligation (SNL) and were monitored for tactile and thermal thresholds. While heat hypersensitivity returned to baseline levels within approximately 35-40 days tactile hypersensitivity was still present at 580 days after SNL. Tactile hypersensitivity at post-SNL day 60 (D60) was reversed by microinjection of (a) lidocaine or (b) a CCK2 receptor antagonist into the rostral ventromedial medulla (RVM) or (c) dorsolateral funiculus (DLF) lesion. RVM lidocaine at D60 or spinal ondansetron, a 5HT₃ antagonist, at post-SNL day 42 produced conditioned place preference (CPP) selectively in SNL treated rats, suggesting long-lasting spontaneous pain. Touch-induced FOS was increased in the spinal dorsal horn of SNL rats at D60 and prevented by prior DLF lesion suggesting that long-lasting tactile hypersensitivity depends upon spinal sensitization, which is mediated in part, by descending facilitation, in spite of resolution of heat hypersensitivity.

Perspective—These data suggest that spontaneous pain is present for an extended period of time and, consistent with likely actions of clinically effective drugs, is maintained by descending facilitation.

Keywords

nerve injury; neuropathic pain; spontaneous pain; central sensitization; RVM; spinal cord

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Introduction

Neuropathic pain is a chronic state that can persist for months, years or even decades following the resolution of the initial precipitating events¹. Nerve injury can elicit spontaneous, stimulus-independent pain that can be constant, often described as burning in quality and intermittent pain described as shooting or electric shock-like^{1, 3, 9}. In addition, patients experience paresthesias and dysesthesias manifesting as abnormal sensations including crawling, numbness, itching, and tingling^{9, 10}. A subpopulation of patients show exaggerated painful responses to non-noxious environmental stimuli, including touch and cold, referred to as allodynia³⁰. Quantitative sensory testing reveals that neuropathic pain patients can be classified using different features of neuropathic pain including the presence or absence of touch and cold allodynia and heat hyperalgesia³⁰.

Our understanding of mechanisms likely to be relevant to neuropathic pain has been aided by the development of pre-clinical models of nerve injury. These models of experimental neuropathic pain reproduce clinical observations to some degree including prolonged periods of hypersensitivity to tactile and cold stimuli, proposed to represent “allodynia,” as well as increased sensitivity to noxious heat, i.e., “hyperalgesia”^{6, 14, 31}. Notably, most studies examining mechanisms underlying pain are performed relatively early (i.e., within the first 2-3 weeks) following injury, limiting examination of possible time-dependency of mechanisms that may be associated with neuropathic pain. We have previously reported that the initiation of behavioral signs of neuropathic pain and central sensitization are initially independent of descending modulation from the rostral ventromedial medulla (RVM), but become dependent on descending facilitation by the second week following injury⁴. At this time, lidocaine inactivation of the RVM, or lesion of putative pain facilitation cells with dermorphin- or CCK-saporin prevents or reverses nerve-injury-induced tactile and heat hypersensitivity^{28, 40}. Additionally, RVM microinjection of a CCK2 receptor antagonist transiently reverses evoked hypersensitivity in nerve-injured rats suggesting that CCK may drive descending facilitation to maintain pain¹⁷. The possible roles of descending modulation in the long-term expression of experimental neuropathic pain and spinal sensitization have not been extensively examined.

Measurement of “spontaneous” or ongoing pain in preclinical settings has been challenging. We recently applied the principle of negative reinforcement to demonstrate that pain relieving treatments will produce conditioned place preference (CPP) selectively in injured rats unmasking the presence of spontaneous neuropathic pain and allowing study of its mechanisms^{7, 29}. The temporal features of spontaneous neuropathic pain in preclinical models have not been determined. Here, we have explored the time course of nerve-injury induced behavioral responses to normally innocuous tactile and noxious thermal stimulation. Additionally, we have determined whether spontaneous pain and spinal sensitization is present for an extended period of time and whether these measures depend on descending pain facilitatory pathways. We found that heat hypersensitivity resolved within approximately 35-40 days following SNL whereas tactile hypersensitivity and spontaneous pain persisted for 60 days post-SNL or longer, and are maintained by descending facilitation arising in the brainstem and ultimately acting via 5HT₃ receptors in the spinal cord.

Materials and Methods

Animals

Male, Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 200-300 g at the start of the experiments, were housed in a 12-h light/dark cycle room. Food and water were available ad libitum. All testing was performed in accordance with the policies and recommendations of the International Association for the Study of Pain (IASP) and the National Institutes of Health (NIH) guidelines for the handling and use of laboratory animals and received approval from the Institutional Animal Care and Use Committee (IACUC) of the University of Arizona.

Spinal nerve ligation

Spinal nerve ligation (SNL) at L₅ and L₆ was induced using the procedure of Kim and Chung¹⁴. The animals were anesthetized with 2% isoflurane delivered in air at 2 l/min. A 3 cm longitudinal incision 5 mm lateral from the midline was made at lower lumbar and sacral levels. The location of the incision is determined by the position of the L₅ spinous process. Connective tissues, remaining muscle and L₆ spinous processes were removed, the L₅ and L₆ spinal nerves freed from the adjacent structure, and the nerves tightly ligated with 4-0 silk suture. Hemostasis was confirmed, the muscles sutured in layers and the wound closed. Sham control rats underwent the same operation and handling as the experimental animals, but without nerve ligation.

Cannulation of the RVM for microinjections

Rats were anesthetized with an i.p. injection of 80 mg/kg of ketamine and 12 mg/kg of xylazine, (Sigma, St. Louis, MO) and placed in a stereotaxic headholder. The skull was exposed and a pair of cannulae, 1.2 mm apart, were directed toward the RVM (AP -11.0 mm from bregma, $\pm$ 0.6 mm; and -7.5 mm from the dura mater). These coordinates were obtained from the rat brain atlas of Paxinos and Watson²⁵. The guide cannulae were cemented in place and secured to the skull by small stainless steel machine screws. The animals were allowed to recover for 5 days post-surgery before behavioral testing. Microinjections were made in a volume of 0.5 μ L injected through a 33 gauge injection cannula that protruded 1 mm beyond the end of the guide cannula and into fresh tissue to prevent backflow. At the termination of all experiments, the animals were sacrificed, Toluidine Blue dye was microinjected into the region and cannula placement was verified histologically. Only those animals with correct cannula placement were included in behavioral analysis.

Intrathecal catheters

Rats were prepared for intrathecal drug injections according to the method described by Yaksh and Rudy³⁸ and used routinely in our laboratories. Rats were anesthetized with ketamine/xylazine (80/12 mg/kg) and the atlanto-occipital membrane was exposed and punctured. A section of PE-10 tubing 8 cm in length was passed caudally from the cisterna magna to the lumbar enlargement. The catheter was secured to the musculature, the wound closed, and a 5 day recovery allowed before any subsequent procedures were performed.

Intrathecal injections of ondansetron (30 µg)³⁴ were made in a volume of 5 µl, followed by a 9 µl flush of saline. Progress of the injection was monitored by the movement of a 1 µl air bubble placed between drug solution and saline flush.

Dorsolateral funiculus (DLF) lesion

Spinal lesions at T₈ were performed in isoflurane-anesthetized rats 55 days following SNL or sham surgery as described and verified previously^{4, 22}. The spinal cord was exposed by laminectomy, and the DLF ipsilateral to the SNL or sham surgery was crushed with fine forceps. Sham spinal surgery was performed by exposing the vertebrae and performing a laminectomy but without damaging neuronal tissue. Animals were allowed to recover for 5 days prior to behavioral testing or induction of touch-evoked FOS.

Spinal FOS evaluation

On day 5 after DLF lesion as described above, awake rats were restrained using a towel and received repetitive non-noxious tactile stimulation of the hindpaw ipsilateral to the SNL as previously described¹⁹. The tactile stimulation consisted of gently rubbing the plantar surface of the hindpaw with the experimenter's thumb for a period of 2 s (“on”) followed by an interval of 2 s (“off”) for a total stimulation time of 10 min in accordance with previous procedures^{36, 37, 39}. The rats were returned to their cages, and, after 2 hr, they were deeply anesthetized and perfused transcardially with 10% buffered formalin. The spinal cords were removed and stored in 10% formalin for immunohistochemistry. The post-fixed spinal cord segments were transferred to 30% sucrose in 0.1M PBS at least overnight, then embedded in optimal cutting temperature (O.C.T.) compound and sliced with a Cryostat at −20°C. The spinal cord was cut in 20 µm and saved for free-floating incubation. After completely washing the O.C.T. compound away, the sections were incubated in 10% normal goat serum for 1 hr, followed by 24–72 hrs in the primary reagent (which included rabbit anti-c-FOS (Chemicon), 2.5% normal goat serum, 0.02% Na Azide and 0.1% X-100 triton in 0.1 M PBS, pH=7.4). After three washes in PBS, the sections were then incubated in reagents with secondary antibody conjugated with fluorescent compound for 2–3 hrs, then rinsed in PBS, mounted with fluorescent mounting medium (Vector labs), and covered with cover slips (Fisher Brand). Quantification of labeled staining was processed as described previously³⁷. Only the Fos positive cells in the ipsilateral dorsal horn (above the horizontal line crossing the central canal) was counted and analyzed.

Behavioral tests

All behavioral tests were carried out by an experimenter blinded to the treatment condition. Tactile hypersensitivity was determined by measuring paw withdrawal thresholds to probing the hindpaw with von Frey filaments. The rats were placed in elevated Plexiglas boxes with wire-mesh floors. A series of 8 von Frey filaments (Stoelting, Wood Dale, IL USA), calibrated in logarithmically spaced increments from 0.41 g to 15 g, were applied to the plantar aspect of the hindpaw until it buckled. Mean paw withdrawal threshold was determined by sequentially increasing and decreasing the stimulus strength and analyzed by the Dixon non-parametric test^{5, 8}. Baseline paw withdrawal thresholds were determined prior to surgery, and post-surgical baselines were obtained after the animals recovered from the SNL or sham surgery. Measurements were performed after drug administration in order

to evaluate blockade of tactile hypersensitivity. A significant ($p = 0.05$) reduction in paw withdrawal threshold from baseline indicated tactile hypersensitivity, and a significant ($p < 0.05$) increase from the SNL baseline indicated attenuation of tactile hypersensitivity.

Behavioral responses to noxious radiant heat were determined by the latency to withdraw the hindpaw from a radiant heat stimulus¹³. The rats were placed on tempered glass plates and a focused infrared beam was directed onto the plantar aspect of the hindpaw. Withdrawal of the hindpaw activated a motion sensor that terminated the heat source and simultaneously stopped a timer. Heat hyperalgesia was defined as a significant ($p < 0.05$) decrease in paw withdrawal threshold relative to baseline values, and reversal of hyperalgesia was indicated by significant ($p < 0.05$) elevations in withdrawal thresholds from the SNL baseline values.

Conditioned place preference was performed using a modification of the single trial conditioning procedure previously described^{16, 29}. Rats underwent a 1 day habituation in which they were placed in the conditioned place preference boxes and allowed to explore all chambers. Behavior was recorded and analyzed to verify that no chamber bias exists to the conditioning chambers. The following day, rats received saline, either intrathecally or by microinjection into the RVM, in the morning followed 4 hours later by drug administration (i.e., RVM lidocaine or spinal ondansetron). On test day, 20 hours following the afternoon pairing, rats were placed in the CPP box with access to all chambers and their behavior recorded for 15 min for analysis for chamber preference. Significantly increased post-conditioning time spent in the drug-paired chamber, as compared to pre-conditioning time, indicates conditioned place preference. Decreased post-conditioning time spent in the drug-paired chamber, as compared to pre-conditioning time, indicates conditioned place aversion. No change between time spent in the drug-paired chamber, as compared to pre-conditioning time, indicates no conditioned place preference or aversion.

Statistics—Tactile and heat hypersensitivity were indicated by a significant reduction in response thresholds relative to the pre-SNL baseline values. These data were analyzed over time by one-factor repeated ANOVA followed by Fisher's Least Significant Difference test. Differences between SNL and sham-operated groups over time were determined by 2-factor ANOVA for repeated measures. Comparisons between the data of multiple groups from the same time point were determined by either one-factor or 2-factor ANOVA. Pairwise comparisons between 2 means were performed by Student's t-test. A significant difference score was determined by Student's paired t-test in order to test the significance of the difference between the baseline value and the treatment-paired chamber after conditioning. Significance was determined at $p = 0.05$.

For CPP experiments, data were analyzed before conditioning (baseline) and after conditioning using 2-factor analysis of variance (chambers vs. treatment). Post-hoc analysis was performed using Bonferroni tests to verify lack of pre-conditioning differences in time spent in the pairing chambers and comparing post-conditioning to pre-conditioning time spent in the drug-paired chamber to determine CPP (increase in post-conditioning time vs. pre-conditioning time) for each group. If significant CPP was determined, group differences were analyzed using difference scores calculated for each rat using the formula: post-

conditioning time spent in chamber – pre-conditioning time spent in chamber. Difference scores for the drug-paired chamber between SNL and sham-operated rats were analyzed using paired t-tests. For all analyses, significance was set at $p < .05$.

Results

Nerve injured rats demonstrated tactile and heat hypersensitivity within 4 days after SNL (Fig. 1A,B). Tactile hypersensitivity was observed throughout 580 days of observation and did not return to pre-injury baseline within this observation period (Fig. 1A). In contrast, heat hypersensitivity gradually diminished over time (Fig 1B) and returned to pre-SNL values between 35 and 40 days post-SNL (Fig. 1C). Accordingly, subsequent studies were performed at 42 or 60 days after SNL, time-points when heat hypersensitivity was no longer observed. Sham-operated rats did not show any changes in behavioral responses to tactile or noxious thermal stimuli at the time points examined across the 580 day observation period (Fig. 1).

Bilateral microinjections of 0.5 μ l of lidocaine (4% w/v) or the CCK2R antagonist, YM-022 (0.25 ng), into the RVM at day 14 or at day 60 after nerve injury had no effect on sham-operated rats but reversed tactile hypersensitivity in rats with SNL (Fig. 2A). Microinjection of saline (0.5 μ l) into the RVM did not produce any changes in withdrawal thresholds (data not shown). In addition, disruption of descending pathways from the RVM by ipsilateral DLF lesions performed at the T8 level, 8 weeks (56 days) following SNL or sham operation abolished the SNL-induced tactile hypersensitivity 60 days post-SNL (Fig. 2B).

To determine whether SNL-induced spontaneous pain was present 60 days after nerve injury, SNL or sham treated rats had RVM cannulas implanted 7 days prior to conditioning day. Pre-conditioning time spent in the conditioning chambers was equivalent across all treatment groups ($p > 0.05$), therefore data were pooled for graphical representation. RVM lidocaine (4% w/v) induced CPP selectively in SNL rats at either day 10 or day 60 (Fig 2C). Difference scores confirm equivalent increase in time spent in the RVM lidocaine paired chamber at both time-points (Fig 2D). RVM lidocaine did not alter time spent in chambers for sham treated rats.

Previous studies have demonstrated that disruption of descending pain facilitatory pathways from the RVM blocks touch-evoked Fos, indicating that descending facilitatory pathways maintain nerve injury-induced spinal sensitization³⁷. Rats with SNL and DLF or sham DLF lesions were prepared for immunofluorescent examination of Fos expression in the lumbar spinal cord 60 days post-SNL. Light touch of the ipsilateral hindpaw increased Fos expression within the ipsilateral dorsal horn of SNL rats with sham DLF lesion (Fig 3A). DLF lesion blocked the touch-induced increase in Fos expression (Fig 3A). Counts of FOS expressing cells within the spinal dorsal horn confirm enhanced touch-evoked FOS in SNL rats (sham lesion) that was blocked by DLF lesion (Fig 3B)

Spinal administration of the 5HT₃ receptor antagonist ondansetron following recovery from thermal hypersensitivity, 42 days after SNL, blocked ongoing pain, further implicating descending pain facilitatory pathways in maintaining persistent spontaneous pain. The

preconditioning time spent in each of the conditioning chamber was equivalent for both groups ($p > 0.05$), therefore data were pooled for graphical representation. Spinal administration of ondansetron resulted in CPP in rats with SNL but not in the sham-operated rats (Fig 3C). The difference in time spent in the ondansetron-paired chamber relative to the saline-paired was significantly ($p < 0.05$) increased for the rats with SNL but not sham surgery (Fig 3D).

Discussion

Animal models of neuropathic pain have demonstrated long-lasting mechanical hypersensitivity that persists across several months^{6, 14}. Here, we confirm and extend these observations and demonstrate that L₅ and L₆ SNL produces long-term tactile hypersensitivity lasting nearly 600 days after SNL. In contrast, heat hypersensitivity has a variable time course that diminishes within approximately 40 days post-SNL. The different time courses suggest apparently divergent mechanisms underlying these commonly observed behavioral measures of experimental neuropathic pain. Additionally, our data show that SNL-induced spontaneous pain persists for at least 60 days following SNL, a time-point after the resolution of SNL-induced heat hypersensitivity. Moreover, descending facilitation from the RVM drives tactile hypersensitivity, spontaneous pain and spinal sensitization following recovery of heat hypersensitivity. The data reveal time-dependent mechanistic events that mediate temporal aspects of nerve-injury induced pain and highlight the persistent role of descending facilitatory pathways from the RVM in long-term maintenance of pain.

We have previously demonstrated that time dependency in the expression of behavioral signs of neuropathic pain that were suggested to represent an “initiation” and a “maintenance” phase of the SNL model⁴. The initiation phase was considered to be driven by increased afferent inputs that are elicited by the nerve injury, and has characteristics that probably include acute injury-induced peripheral activity. Indeed, previous studies have demonstrated that spontaneous afferent activity correlates with the initial expression of neuropathic pain. The onset of tactile hypersensitivity occurs with the development of afferent discharge that is most pronounced one week following injury but diminish over time suggesting temporal evolution of mechanisms that underlie neuropathic behaviors^{12, 18, 33}. During the first week post-SNL, evoked pain is not blocked by manipulations that disrupt descending pain facilitation possibly suggesting a dominant role of peripheral input without a requirement for central modulation for the early initiation of neuropathic pain⁴. Our studies also showed that descending facilitation becomes more prominent at later time points following injury, suggesting the possibility that diminished, but persistent enhanced afferent inputs lead to central amplification reflected in part by engagement of descending facilitation to “maintain” neuropathic pain^{4, 7}. These and later studies suggested that pronociceptive neuroplastic changes mediate SNL induced evoked pain^{11, 17, 20, 23, 40} and also contribute to spontaneous pain^{16, 29}.

Within the clinical setting, chronic neuropathic pain can last months to years, and is commonly characterized as spontaneous pain that is often described as burning^{2, 3}, with some neuropathic pain patients also suffering from pain elicited by normally innocuous

touch or cold, termed allodynia²⁶. Here, we examined the characteristics of experimental neuropathic pain at extended time points that may have relevance to the chronic clinical nature of such pain. We used a novel preclinical approach to evaluate the presence of nerve-injury induced spontaneous pain. As pain is unpleasant, animals will associate a context with pain relief^{15, 16}, and this can be captured using conditioned place preference. We examined the role of descending facilitation at time points following the resolution of a key behavioral endpoint, heat hyperalgesia, and found that spontaneous pain is persistent. The differential time course of heat hyperalgesia and tactile hypersensitivity might be related to the contributions of different fiber types to these behavioral measures. Our earlier observations^{21, 24, 29, 32} showed that desensitization of TRPV1 positive afferent fibers by a systemic injection of resiniferatoxin (RTX) abolished heat hyperalgesia but not tactile hypersensitivity in rats with SNL²¹. Conversely, ablation of the ascending touch pathway by physical disruption of the dorsal columns or microinjection of lidocaine into the nucleus gracilis abolished tactile hypersensitivity but not heat hyperalgesia³². SNL was associated with marked up-regulation of NPY in large-diameter myelinated DRG profiles as well as in the n. gracilis²⁴ reflecting injury-induced adaptations in the touch pathways. On the other hand, tactile hypersensitivity may also reflect changes in central processing that are dependent upon C-fiber inputs as demonstrated in human subjects^{26, 27}. Collectively, these data suggest that the interpretation of the behavioral response to normally innocuous touch following nerve injury is complex and may, or may not, be interpreted as a measure that is relevant to painful “allodynia” in humans.

Our previous observations also suggest that spontaneous pain is dependent on afferent input that differs from tactile hypersensitivity^{15, 21, 32}. Functional desensitization of TRPV1 positive fibers with systemic resiniferatoxin (RTX) abolished responses to noxious thermal stimuli as well as spontaneous pain while having no effect on tactile hypersensitivity¹⁵. Moreover, blockade of NPY signaling within the n. gracilis failed to block heat hypersensitivity or spontaneous pain, but fully reversed tactile hypersensitivity¹⁵. Importantly, administration of NPY into the n. gracilis produces robust tactile hypersensitivity^{15, 24}, but fails to induce conditioned place aversion¹⁵ suggesting that spontaneous pain likely requires an injury-induced afferent drive. Based on these observations, it was suggested that nerve-injury induced heat hyperalgesia and spontaneous pain are mediated through activation of small diameter, unmyelinated nociceptors that terminate in the dorsal horn of the spinal cord and communicate with ascending projections. Here, we extend these observations to show that spontaneous pain persists even after recovery from evoked heat hypersensitivity. The reasons for this divergence in time course are not known. Possibly relevant to these preclinical observations is that patients can be subdivided on the basis of their neuropathic syndromes that can include long-lasting spontaneous pain in the absence of signs of sensory gain including evoked hyperalgesia^{1, 2}.

Our studies show that descending facilitation from the RVM is important in the expression of spontaneous pain even after the resolution of heat hypersensitivity, perhaps contributing to a sensitized spinal cord that can promote enhancement of injury induced sensory inputs³⁵. Previous studies have demonstrated that peripheral nerve injury enhances availability of CCK within the RVM, which drives descending facilitation^{17, 40}. Here, microinjection of the CCK2 receptor antagonist into the RVM transiently reversed behavioral signs of tactile

hypersensitivity following resolution of heat hypersensitivity confirming a persistent role of descending facilitation in maintaining long-term aspects of neuropathic pain. Disruption of descending facilitation by either microinjection of lidocaine in the RVM or surgical lesions of the descending tracts of the dorsolateral funiculus was previously shown to eliminate behavioral signs of spontaneous and evoked neuropathic pain during the maintenance phase, i.e., prior to recovery of SNL induced heat hypersensitivity^{4, 16, 17, 23, 28, 29}. Here, disrupting these descending pain facilitatory pathways also prevented both spontaneous pain and tactile hypersensitivity at 60 days after SNL, indicating that descending facilitation maintains long-term neuropathic pain.

It is possible that spinal sensitization following recovery of heat hypersensitivity is maintained through activation of descending pain facilitatory pathways from the RVM. Supporting this, enhanced spinal Fos expression in the spinal dorsal horn, indicative of spinal sensitization^{37, 39}, was present 60 days after SNL and was blocked by lesions of the DLF. These results are consistent with our previous observations that disruption of descending facilitation blocks touch-evoked Fos during the early “maintenance” phase of neuropathic pain³⁷. Further evidence for the role of the descending pain facilitatory pathways is that spinal administration of ondansetron, the 5HT₃ receptor antagonist, blocked SNL-induced ongoing pain. Previous studies by Suzuki, Dickenson and colleagues have implicated spinal serotonergic actions through the 5HT₃ receptor in enhanced nociceptive transmission at the spinal cord level^{34, 35}. Spinal administration of the specific 5HT₃ receptor antagonist ondansetron has been demonstrated to block nerve-injury induced hypersensitivity to noxious thermal and tactile stimuli as well as electrophysiological signs of spinal sensitization^{34, 35}. Our data extend these observations, demonstrating that spinal ondansetron blocks nerve-injury induced spontaneous pain. Moreover, these observations further indicate a significant role for descending pain facilitatory pathways in maintaining spontaneous pain following recovery of from heat hypersensitivity.

Patients with chronic neuropathic pain experience persistent pain lasting months to years following injury that is characterized by ongoing spontaneous pain (pain at rest) and, in some patients, hypersensitivity to environmental stimuli including pain to normally non-noxious stimuli (tactile allodynia). Our preclinical data raise the possibility of likely evolution of mechanisms mediating nerve-injury induced pain, with increased reliance on central mechanisms such as descending pain facilitatory pathways and spinal sensitization at times that may have relevance to chronic neuropathic pain reported in humans^{1, 9}. It should be noted that persistent changes in peripheral mechanisms may also contribute to late phase chronic pain; this possibility requires further investigation. These conclusions are consistent with reports of changes within the brain in rats several months following nerve injury (SNI)³¹. Notably, in that study, the degree of mechanical hypersensitivity was associated with alterations in areas associated with processing and/or modulation of pain sensation or affect (i.e., the somatosensory and anterior cingulate cortices)³¹ as well as corresponding temporally with behavioral signs of anxiety³¹. Collectively, these observations point to central sites as relevant targets for the treatment of chronic neuropathic pain.

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Figure 1. Time-dependent changes in SNL-induced evoked hypersensitivity

(A) SNL caused tactile hypersensitivity in rats indicated by significant ($p < 0.05$) reductions in paw withdrawal thresholds to von Frey filaments within 3 days post-surgery. The SNL-induced tactile hypersensitivity was maintained throughout the 580 day testing period. (B) SNL induced heat hypersensitivity in rats indicated by significant ($p < 0.05$) reductions in paw withdrawal latencies to noxious radiant heat within 3 days after SNL. (C) SNL-induced reduction in paw withdrawal latencies to radiant heat recovered between 35-40 days post-SNL, with paw withdrawal latencies returning to non-injured values at the day 40 time-point, $*p < 0.05$ vs baseline (BL). All graphs indicate mean $\pm$ SEM, $n=36$.

Figure 2. Descending Facilitation from the RVM maintains SNL-induced evoked and spontaneous pain two months following SNL

(A) Microinjection of lidocaine (4% w/v in 5 μ l) or of the CCK2 receptor antagonist, YM022 (0.25 ng/5 μ l) into RVM reversed mechanical hypersensitivity 14 days as well as 60 days post SNL, * p <0.05 vs baseline, n =8-12. (B) Ipsilateral lesions of the dorsolateral funiculus (DLF) resulted in a complete blockade of SNL-induced tactile hypersensitivity 60 days post-SNL, * p <0.05 vs baseline n =6-8. (C) Microinjection of lidocaine into the RVM produced CPP in rats 10 days as well as 60 days post SNL, * p <0.05 vs baseline, n =6-7. (D) Difference scores indicate that RVM lidocaine induce robust preference for the lidocaine paired chamber at both time-points, * p <0.05 vs sham.

Figure 3. Spinal Sensitization is maintained by descending facilitatory pathways

(A) Representative images demonstrating touch-evoked Fos expression in the ipsilateral spinal dorsal horn 60 days following SNL, compared to unstimulated controls (top panels). DLF lesions blocked touch-evoked Fos in SNL treated rats 60 days following surgery (bottom panels). (B) Quantification confirmed that lesions of the dorsolateral funiculus (DLF) ipsilateral to the SNL blocked touch-evoked Fos expression within the spinal dorsal horn 60 days post-SNL, $*p < 0.05$ vs. unstimulated. (C) Spinal administration of ondansetron (30 μ g) produced conditioned place preference in SNL rats 42 days post SNL, $*p < 0.05$ vs baseline, $n = 8-10$. (D) Difference scores confirm that SNL rats demonstrate increased time spent in the ondansetron paired chamber, $*p < 0.05$ vs. sham.