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A partial L5 spinal nerve ligation induces a limited prolongation of mechanical allodynia in rats: an efficient model for studying mechanisms of neuropathic pain

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Abstract

The relationship between pain severity and the extent of injury to a peripheral nerve remains elusive. In this study, we compared the pain behavior resulting from partial (1/3–1/2 thickness) and full L5 spinal nerve ligation (SNL) in rats. The decrease in paw withdrawal threshold (PWT) to mechanical stimuli in the hindpaw ipsilateral to the injury was comparable in the two groups on days 3–21 post-injury. However, the decreased PWT recovered earlier in the partial SNL group than in the full SNL group. These observations suggest that the duration of neuropathic pain behavior, but not the early development of mechanical allodynia, is dependent on the extent of nerve injury. On days 6 and 15 post-injury, when the mechanical allodynia was similar in the two groups, systemic morphine induced a greater reduction of mechanical allodynia in the partial SNL group than in the full SNL group. Furthermore, in partial SNL rats, at post-injury time points when they had largely recovered from the neuropathic pain state, systemic administration of naloxone hydrochloride (day 53) or naloxone methiodide (a non-selective peripherally acting opioid receptor antagonist; day 64) or intra-plantar injection of naloxone methiodide rekindled mechanical pain hypersensitivity in the ipsilateral hindpaw, suggesting a prolonged activation of endogenous opioidergic pain-inhibition. Therefore, partial SNL in rats may represent an efficient model for studying the mechanisms of neuropathic pain, testing effects of analgesic/antihyperalgesic drugs, and understanding endogenous pain inhibitory mechanisms that lead to reversal of the pain behavior with time.

Keywords

partial spinal nerve injury; mechanical allodynia; neuropathic pain; morphine; rat

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The extent of peripheral nerve injury may affect the manifestation of neuropathic pain. The risk and severity of post-herpetic neuralgia was suggested to correlate highly with the magnitude of injury to unmyelinated cutaneous afferents [15]. However, in other pain states this relationship is less clear. For example, pain can be severe in patients with complex regional pain syndrome type I who have only subtle axonal losses [1;16]. In experimental animals, the

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incidence and severity of post-traumatic pain resulting from distal nerve injuries were also independent of the lesion-size [11;18]. Therefore, the relationship between the pain severity and the lesion size to a peripheral nerve remains a topic of debate. In most previous studies, the full time courses (i.e., onset, maintenance, and recovery) of neuropathic pain were not compared between animals with different degrees of injury.

To determine the effects of lesion size on the expression of neuropathic pain, we examined whether a partial ligation of the L5 spinal nerve (1/2–1/3 thickness) in male Sprague-Dawley rats would cause a less intense and shorter lasting mechanical allodynia than one induced by a full L5 spinal nerve ligation (SNL) model. The L5 SNL model has been widely used to study the mechanisms of neuropathic pain and to test the effects of drugs on neuropathic pain behavior. Compared to other preclinical nerve injury models (e.g., chronic constriction injury of the sciatic nerve, partial sciatic nerve injury, spared nerve injury), the L5 SNL model is advantageous because 1) the nerve injury is standardized and highly repeatable and 2) the differences in effect on injured and uninjured afferents, dorsal root ganglia (DRG), and spinal segments can be investigated relatively easily. Morphine analgesia is compromised after nerve injury and that a consequent reduction of endogenous opioidergic inhibition might contribute to the expression of neuropathic pain [19;21]. In separate studies, we compared the effectiveness of systemic morphine in attenuating mechanical allodynia, a well-characterized manifestation of neuropathic pain, in partial and full L5 SNL models at a time point when the levels of mechanical allodynia were comparable. We also examined whether blockade of opioid receptors can rekindle the mechanical pain hypersensitivity in the partial SNL rats following recovery from the neuropathic pain state.

Ligation of L5 spinal nerve was carried out in adult male Sprague-Dawley rats (200–350 g, Harlan Bioproducts for Science, Indianapolis, IN). The animals were deeply anesthetized with isoflurane (2.0%, Abbott Laboratories, North Chicago, IL) delivered through a nose cone. Under aseptic conditions, the skin was incised at the midline over the lumbar spine, and the left transverse process of the L6 vertebra was removed. For a full spinal nerve injury, the left L5 spinal nerve was tightly ligated with a 9-0 nylon suture (S&T, Neuhausen, Switzerland). To induce a partial SNL, approximately 1/3–1/2 thickness of the L5 spinal nerve was ligated with the same 9-0 nylon suture under the dissecting microscope. Care was taken not to pull the nerve or contact the intact L4 spinal nerve. After hemostasis was achieved, the muscle layer was approximated with 4-0 synthetic absorbable suture (Look, Reading, PA) and the skin closed with metal clips. In a sham control group, the surgical procedure was identical to that described above, except that the left L5 spinal nerve was not injured. After the surgery, the rats were returned to their cages, kept warm under a heat lamp, and monitored during recovery. Skin staples were removed approximately 1 week after surgery. All procedures were approved by the Johns Hopkins University Animal Care and Use Committee as consistent with the National Institutes of Health Guide for the Use of Experimental Animals to ensure minimal animal use and discomfort. To minimize experimenter bias, the investigator who performed the behavioral tests was blinded to the animals' surgery conditions. To minimize variability of the behavioral outcome measures, animals were trained for 3 to 5 days before baseline data were obtained. All experimental conditions (e.g., animal age, gender, drug preparation, drug injection, etc.) were consistent across groups. Animals were habituated to the test environment for ≥ 30 min before testing was begun on a given day.

To test for signs of mechanical allodynia, animals were placed under plastic domes on a mesh floor that allowed full access to the plantar surface of the paws. The area tested was the region between the foot-pads in the plantar aspect of the hindpaw. The up-down method was used to quantify the allodynia to mechanical stimuli [3]. To determine the paw-withdrawal thresholds (PWTs), the pattern of positive and negative responses to the von Frey filament stimulation was converted to a 50% threshold value using the formula provided by Dixon [6]. A series of

von Frey filaments that deliver approximately logarithmic incremental forces (0.38, 0.57, 1.23, 1.83, 3.66, 5.93, 9.13, 13.1-g) were applied for 4 to 6 s to the test area between the footpads on the plantar surface of the hindpaw. The 1.83-g stimulus was applied first. If a positive response occurred, the next smaller von Frey filament was used; if a negative response was observed, the next higher force was used. The test was continued until (1) the responses to five stimuli were assessed after the first crossing of the withdrawal threshold or (2) the upper/lower end of the von Frey set was reached before a positive/negative response had been obtained. Abrupt paw withdrawal, licking, and shaking were regarded as positive responses. The PWTs in most naïve rats were at or near the cut-off threshold (calculated from the highest force of 13.1-g when it induced no withdrawal response). Rats with a low PWT that was less than 50% of cut-off value at pre-injury condition were excluded from the study.

Naloxone hydrochloride (a non-selective opioid receptor antagonist that can have peripheral and central effects), naloxone methiodide (a non-selective peripherally acting opioid receptor antagonist that does not cross the blood-brain barrier), and morphine sulfate were purchased from Sigma-Aldrich (St. Louis, MO). Stock solutions were freshly prepared. All other drugs were dissolved initially in distilled water and then further diluted to the final concentration with saline (0.9%). The PWT was normalized as percent of pre-injury baseline value for each animal at different post-injury and post-drug treatment time points, and data were analyzed by two-way mixed model ANOVA (factors: time/drug treatment, injury group). The least significant difference *post hoc* test was used to compare data between the two injury groups at each time point and between different time points in each group. Data are presented as mean $\pm$ SEM. $P < 0.05$ was considered statistically significant.

According to our previous study, days 3–7 represent the developmental phase, days 14–35 reflect the peak-maintenance phase, and the period after day 35 or 42 represents the recovery phase of neuropathic pain after a full SNL in rats [8]. In current study, the averaged PWT of the hindpaw ipsilateral to the injury was significantly decreased compared to the baseline PWT from day 3 to day 70 in rats after full SNL (Fig. 1A). The PWTs in rats with partial SNL ($n=17$) and full SNL ($n=11$) were not significantly different on days 3, 7, 14, or 21 post-injury, suggesting that the development and the peak level of mechanical allodynia are comparable between the two groups. However, PWT in the partial SNL group gradually returned toward pre-injury level beginning on day 25 post-injury. Compared to those in the full SNL group, the PWTs were significantly higher in the partial SNL group from day 25 to day 70 post-injury, suggesting that a smaller lesion of the spinal nerve led to a shorter lasting mechanical pain hypersensitivity. No significant changes in PWTs of the contralateral hindpaws were observed in either group after injury (Fig. 1B). Rats in both groups appeared healthy and were well groomed, showed no foot drop or autotomy behavior. A few rats (1 from the full SNL group and 2 from the partial SNL group) did not show a remarkable reduction in ipsilateral PWT ($>50\%$ decrease from the pre-injury baseline) after nerve injury. Regardless, they were included in the post-injury time course study in order to compare the group effect of full and partial SNL on mechanical pain sensitivity.

In separate studies, we compared the ability of systemic morphine to attenuate mechanical allodynia in the partial SNL ($n=10$) and full SNL rats ($n=6$) on days 6 and 15 post-injury, assuming that those time points represent the late developmental phase and early maintenance phase of neuropathic pain, respectively, and when the mechanical pain hypersensitivity was comparable between the two groups. All rats in this cohort showed a decrease in PWT that was $>50\%$ from their respective pre-injury baseline on day 6 post-injury before the drug injection. After the pre-drug PWT test, morphine sulfate (1.5 mg/kg) was injected subcutaneously in the back, and the PWT was measured again at 30 min, 120 min, and 24 h post-injection. This dose of morphine was shown in a previous study to partially reverse the mechanical allodynia in the SNL model [24] and was chosen to avoid the ceiling effect. Morphine significantly increased

the ipsilateral PWT at 30 min post-injection in both groups (Fig. 1C and D). However, on both days, the PWT was significantly higher in the partial SNL group than in the full SNL group at this 30-min time point ($P<0.05$). On day 6, the analgesic effect of morphine had dissipated by 120 min post-injection in the full SNL group but remained significant in the partial SNL group. There were no significant changes in contralateral PWT after morphine injection (data not shown).

On day 53 post-injury, the PWT in the partial SNL group ($n=7$) was not significantly different from the pre-injury level but was higher than that in the full SNL group ($n=6$). The systemic injection of naloxone hydrochloride (10 mg/kg, i.p.) rekindled mechanical allodynia in the partial SNL group, as indicated by significant decreases in PWT from the pre-drug value at 30 min and 90 min post-injection ($P<0.05$, Fig. 2A). After rats in the partial SNL group received naloxone, their ipsilateral PWTs decreased to a level that was near the pre-drug PWT of the full SNL group. Naloxone produced a small, but statistically insignificant, decrease in the ipsilateral PWT of the full SNL group and produced no significant change in contralateral PWT in either group. In the same groups of rats on day 64 post-injury, systemic administration of naloxone methiodide (5 mg/kg, i.p.) also significantly reduced ipsilateral PWT from the pre-drug value at 30 min post-injection in the partial SNL group but not in the full SNL group (Fig. 2B). The decreased PWT in the partial SNL group returned to near pre-drug level 6 h later. At this time point, a local intra-plantar injection of naloxone methiodide (50 μ g/50 μ l) rekindled mechanical allodynia in the partial SNL rats, significantly reducing PWT at 30 min post-injection ($P<0.05$). Neither systemic nor local injection of naloxone methiodide significantly changed the PWT in the full SNL group, and no significant changes in contralateral PWT were observed after the drug treatment. In a small group of sham-injured rats ($n=4$), neither systemic (i.p.) nor intra-plantar injection of vehicle (saline, 50 μ l) changed the PWT (data not shown).

The time course and magnitudes of decrease in the PWT from day 3 to day 21 post-injury were comparable between the partial and full SNL groups, suggesting that the onset and peak magnitude of mechanical allodynia are similar in the two groups. However, the mechanical allodynia was of a shorter duration in the partial SNL group than in the full SNL group. These findings suggest that the initial development phase (e.g., onset and peak level) of neuropathic pain is independent of the extent of nerve injury. In contrast, the time course of the maintenance of mechanical allodynia, which might reasonably be presumed to reflect the prolongation of neuropathic pain experience, appears to be related to the magnitude of the injury to the nerve. Nevertheless, it is possible that the severity and duration of spontaneous pain and thermal pain hypersensitivity may be independent of the extent of L5 spinal nerve lesion. In addition, this correlation between lesion size and the duration of mechanical allodynia may be different or nonexistent in neuropathic pain conditions of other etiologies (e.g., virus infection, ischemia, diabetic neuropathy). We are aware that the severity and prolongation of neuropathic pain may be related not only to the pathologic condition and changes in the fundamental pain signal itself, but also to other factors (e.g., genetic polymorphisms, gender, age, reorganization of the central nervous system, site of injury, and intervening variables) [2;4;9;10;12;17]. Accordingly, these factors may account, at least in part, for the differences between current observations and previous findings [11;18].

Mechanical allodynia is the most common and disabling stimulus-evoked symptom of neuralgia and is often difficult to treat. Although partial SNL rats expressed a magnitude of mechanical allodynia similar to that of full SNL rats on days 6 and 15 post-injury, they showed an increased sensitivity to systemic morphine analgesia; the anti-allodynic effect of morphine was significantly higher in the partial SNL than in the full SNL group. We did not examine the dose-response function of morphine, but the current finding suggests that systemic morphine is more potent at attenuating mechanical allodynia in the partial SNL rats than in the full SNL rats. This observation indicates that the extent of injury to a peripheral nerve may affect the

dose of morphine needed to attenuate the resultant mechanical pain hypersensitivity. An earlier study showed that total axotomy reduced markers of primary afferents more than did partial axotomy [11]. The greater analgesic potency of morphine in the partial SNL rats may be caused in part by less reduction in opioid receptor expression or function, as compared to that after a full SNL [5;23].

An endogenous opioidergic mechanism was suggested to be activated after repetitive noxious inputs and to counteract the development of spinal neuronal sensitization and persistent pathological pain [5;7;13]. Yet, the extent to which endogenous opioid inhibitory mechanisms remain tonically active after the pain behavior has dissipated is unclear. We observed that systemic naloxone hydrochloride unmasked the tonic endogenous opioidergic inhibition of mechanical pain sensitivity in partial SNL rats on day 53 and day 70 (data not shown) post-injury, when animals have largely recovered from the neuropathic pain state. Furthermore, blocking of peripheral opioid receptors with naloxone methiodide, a non-selective peripherally acting opioid receptor antagonist that does not cross the blood-brain barrier, also invoked mechanical pain hypersensitivity in the same group of partial SNL rats on day 64 post-injury. Surprisingly, both systemic administration and local injection (at a systemically inactive dose of 50 µg) of naloxone methiodide reduced PWT to a similar degree as systemic naloxone hydrochloride. These findings suggest that a partial SNL leads to prolonged activation of an endogenous opioidergic pain-inhibitory mechanism and that activation of peripheral opioid receptors is an important component. The reductions in PWT in the full SNL group after the opioid antagonist treatments at the same post-injury time points were small, likely due to a flooring effect of neuropathic pain in these rats.

The partial SNL model mimics the development and peak magnitude of mechanical allodynia in the full SNL model, but recovered earlier. The fundamental cellular and molecular substrates for neuropathic pain induced by a partial and full SNL may be similar. Accordingly, the partial SNL represents a useful addition to the toolbox of preclinical mono-neuropathy models that is efficient for unraveling the evolving nature of anatomical and functional changes in the nervous system associated with different phases of neuropathic pain (i.e., development, maintenance, and recovery) in a shortened time scale [20;22;25]. Therefore, it may provide an alternative model that reduces the overall cost and effort in animal care. As animals subjected to partial SNL are sensitive to analgesics (e.g., morphine), the model is useful for preclinical testing of drug actions on neuropathic pain and for studying endogenous pain inhibitory mechanisms. We are aware that the injury size may not be standardized between animals with partial SNL. This limitation may lead to a less reproducible nerve injury and increase the variance of the data, as compared to that from the full SNL. We have attempted to minimize this variability by meticulous attention to the technique and by carrying out the ligation under the dissecting microscope. In addition, we did take into account the potential for increased variability in the data and compensated for this by increasing the sample size and appropriately randomizing and blinding the experiments. Future studies may be needed to examine how different extents of SNL might differently regulate neurotransmitter and receptor expression in the DRGs (e.g., injured and uninjured) and dorsal horn of the spinal cord and to identify the mechanisms that contribute to the earlier recovery from neuropathic pain in the partial SNL animal. Use of this model may provide important directions for developing therapeutic strategies that enhance axonal regeneration and promote the positive adaptive changes to help functional and sensory recovery [14;25].

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Reference List

1. Albrecht PJ, Hines S, Eisenberg E, Pud D, Finlay DR, Connolly MK, Pare M, Davar G, Rice FL. Pathologic alterations of cutaneous innervation and vasculature in affected limbs from patients with complex regional pain syndrome. *Pain* 2006;120:244–266. [PubMed: 16427199]
2. Basbaum AI. A new way to lose your nerve. *Sci Aging Knowledge Environ* 2004;2004:e15.
3. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* 1994;53:55–63. [PubMed: 7990513]
4. Costigan M, Scholz J, Woolf CJ. Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu Rev Neurosci* 2009;32:1–32. [PubMed: 19400724]
5. deGroot JF, Coggeshall RE, Carlton SM. The reorganization of mu opioid receptors in the rat dorsal horn following peripheral axotomy. *Neurosci Lett* 1997;233:113–116. [PubMed: 9350845]
6. Dixon WJ. Efficient analysis of experimental observations. *Annu Rev Pharmacol Toxicol* 1980;20:441–462. [PubMed: 7387124]
7. Guan Y, Borzan J, Meyer RA, Raja SN. Windup in dorsal horn neurons is modulated by endogenous spinal mu-opioid mechanisms. *J Neurosci* 2006;26:4298–4307. [PubMed: 16624950]
8. Guan Y, Johaneck LM, Hartke TV, Shim B, Tao YX, Ringkamp M, Meyer RA, Raja SN. Peripherally acting mu-opioid receptor agonist attenuates neuropathic pain in rats after L5 spinal nerve injury. *Pain* 2008;138:318–329. [PubMed: 18276075]
9. Ji RR, Strichartz G. Cell signaling and the genesis of neuropathic pain. *Sci STKE* 2004;2004:reE14. [PubMed: 15454629]
10. Langford DJ, Cragger SE, Shehzad Z, Smith SB, Sotocinal SG, Levenstadt JS, Chanda ML, Levitin DJ, Mogil JS. Social modulation of pain as evidence for empathy in mice. *Science* 2006;312:1967–1970. [PubMed: 16809545]
11. Lee JW, Siegel SM, Oaklander AL. Effects of distal nerve injuries on dorsal-horn neurons and glia: relationships between lesion size and mechanical hyperalgesia. *Neuroscience* 2009;158:904–914. [PubMed: 18992304]
12. Liu CN, Michaelis M, Amir R, Devor M. Spinal nerve injury enhances subthreshold membrane potential oscillations in DRG neurons: relation to neuropathic pain. *J Neurophysiol* 2000;84:205–215. [PubMed: 10899197]
13. Mansikka H, Zhao C, Sheth RN, Sora I, Uhl G, Raja SN. Nerve injury induces a tonic bilateral mu-opioid receptor-mediated inhibitory effect on mechanical allodynia in mice. *Anesthesiology* 2004;100:912–921. [PubMed: 15087627]
14. Navarro X, Vivo M, Valero-Cabre A. Neural plasticity after peripheral nerve injury and regeneration. *Prog Neurobiol* 2007;82:163–201. [PubMed: 17643733]
15. Oaklander AL. The density of remaining nerve endings in human skin with and without postherpetic neuralgia after shingles. *Pain* 2001;92:139–145. [PubMed: 11323135]
16. Oaklander AL, Rissmiller JG, Gelman LB, Zheng L, Chang Y, Gott R. Evidence of focal small-fiber axonal degeneration in complex regional pain syndrome-I (reflex sympathetic dystrophy). *Pain* 2006;120:235–243. [PubMed: 16427737]
17. Raber P, Devor M. Social variables affect phenotype in the neuroma model of neuropathic pain. *Pain* 2002;97:139–150. [PubMed: 12031787]
18. Siegel SM, Lee JW, Oaklander AL. Needlestick distal nerve injury in rats models symptoms of complex regional pain syndrome. *Anesth Analg* 2007;105:1820–9. table. [PubMed: 18042888]
19. Stevens CW, Kajander KC, Bennett GJ, Seybold VS. Bilateral and differential changes in spinal mu, delta and kappa opioid binding in rats with a painful, unilateral neuropathy. *Pain* 1991;46:315–326. [PubMed: 1661886]
20. Stubbley LA, Martinez MA, Karmally S, Lopez T, Cejas P, Eaton MJ. Only early intervention with gamma-aminobutyric acid cell therapy is able to reverse neuropathic pain after partial nerve injury. *J Neurotrauma* 2001;18:471–477. [PubMed: 11336447]
21. Suzuki R, Chapman V, Dickenson AH. The effectiveness of spinal and systemic morphine on rat dorsal horn neuronal responses in the spinal nerve ligation model of neuropathic pain. *Pain* 1999;80:215–228. [PubMed: 10204734]

22. Wang X, Ratnam J, Zou B, England PM, Basbaum AI. TrkB signaling is required for both the induction and maintenance of tissue and nerve injury-induced persistent pain. *J Neurosci* 2009;29:5508–5515. [PubMed: 19403818]
23. Zhang X, Bao L, Shi TJ, Ju G, Elde R, Hokfelt T. Down-regulation of mu-opioid receptors in rat and monkey dorsal root ganglion neurons and spinal cord after peripheral axotomy. *Neuroscience* 1998;82:223–240. [PubMed: 9483516]
24. Zhao C, Tall JM, Meyer RA, Raja SN. Antiallodynic effects of systemic and intrathecal morphine in the spared nerve injury model of neuropathic pain in rats. *Anesthesiology* 2004;100:905–911. [PubMed: 15087626]
25. Zimmermann M. Pathobiology of neuropathic pain. *Eur J Pharmacol* 2001;429:23–37. [PubMed: 11698024]

Fig. 1.

Rats showed less persistent mechanical allodynia and increased sensitivity to morphine analgesia after a partial L5 spinal nerve ligation (SNL) than after a full SNL. (A) A full L5 SNL ($n=11$) induced a remarkable decrease in PWT in the hindpaw ipsilateral to the injury that lasted for more than 70 days, whereas a partial L5 SNL ($n=17$) induced mechanical allodynia that lasted only to day 42 post-injury. The PWTs were significantly higher in the partial SNL group than in the full SNL group from day 25 to day 70 post-injury. (B) There were no significant changes in PWT of the contralateral hindpaw in either group after injury. (C-D) Morphine sulfate (1.5 mg/kg, s.c.) significantly increased the PWT of the ipsilateral hindpaw in both partial SNL ($n=10$) and full SNL ($n=6$) groups at 30 min post-injection on day 6 (C) and day 15 (D) post-injury. Although the PWTs were comparable between the two groups before morphine injection, the PWT at 30 min post-morphine injection was significantly higher in the partial SNL group than in the full SNL group. # $P < 0.05$ versus the pre-drug baseline. * $P < 0.05$ versus the full SNL group. Data are presented as means $\pm$ SEM.

Fig. 2.

Blocking systemic and peripheral opioid receptors rekindled mechanical pain hypersensitivity in the partial SNL rats after they had recovered from the neuropathic pain state. (A) On day 53 post-injury, the PWT in the partial SNL group ($n=7$) was significantly higher than that in the full SNL group ($n=6$). The intraperitoneal injection of naloxone hydrochloride (10 mg/kg, i.p.) significantly decreased the PWT in the partial SNL group but not in the full SNL group. (B) On day 64 post-injury, systemic administration (i.p.) of naloxone methiodide (MB-Naloxone; 5 mg/kg) also significantly reduced PWT at 30 min post-injection in the partial SNL group. Six hours later, when the PWT had returned to near pre-drug level, an intra-plantar (i.pl.) injection of naloxone methiodide (50 µg/50 µl) again invoked mechanical pain hypersensitivity in the partial SNL rats. There were no significant changes in PWT of the contralateral hindpaw after drug treatments. # $P < 0.05$ versus the pre-drug baseline. * $P < 0.05$ versus the full SNL group. Data are presented as means $\pm$ SEM.