Lack of correlation between spinal microgliosis and long-term development of tactile hypersensitivity in two different sciatic nerve crush injury

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Abstract
Microglia activation following peripheral nerve injury has been shown to contribute to central sensitization of the spinal cord for the development of neuropathic pain. In a recent study, we reported that the amount of nerve damage does not necessarily correlate with chronic pain development. Here we compared the response of spinal microglia, using immunohistochemistry as a surrogate of microglial activation, in mice with two different types of crush injury of the sciatic nerve. We confirmed that incomplete crush of the sciatic nerve (partial crush injury, PCI) resulted in tactile hypersensitivity after the recovery of sensory function (15 days after surgery), whereas the hypersensitivity was not observed after the complete crush (full crush injury, FCI). We observed that immunoreactivity for Iba-1, a microglial marker, was greater in the ipsilateral dorsal horn of lumbar (L4) spinal cord of mice 2 days after FCI compared to PCI, positively correlating with the intensity of crush injury. Ipsilateral Iba-1 reactivity was comparable between injuries at 7 days with a significant increase compared to the contralateral side. By day 15 after injury, ipsilateral Iba-1 immunoreactivity was much reduced compared to day 7 and was not different between the groups. Our results suggest that the magnitude of the early microgliosis is dependent on injury severity, but does not necessarily correlate with the long-term development of chronic pain-like hypersensitivity after peripheral nerve injury.

Keywords
Chronic pain, microglia, neuropathic pain, partial crush injury

Date Received: 29 January 2021; Revised 10 February 2021; accepted: 25 March 2021

Chronic pain is a common maladaptive response after peripheral nerve injury. Activation of microglia in the dorsal horn of spinal cord is now established as a significant mechanism underlying central sensitization for the development of chronic pain. It is widely considered that microglia activation after nerve damage is responsible for neuropathic pain development. In our recent study, we found that the degree of nerve damage does not necessarily correlate with chronic pain development: long-term tactile hypersensitivity was induced by an incomplete partial crush injury (PCI), but not by complete full crush injury (FCI) of the sciatic nerve in adult mice. Here we asked whether the activation of spinal microglia might underlie the difference in pain-like behavior between the two crush injury conditions (PCI versus FCI). We examined the time course of spinal microgliosis in the two crush models using immunohistochemistry.

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Male C57BL/6 mice aged over 6 weeks were used for this study. Experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Seoul National University. Full and partial crush injury were performed as previously described.10 Surgeries were performed in right side of the sciatic nerve under isoflurane anesthesia (2% induction in 100% oxygen maintained at 1.5% using a face mask). The depth of anesthesia was constantly assessed by monitoring the breathing rate and adjusted during surgery accordingly. The sciatic nerve was crushed for 15 s using an ultra-fine hemostat (Cat no. 13020–12, Fine Science Tools). For partial crush in the sciatic nerve, the hemostat was fitted with a custom spacer created from two layers of aluminum foil (15 μm thick) to create 30 μm gap when fully closed. Full sciatic nerve crush was performed in the same way except for closing the hemostat on the third locking position without a spacer. The wound was closed in a suture of the overlying muscle facia and then closed with a skin clip. Aseptic technique was maintained throughout. Mice were recovered in a warm, darkened cage and monitored until regaining conscious and locomotion.

Pinprick sensory recovery testing was performed as previously described.10,11 Briefly, the lateral side of the affected hind paw was separated into five regions from the toe to the heel and stimulated with a stainless-steel Austerlitz insect pin (Size 000, FST, Germany). A sensory response was confirmed by rapid lifting or flinching of the paw. The number of responses to two consecutive pin applications to the skin was recorded per region providing a score out of 10 (100% response equals to score 10). The pinprick analysis was conducted every two days up to 15 days post-injury (dpi). Mechanical sensitivity was assessed in both ipsilateral and contralateral hind paws at dpi 15, when sensory response to pin prick stimuli is fully recovered in both groups, using a series of von Frey filaments. The 50% withdrawal threshold was determined using the ‘up-down’ method.12,13 All behavioral testing were performed by an investigator who was blind to the injury group.

In all mice used for immunohistochemical analysis, we performed pinprick tests on dpi 1 to confirm the surgery as described above. For dpi 15 samples for IHC analysis, we measured the tactile hypersensitivity with von Frey test before the sampling. The mice were then perfused with PBS and then fixed with 4% paraformaldehyde transcardially after terminal anesthesia with pentobarbital (>150mg/kg). L3-5 spinal segments were isolated,14 and then protected in 30% sucrose solution for frozen tissue section. Spinal cords were sliced into 30 μm sections and every third sections were collected for the staining steps using free-floating methods, as previously described.15–17 For the staining procedure, the spinal cord sections were washed with PBS several times and pre-incubated with 5% normal donkey serum in PBS containing 0.3% Triton-x (PBST) for 1 h before the antibody application. Primary antibody against ionized calcium binding adaptor molecule-1 (Iba-1) (1:1,000; Cat no. 019–19741, Wako) was diluted in 1% normal donkey serum and incubated overnight at 4°C. Donkey anti-rabbit-Cy3 (1:200; Cat no. 711–165-152, Jackson laboratory) secondary antibody was diluted in 1% normal donkey serum and incubated for 1 h at room temperature (RT). The stained sections were then mounted on a slide glass and covered with coverslips after applying anti-fade mounting medium (Cat no. H-1000, Vector) for image analyses. After visual assessment, three sections with the brightest fluorescence that have L4 spinal anatomy were selected for further analyses.18,19 Fluorescence images (512 × 1024) were acquired on a laser-scanning confocal microscope (LSM 700 Zeiss) with Zen 2010 software (v8.1 SP1, Zeiss). Five z-stack images (2 μm interval) were merged as a maximum projection for the analysis. Ipsilateral and contralateral spinal dorsal horn areas were selected from each image by referring to the accompanying phase image and the mean Iba-1 fluorescence intensity was analyzed using ImageJ software. Both image acquisition and the analysis were performed by an investigator who was blind to the injury group. The mean fluorescence intensity from three sections was calculated for each animal. Individual data points represent a single animal and data are presented as mean ± standard error of the mean. Two-group analyses were performed by Student’s t-test; behavioral time course data were analyzed by either two-way or one-way ANOVA; spinal Iba1 immunoreactivity intensities were compared by one-way ANOVA using Prism 5 (GraphPad).

We measured pinprick responses in the affected hind paw after FCI and PCI to test sensory function every two days (Figure 1(a)). One day after crush injury, the pinprick response score was zero in FCI, whereas sensory function remained partially intact in the PCI group due to the incomplete crush injury in the sciatic nerve. The pinprick response began to recover from 7 days post-injury (dpi) and reached full recovery of pinprick response at dpi 15 in PCI. The PCI group also took 15 days for full recovery. This observation confirms our previous report.10 We next measured withdrawal thresholds to tactile stimuli applied to the hind paws at dpi 15, when pinprick sensory recovery is complete (Figure 1(a)). Compared to FCI, the PCI group displayed greater tactile hypersensitivity in the ipsilateral hind paw (dpi 15, Full ipsi × Partial ipsi, P = 0.0001) (Figure 1(b)) which is consistent with our previous report.10 Also, when compared to the baseline threshold, the PCI group developed tactile hypersensitivity in the ipsilateral hind paw at dpi 15 whereas FCI did not (Figure 1(b)). Both groups showed a significant decrease
Figure 1. (a) Pinprick response score measured every 2 days after crush injury. Repeated measures of Two-way ANOVA; Effect of surgery, $F(1, 56) = 183.95, P$ value $< 0.0001$ n = 5 mice per group. Bonferroni post-test, *$P < 0.05$, ***$P < 0.001$ ($t = 3.054 \sim 7.126$). ns; $P > 0.05$ ($t = 1.23$). Data are presented as means $\pm$ SEM. (b) 50% paw withdrawal threshold measured before the surgery and on dpi 15 in FCI and PCI groups in both hind paws. Repeated measures of One-way ANOVA between the time points. Tukey's multiple comparison test, ns $> 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Paired Student's t-test (Full ipsi x contra: $P = 0.0254$; Partial ipsi x contra: $P = 0.0127$) and unpaired Student's t-test (Full ipsi x Partial ipsi: $P = 0.0001$), *$P < 0.05$. Data are presented as means $\pm$ SEM. Each data point represents a single animal. FCI n = 8, PCI n = 7. (c) Immunofluorescence of Iba-1 in spinal dorsal horn (L4) after FCI and PCI. White dashed lines indicate regions of interest for image analyses. Scale bars, 500 $\mu$m. Quantitative analyses of Iba-1 immunofluorescence intensity within the ipsi- and contralateral dorsal horn at (d) dpi 2, (e) dpi 7 and (f) dpi 15. One-way ANOVA. Tukey's multiple comparison test, *$P < 0.05$; ***$P < 0.001$. Data are presented as means $\pm$ SEM. A dot represents an animal. n = 4-5 each.
in the mechanical threshold in the contralateral side after injury compared to baseline, which was not different between two groups (dpi 15, Full contra x Partial contra, P = 0.8832) (Figure 1(b)).

We next assessed the fluorescence intensity of immunoreactivity against Iba-1 in the dorsal horn of L4 lumbar spinal cord as a surrogate of microglial activation at three time points after injury in the two models. These time points were chosen according to the behavior phenotypes: dpi 2 (early time), dpi 7 (end of the axonal degeneration) and dpi 15 (full recovery of sensory function, development of tactile hypersensitivity) (Figure 1(c)). The expression of Iba-1 in the ipsilateral dorsal horn was significantly upregulated 2 days after FCI and PCI when compared to contralateral dorsal horn; however, Iba-1 reactivity in the FCI group was significantly greater than the PCI group (Figure 1(d)). The Iba-1 immunoreactivity was the most prominent at dpi 7 and was comparable in both groups with a significant increase in intensity compared to the contralateral side (Figure 1(e)). At dpi 15, Iba-1 immunoreactivity was reduced, although still significantly higher than the contralateral side in both groups (Figure 1(f)).

Our results show that initial microglial reactivity in the spinal cord is more prominent after more severe nerve damage (FCI) compared to an incomplete crush injury (PCI), despite the fact that the PCI group developed greater mechanical sensitivity - a surrogate of neuropathic pain in mice. Although some studies have reported graded nerve constriction injury influences the extent of spinal glial reactivity,20,21 and numerous studies support that microglia function is required for pain development,22–24 the correlation of the magnitude of spinal microglial reactivity with pain behavior is less clear.21,25,26 Our observations from two crush models add to growing evidence that early and predominant microgliosis is neither necessary nor sufficient for neuropathic pain-like behavior in mice.5 Nevertheless, the role of microglial function in the development of neuropathic pain after PCI requires further investigation.

Spinal microgliosis after peripheral nerve injury is considered a transient event, which increases in few days after the injury and the maximal activity is observed within a week in various animal models of neuropathic pain.5,27,28 The peripheral nerve injury models that are commonly used for studying chronic pain in rodents typically develop pain within a week after nerve injury.29–31 As a consequence, the linkage between chronic pain and spinal microgliosis activation has been studied at relatively early time points (within 2 weeks post-injury).6,32,33 The partial crush injury, representing a novel animal model of neuropathic pain, showed maximal microgliosis at dpi 7, which significantly decreased at dpi 15. The observation is consistent with other previous peripheral nerve injury (PNI) models.27,34 However, unlike other PNI models with pain, the PCI model develops pain after the sensory function is recovered (dpi 15). This may imply that the spinal microgliosis is rather injury-dependent than being associated with long-term development of neuropathic pain.

From our immunohistochemical images (Figure 1(c)), we consistently observed the Iba-1 reactivity in the ventral horn where the cell bodies of motor neurons exist, and the pattern of the microgliosis in the ventral horn was similar to that of the dorsal horn. Given that the sciatic nerve contains sensory afferents and motor efferents, our results may also suggest the response of microglial reactivity is an injury-dependent event, irrespective of nerve types. However, as it has been shown that dysfunctions of motor neurons elicit pain behavior,35,36 further investigation is required whether microglial activity in the ventral horn is indeed involved in the development of neuropathic pain.

Microglial activity is considered as a key mediator of sex-dimorphism in pain,37 and the activation of spinal microglia after nerve injury plays a limited role in female mice, unlike in the male mice.38–41 Interestingly, recent studies have reported that Iba-1 immunoreactivity is not different between male and female mice after peripheral nerve injury,5,39,40 suggesting the limited involvement of microgliosis in the generation of neuropathic pain. Although it remains to be tested in the crush models, our finding seems to be in line with these reports.

It has been reported that the activation of spinal microglia not only modulates neuronal synapses but also augments astrocyte activity.7,42–44 Whether the subsequent activation of astrocytes after microglia activation is also injury-dependent or not is worthy of further investigation. Comparing the time-dependent changes of astrocyte activation in the spinal cord between the FCI and PCI groups may provide clues as to whether astrocytes are functionally relevant to pain independently of microglial reactivity.

Our results suggest that while spinal microgliosis is injury severity-dependent, it does not necessarily correlate with long-term development of neuropathic pain. A small number of clinical studies have tested compounds that interfere with glial function for neuropathic pain,45 however microglial modulatory agents are yet to show robust or consistent results in trials.46–49 Greater understanding of the time-dependence of microglial contributions to persistent pain may improve the translation of pre-clinical animal studies on microglia as targets in chronic pain.50 The ability to induce contrasting neuropathic pain phenotypes despite a similar nerve injury makes the PCI model useful in the optimization of drug administration relative to the injury and for further validation of the molecular mechanisms of microglia activity in chronic pain.
Acknowledgment
The authors would like to thank Dr. Alexander J Davies for his critical reading and editing the manuscript, and Yoon Kyung Lee, Hyunjoon Rhee, Sangwook Shim for their assistance in immunohistochemical analysis.

Author Contributions
HWK and SBO designed the experiment and wrote the manuscript. HWK and CHW have performed the experiments and analysis.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was supported by the National Research Foundation of Korea grants (NRF-2017M3C7A1025602, NRF-2018R1A5A204418 and NRF-2021R1A2C3003334) funded by the Korean government MSIT (Ministry of Science and ICT).

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References
1. Watkins LR, Milligan ED, Maier SF. Glial activation: a driving force for pathological pain. Trends Neurosci 2001; 24: 450–455.
2. Suter MR, Wen YR, Decosterd I, Ji RR. Do glial cells control pain? Neuron Glia Biol 2007; 3: 255–268.
3. Scholz J, Woolf CJ. The neuropathic pain triad: neurons, immune cells and glia. Nat Neurosci 2007; 10: 1361–1368.
4. Inoue K, Tsuda M. Microglia in neuropathic pain: cellular and molecular mechanisms and therapeutic potential. Nat Rev Neurosci 2018; 19: 138–152.
5. Chen G, Zhang YQ, Qadri YJ, Serhan CN, Ji RR. Microglia in pain: detrimental and protective roles in pathogenesis and resolution of pain. Neuron 2018; 100: 1292–1311.
6. Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, Inoue K. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature 2003; 424: 778–783.
7. Zhuang ZY, Gerner P, Woolf CJ, Ji RR. ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model. Pain 2005; 114: 149–159.
8. Gruber-Schoffnegger D, Drdla-Schutting R, Honigsperger C, Wunderbaldinger G, Gassner M, Sandkühler J. Induction of thermal hyperalgesia and synaptic long-term potentiation in the spinal cord lamina I by TNF-alpha and IL-1beta is mediated by glial cells. J Neurosci 2013; 33: 6540–6551.
9. Clark AK, Gruber-Schoffnegger D, Drdla-Schutting R, Gerhold KJ, Malcangio M, Sandkühler J. Selective activation of microglia facilitates synaptic strength. J Neurosci 2015; 35: 4552–4570.
10. Davies AJ, Kim HW, Gonzalez-Cano R, Choi J, Back SK, Roh SE, Johnson E, Gabraic 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 2019; 176: 716–728.e718.
11. Latremoliere A, Cheng L, DeLisle M, Wu C, Chew S, Hutchinson EB, Sheridan A, Alexandre C, Latremoliere F, Sheu SH, Golidy S, Omura T, Huebner EA, Fan Y, Whitman MC, Nguyen E, Hermawan C, Pierpaoli C, Tischfeld MA, Woolf CJ, Englue EC. Neuronal-specific TUBB3 is not required for normal neuronal function but is essential for timely axon regeneration. Cell Rep 2018; 24: 1865–1879.e1869.
12. Dixon WJ. Efficient analysis of experimental observations. Annu Rev Pharmacol Toxicol 1980; 20: 441–462.
13. 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.
14. Harrison M, O’Brien A, Adams L, Cowin G, Ruitenberg MJ, Sengul G, Watson C. Vertebral landmarks for the identification of spinal cord segments in the mouse. Neuroimage 2013; 68: 22–29.
15. Ji RR, Befort K, Brenner GJ, Woolf CJ. ERK MAP kinase activation in superficial spinal cord neurons induces prodynorphin and NK-1 upregulation and contributes to persistent inflammatory pain hypersensitivity. J Neurosci 2002; 22: 478–485.
16. Lee YJ, Yoon SY, Won J, Kim HB, Kang Y, Oh SB. Sinomenine produces peripheral analgesic effects via inhibition of voltage-gated sodium currents. Neuroscience 2017; 358: 28–36.
17. Davies AJ, Kim D, Park J, Lee JY, Vang H, Pickering AE, Oh SB. Hedonic drinking engages a supraspinal inhibition of thermal nociception in adult rats. Pain 2019; 160: 1059–1069.
18. Rigaud M, Gemes G, Barabas ME, Chernoff DI, Abram SE, Stucky CL, Hogan QH. Species and strain differences in rodent sciatic nerve anatomy: implications for studies of peripheral nerve injury. Brain Behav Immun 2010; 193: 47–53.
22. Narita M, Yoshida T, Nakajima M, Narita M, Miyatake M, Takagi T, Yajima Y, Suzuki T. Direct evidence for spinal cord microglia in the development of a neuropathic pain-like state in mice. J Neurochem 2006; 97: 1337–1348.

23. Guan Z, Kuhn JA, Wang X, Colquitt B, Solorzano C, Vaman S, Guan AK, Evans-Reinsch B, Braz J, Devor M, Abboud-Werner SL, Lanier LL, Lomvardas S, Basbaum AI. Injured sensory neuron-derived CSF1 induces microglial proliferation and DAP12-dependent pain. Nat Neurosci 2016; 19: 94–101.

24. Gu N, Peng J, Murugan M, Wang X, Eyo UB, Sun D, Ren Y, DiCicco-Bloom E, Young W, Dong H, Wu LJ. Spinal microgliosis due to resident microglial proliferation is required for pain hypersensitivity after peripheral nerve injury. Cell Rep 2016; 16: 605–614.

25. Colburn RW, DeLeo JA, Rickman AJ, Yeager MP, Kwon P, Hickey WF. Dissociation of microglial activation and neuropathic pain behaviors following peripheral nerve injury in the rat. J Neuroimmunol 1997; 79: 163–175.

26. Colburn RW, Rickman A, DeLeo JA. The effect of site and type of nerve injury on spinal glial activation and neuropathic pain behavior. Exp Neurol 1999; 157: 289–304.

27. Echeverry S, Shi XQ, Zhang J. Characterization of cell proliferation in rat spinal cord following peripheral nerve injury and the relationship with neuropathic pain. Pain 2008; 135: 37–47.

28. Inoue K, Tsuda M. Microglia and neuropathic pain. Glia 2009; 57: 1469–1479.

29. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988; 33: 87–107.

30. Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 1992; 50: 355–363.

31. Decosterd I, Woolf CJ. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain 2000; 87: 149–158.

32. Masuda T, Iwamoto S, Yoshinaga R, Tozaki-Saitoh H, Nishiyama A, Mak TW, Tamura T, Tsuda M, Inoue K. Transcription factor IRF5 drives P2X4R- reactive microglia gating neuropathic pain. Nat Commun 2014; 5: 3771.

33. Yu T, Zhang X, Shi H, Tian J, Sun L, Hu X, Cui W, Du D. P2Y12 regulates microglia activation and excitatory synaptic transmission in spinal lamina II neurons during neuropathic pain in rodents. Cell Death Dis 2019; 10: 165.

34. Peng J, Gu N, Zhou L, B Eyo U, Murugan M, Gan W-B, Wu L-J. Microglia and monocytes synergistically promote the transition from acute to chronic pain after nerve injury. Nat Commun 2016; 7: 12029.

35. Wu G, Ringkamp M, Hartke TV, Murinson BB, Campbell JN, Griffin JW, Meyer RA. Early onset of spontaneous activity in uninjured C-fiber nociceptors after injury to neighboring nerve fibers. J Neurosci 2001; 21: RC140.

36. Wu G, Ringkamp M, Murinson BB, Pogatzki EM, Hartke TV, Weerahandi HM, Campbell JN, Griffin JW, Meyer RA. Degeneration of myelinated efferent fibers induces spontaneous activity in uninjured C-fiber afferents. J Neurosci 2002; 22: 7746–7753.

37. Mapplebeck JCS, Beggs S, Salter MW. Sex differences in pain: a tale of two immune cells. Pain 2016; 157 Suppl 1: S2–S6.

38. Sorge RE, LaCroix-Fralish ML, Tuttle AH, Sotocinal SG, Austin JS, Ritchie J, Chanda ML, Graham AC, Topham L, Beggs S, Salter MW, Mogil JS. Spinal cord toll-like receptor 4 mediates inflammatory and neuropathic hyper-sensitivity in male but not female mice. J Neurosci 2011; 31: 15450–15454.

39. Sorge RE, Mapplebeck JC, Rosen S, Beggs S, Taves S, Alexander JK, Martin LJ, Austin JS, Sotocinal SG, Chen D, Yang M, Shi XQ, Huang H, Pillon NJ, Bilan PJ, Tu Y, Klip A, Ji RR, Zhang J, Salter MW, Mogil JS. Different immune cells mediate mechanical pain hypersensitivity in male and female mice. Nat Neurosci 2015; 18: 1081–1083.

40. Taves S, Berta T, Liu DL, Gan S, Chen G, Kim YH, Van de Ven T, Laufer S, Ji RR. Spinal inhibition of p38 MAP kinase reduces inflammatory and neuropathic pain in male but not female mice: sex-dependent microglial signaling in the spinal cord. Brain Behav Immun 2016; 55: 70–81.

41. Chen G, Luo X, Quadri MY, Berta T, Ji RR. Sex-dependent glial signaling in pathological pain: distinct roles of spinal microglia and astrocytes. Neurosci Bull 2018; 34: 98–108.

42. Miyoshi K, Obata K, Kondo T, Okamura H, Noguchi K. Interleukin-18-mediated microglia/astrocye interaction in the spinal cord enhances neuropathic pain processing after nerve injury. J Neurosci 2008; 28: 12775–12777.

43. Liu W, Tang Y, Feng J. Cross talk between activation of microglia and astrocytes in pathological conditions in the central nervous system. Life Sci 2011; 89: 141–146.

44. Ji RR, Berta T, Nedergaard M. Glia and pain: is chronic pain a gliopathy? Pain 2013; 154 Suppl 1: S10–S28.

45. Romero-Sandoval EA, Horvath RJ, DeLeo JA. Neuroimmune interactions and pain: focus on glial-modulating targets. Curr Opin Investig Drugs 2008; 9: 726–734.

46. Landry RP, Jacobs VL, Romero-Sandoval EA, DeLeo JA. Propentofylline, a CNS glial modulator does not decrease pain in post-herpetic neuralgia patients: in vitro evidence for differential responses in human and rodent microglia and macrophages. Exp Neurol 2012; 234: 340–350.

47. Smith AM, Dragunow M. The human side of microglia. Trends Neurosci 2014; 37: 125–135.

48. Vanelderen P, Van Zundert K, Kozicz T, Puyalbert M, De Vooght P, Mestrum R, Heylen R, Roubos E, Vissers K. Effect of minocycline on lumbar radicular neuropathic pain: a randomized, placebo-controlled, double-blind clinical trial with amitriptyline as a comparator. Anesthesiology 2015; 122: 399–406.

49. Sumitani M, Ueda H, Hozumi J, Inoue R, Kogure T, Yamada Y, Kogure T. Minocycline does not decrease the intensity of neuropathic pain intensity, but does improve its affective dimension. J Pain Palliat Care Pharmacother 2016; 30: 31–35.

50. Haight ES, Forman TE, Cordonnier SA, James ML, Tawfik VL. Microglial modulation as a target for chronic pain: from the bench to the bedside and back. Anesth Analg 2019; 128: 737–746.