Published in final edited form as:
J Formos Med Assoc. 2011 August ; 110(8): 487–494. doi:10.1016/S0929-6646(11)60074-0.

Role of microglia in neuropathic pain, postoperative pain, and morphine tolerance

Yeong-Ray Wen[1,2,3], **Ping-Heng Tan**[1,4], **Jen-Kun Cheng**[1,5,6,7], **Yen-Chin Liu**[1,8], and **Ru-Rong Ji**[1]

[1]Sensory Plasticity Laboratory, Pain Research Center, Department of Anesthesiology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA

[2]Department of Anesthesiology, China Medical University Hospital, Taichung, Taiwan

[3]School of Medicine, China Medical University, Taichung, Taiwan

[4]Department of Anesthesia and Critical Care and Department of Biomedical Engineering, E-DA Hospital/I-Shou University, Kaohsiung, Taiwan

[5]Department of Anesthesiology, Mackay Memorial Hospital, Taipei, Taiwan

[6]Mackay Medicine, Nursing and Management College, Taipei, Taiwan

[7]Department of Anesthesiology, School of Medicine, Taipei Medical University, Taipei, Taiwan

[8]Department of Anesthesiology, College of Medicine, National Cheng Kung University, Tainan City, Taiwan

Abstract

Management of chronic pain such as nerve injury-induced neuropathic pain associated with diabetic neuropathy, viral infection, and cancer is a real clinical challenge. Major surgeries such as breast and thoracic surgery, leg amputation, and coronary artery bypass surgery also lead to chronic pain in 10–50% of individuals after acute postoperative pain, in part due to surgery-induced nerve injury. Current treatments mainly focus on blocking neurotransmission in the pain pathway and have only resulted in limited success. Ironically, chronic opioid exposure may lead to paradoxical pain. Development of effective therapeutic strategies requires a better understanding of cellular mechanisms underlying the pathogenesis of neuropathic pain. An important progress in pain research points to important role of microglial cells in the development of chronic pain. Spinal cord microglia are strongly activated after nerve injury, surgical incision, and chronic opioid exposure. Increasing evidence suggests that under all these conditions the activated microglia not only exhibit increased expression of microglial markers CD11b and Iba1 but also display elevated phosphorylation of p38 MAP kinase. Inhibition of spinal cord p38 has been shown to attenuate neuropathic pain and postoperative pain, as well as morphine-induced antinociceptive tolerance. Activation of p38 in spinal microglia results in increased synthesis and release of the neurotrophin BDNF and the proinflammatory cytokines IL-1β, IL-6, and TNF-α. These microglia-released mediators can powerfully modulate spinal cord synaptic transmission, leading to increased excitability of dorsal horn neurons, i.e. central sensitization, in part via suppressing inhibitory synaptic transmission. We review the studies that support the pronociceptive role of microglia in conditions of neuropathic pain, post-surgical pain, and opioid

Corresponding author: Correspondence should be addressed to Ru-Rong Ji, Department of Anesthesiology, Brigham and Women's Hospital, 75 Francis Street, MRB 611, Boston, MA 02115, USA, Tel: (617) 732-8852; Fax: (617) 730-2801 rrji@zeus.bwh.harvard.edu.

Conflict of Interest:
All the authors have no financial interests related to the material in the manuscript.

tolerance. Some of these studies have been accomplished by four Taiwanese anesthesiologists who are also co-authors of this review during their training at Harvard Medical School. We conclude that targeting microglial signalling may lead to more effective treatments for devastating chronic pain after diabetic neuropathy, viral infection, cancer, and major surgeries in part via improving the analgesic efficacy of opioids.

Keywords

Central sensitization; neuronal-glial interactions; proinflammatory cytokines; p38 MAP kinase; spinal cord

Microglia activation and neuropathic pain

Microglial cells originate from bone marrow-derived monocytes migrating to the central nervous system (CNS) during perinatal time and account for 5–12% of total cells in the CNS. In normal conditions, microglia are ramified and were thought to be “quiescent”. However, microglia in the non-injured conditions are not really quite, because they can actively sense their environment with their ramified processes.[1] After peripheral nerve injury, microglia in the spinal cord become activated showing dramatic changes in morphology (from ramified to amoeboid) and robust increases in the expression of microglial markers such as CD11b and Iba1 (Fig. 1).[2] Proliferation of microglia in the spinal cord after nerve injury is also a feature of microglia activation. In the normal conditions, glial cell proliferation is rarely detected. However, robust microglial proliferation occurs under several neuropathic pain conditions after sciatic nerve constriction, partial sciatic nerve ligation, or spared nerve injury (SNI),[2–3] in which two of the three terminal branches of the sciatic nerve are ligated leaving the third brand sural nerve intact.[4] Notably, nerve injury-induced cell proliferation in the spinal cord is largely restricted to microglial cells, although proliferation of other cell types such as astrocytes was also reported.[5] The specific role of microglial proliferation for the control of neuropathic pain has not been clearly demonstrated. But more microglia could result in increased production of pain mediators from microglia.

While nerve injury-induced morphological changes of microglia are very striking, biochemical changes after nerve injury are more important for microglia to induce pain. Nerve injury results in a dramatic up-regulation of the ATP receptor P2X4[6] and the chemokine receptor CX3CR1 in spinal cord microglia.[7–8] Spinal blockade of P2X4 and CX3CR1 signaling attenuates nerve injury-induced neuropathic pain.[6,8] The chemokine receptor CCR2 and the Toll-like recepotor-4 (TLR4) also contribute to neuropathic pain sensitization via microglial activation,[9–10] although CCR2 and TLR4 localization in microglia has not been clearly demonstrated.

Studies from many laboratories in the world have demonstrated that nerve injury causes phosphorylation of p38 mitogen-activated protein kinase (MAPK) in spinal cord microglia.[11–12] Phsopho-p38 (p-p38) levels are low in the spinal cord of non-injured rats. Spinal nerve ligation induces a substantial increase in p-p38 levels in the injured side of the spinal cord, which is accompanied by an increase in p38 activity.[11] Strikingly, p38 is primarily if not exclusively activated in spinal cells expressing the microglial markers CD11b/OX-42 and Iba1.[13–14] In contrast, p-p38 is barely detected in NeuN-expressing neurons, although low-level of p-p38 may be seen occasionally. We confirmed microglia activation of p38 in the spared nerve injury model.[15] p38 activation in spinal microglia was also reported after ventral root lesion[16] and spinal cord injury.[17] Although p38 activation peaks in the first week of nerve injury, the activation is still maintained even 3 weeks later.[18]

Thus, either intrathecal pre-treatment of p38 inhibitor (e.g., SB203580 and FR167653) or intrathecal post-treatment of p38 inhibitor, at early and late time of nerve injury, can effectively reduce nerve injury-mechanical allodynia, a cardinal feature of neuropathic pain.[13–15] Consistently, minocycline, a non-selective microglial inhibitor attenuates neuropathic pain by inhibiting p38.[17,19] Since minocycline only inhibits neuropathic pain in the early phase, it may not inhibit p38 activation in the late-phase.

What is causing the activation of p38 and microglia in the spinal cord? We have shown that matrix metalloproteinase-9 (MMP-9) can cause microglial activation via neuronal-glial interaction. Spinal nerve ligation elicits a rapid increase in MMP-9 protein and activity in DRG neurons.[20] Intrathecal administration of MMP-9 induces persistent mechanical allodynia for many days.[20] Intrathecal MMP-9 also induces a drastic activation of spinal microglia, as revealed by increased p38 phosphorylation and OX-42 expression in the spinal cord.[20] A critical issue to study MMP-9 function is how to persistently suppress MMP-9 expression in the DRG. Tan and coauthors[21] developed an RNA interference strategy to target gene expression in the pain system, using a cationic polymer, polyethyleneimine (PEI) to form a 'proton sponge' due to its buffering capacity, which enables PEI to buffer endosomes and induce their rupture to release small interfering RNA (siRNA) into the cytoplasm.[21–22] We employed this siRNA strategy to target MMP-9 in the DRGs after nerve injury. Intrathecal injections of MMP-9 specific siRNA (2 × 5 μg) in rats effectively suppressed spinal nerve ligation-induced MMP-9 up-regulation by >70% in the DRG without affecting MMP-2 levels.[20] Importantly, this siRNA treatment also suppressed microglia activation in the spinal cord and delayed the development of mechanical allodynia.[20] We found that Cy3-labeleld siRNA was heavily taken up by many DRG cells 3 hours after intrathecal injection.[22] These results suggest that siRNA knockdown is an effective way to study gene functions in neuropathic pain. An association of MMP-9 with microglia activation of p38 is further validated by the finding that intrathecal p38 inhibitor can block MMP-9-induced neuropathic pain symptom, mechanical allodynia.[20]

MMP-9, as well as ATP and chemokines (e.g., CCL2 and fractalkine (FKN)/CX3CL1) could be released from DRG neurons by nerve injury-induced discharge, causing the activation of microglia in the spinal cord (Fig. 2). It is generally believed that nerve injury-induced spontaneous discharge in the axons and cell bodies of DRG neurons can drive neuropathic pain.[23] Indeed, blocking neural activity in the sciatic nerve by the local anesthetic bupivacaine can prevent nerve injury-induced spinal microglia activation of p38 in the spared nerve injury model.[15] In contrast, blocking C-fiber activity in the sciatic nerve with an ultrapotent capsaicin analogue resiniferatoxin fails to inhibit p38 activation in this model.[24] Thus, activity from large myelinated A-fibers is also important for microglia activation after nerve injury.

Microglia activation and postoperative pain

Growing evidence has indicated that post-surgical pain, traditionally regarded as acute pain and resolved spontaneously, could become chronic and persistent under similar processes. For example, groin hernia repair, breast and thoracic surgery, leg amputation, thoracotomy and coronary artery bypass surgery result in chronic pain in 10–50% individuals after acute post-surgical pain, in part due to surgery-induced nerve injury.[25] In light of various types of surgeries in human, an optimal animal model is essential for investigating the mechanisms and treatments of postoperative pain. The most widely-used surgical pain model in rodents was developed by Brennan, et al.[26] In this incisional pain model, a longitudinal incision (1 cm) was made in the plantar surface deep to muscle layers in a hind limb.[26] Behaviorally, hypersensitivity to mechanical touch and radiant heat were shown to develop immediately after surgery and last for 2–3 days. Many studies demonstrated that this model is compatible

to human incisional pain in terms of behavioural, pharmacological, and molecular changes.[26–28] Other surgical models have also been developed since then to mimic different conditions of human surgeries, such as back incision,[29] hindlimb incision,[30] and gastrocnemius incision as models of incisional pain, a thoracotomy model[31] to study surgeries in nerve-rich tissues, a laparotomy model[32] to mimic abdominal surgical consequences, and a skin/muscle incision and retraction (SMIR) model[33] to explore potentially persistent pain following an operation at somatic tissues.

In our previous study[28] in the plantar incision model, we found that a simple brief incision at the paw induced a marked up-regulation of p38 phosphorylation in the spinal dorsal horn, starting within 1 hour, reaching peak at 1 day, and declining after 3–5 days. The time course of p38 activation was compatible with that of early pain progression after operation. Except very few neurons expressing p-p38 within the first 1 hour, we observed that activated p38 is exclusively expressed in microglia. However, change of microglia surface marker (e.g., CD11b/OX-42) after incision was found 2–3 days later, with a remarkable increase from day 3 to 7 after incision.[28] The function of the delayed microglial reaction remains to be investigated.

Involvement of p38 MAPK in postoperative pain development is further confirmed by pharmacological inhibition of p38 via intrathecal route. The p38 inhibitor FR167653 produces a potent anti-inflammatory action by inhibiting the production of interleukin-1beta (IL-1β) and tumour necrosis factor-alpha (TNF-α). We found that intrathecal FR167653 prevented incision-induced mechanical allodynia and also reduced p-p38 levels in the spinal dorsal horn, but only had a mild effect on reducing thermal hyperalgesia.[28] These results support the hypothesis that p38 activation in spinal microglial cells plays a critical role in the development and maintenance of postoperative mechanical hypersensitivity. This study also suggests that targeting p38 in microglia may offer a novel way of preventing persistent postoperative pain by inhibiting microglia-driven “central sensitization”, i.e. hyperactivity in spinal cord dorsal horn neurons, a critical mechanism underlying the development of persistent pain.[34] In addition, Eisenach and collaborator also showed that cyclooxygenase-1 (COX-1) is dramatically up-regulated in spinal microglia after incision and intrathecal administration of a COX-1 inhibitor can attenuate post-incisional pain for several days.[35] It is attempting to postulate that p38 activation in microglia induces COX-1 expression to drive incisional pain, although p38 can regulate many other targets (Fig. 2).

Microglia activation and morphine tolerance

Opioids are the primary treatment for chronic pain. Medical practice has shifted over the past decades, and long-term use of opioids is common. However, long-term administration of opioids produces negative health consequences, such as increased risk of abuse and addiction. Prolonged administration of opioids is also associated with the development of antinociceptive tolerance, wherein higher doses of the drug are required over time to elicit the same degree of analgesia. Numerous animal studies have demonstrated that sustained exposure to systemic or spinal opioids, including morphine, DAMGO, fentanyl, or heroin produces paradoxical pain, characterized as heat hyperalgesia and mechanical allodynia. Opioid-induced hyperalgesia was also found in chronic pain patients.[36–37] Chronic morphine exposure results in a strong upregulation of the microglia markers CD11b and Iba1, as well as the ATP receptors P2X4 and P2X7 in spinal microglia.[38–39] Intrathecal injections of antisense oligodeoxynucleotides against P2X4 or P2X7 antagonist prevent the development of morphine tolerance and microglial reaction.[38–39] In particular, chronic morphine induces p38 activation in spinal microglia, and intrathecal treatment of p38 inhibitor or minocycline prevents the development of morphine tolerance.[40–41]

Mechanisms of microglia-evoked pain

Figure 2 illustrates how microglia activation causes pain hypersensitivity after nerve injury, surgical procedures, and chronic opioid exposure. Phosphorylation of p38 in microglia via activation of P2X4 receptor was shown to increase the synthesis and release of the neurotrophin BDNF, and BDNF could enhance neuropathic pain via suppressing inhibitory synaptic transmission in the spinal cord.[42] Phosphorylation of p38 in microglia also results in increased synthesis of the proinflammatory cytokines IL-1β, IL-6, and TNF-α in part through the activation of the transcription factor NF-κB.[11,43] lipopolysaccharide (LPS), a potent microglia activator and also a TLR4 ligand, has been shown to induce IL-1β release via p38 activation in spinal microglia.[44]

Accumulating evidence indicates a critical role of IL-1β, IL-6, and TNF-α in inducing hyperactivity of dorsal horn neurons, i.e. central sensitization, leading to pain hypersensitivity.[45–46] Intrathecal administration of IL-1β, IL-6, and TNF-α induce robust heat hyperalgesia and mechanical allodynia.[46–47] Conversely, spinal blockade of these cytokines has been shown to attenuate inflammatory pain, neuropathic pain, and morphine tolerance.[48–52] Intrathecal administration of IL-1β induces a substantial increase in Cox-2 mRNA levels in the spinal cord.[53] Perfusion of spinal cord slices IL-1β, IL-6, and TNF-α also activate the transcription factor CREB (cAMP response element-binding protein),[46] which is critical for the transcription of pronociceptive genes such as neurokinin-1 and Cox-2 and long-term neuronal plasticity in dorsal horn neurons.[34] In particular, we found that these proinflammatory cytokines also have non-transcriptional role in pain control. They can powerfully regulate synaptic transmission via enhancing excitatory synaptic transmission and suppressing inhibitory synaptic transmission.[46] Our patch clamp recordings in isolated spinal cord slices have revealed the following findings. First, IL-1β and TNF-α increase spontaneous excitatory postsynaptic currents (sEPSCs) in dorsal horn neurons and enhance AMPA- or NMDA-induced currents. Second, IL-1β and IL-6 decrease spontaneous inhibitory postsynaptic currents (sIPSCs) in dorsal horn neurons and suppress GABA- or glycine-induced currents.[46–47,51] Similar findings on IL-1β's actions were also reported in cultures dorsal horn neurons,[54] and TNF-α causes disinhibition in GABAergic neurons in spinal cord slices.[55] In addition to direct effects on synaptic transmission, TNF-α could further activate astrocytes via c-Jun N-terminal kinase (JNK) to produce monocyte chemoattractant protein-1 (MCP-1/CCL2), an important chemokine for central sensitization,[56] whereas IL-1β can activate spinal microglia via p38 phosphorylation.[24,57] Of note morphine metabolite morphine-3-glucuronide has been shown to facilitate pain via TLR4 activation and IL-1β release; and conversely, intrathecal injection of IL-1β antagonist and TLR4 inhibitor can potentiate morphine analgesia.[58]

Conclusions and future directions

Chronic pain is an increasing burden for the society, affecting 20% of the population worldwide. Current treatments that focus mostly on targeting neuronal excitability and transmission are not satisfying. The emerging role of microglia in pain control brought great excitement to the pain research field. We have discussed the pronociceptive role of microglia in neuropathic pain, postoperative pain, and opioid tolerance. It is important to point out that some of these studies have been accomplished by four Taiwanese anesthesiologists as co-authors of this review during their training at Harvard Medical School. Apparently, microglia regulate chronic pain and opioid tolerance via neuronal-glial interactions (Fig. 2). First, primary sensory neurons exhibit hyperactivity after nerve injury, surgical procedures, and chronic opioid treatment and release potential microglial activators such as ATP, MMP-9, and the chemokines (e.g., FKN and MCP-1). Second, p38 activation in microglia leads to the production of pain mediators such as neurotrophin and cytokines to

modulate synaptic transmission and enhance pain. Thus, targeting microglial signaling via inhibiting the actions of chemokines (FKN, CCL2), ATP receptors (P2X4, P2X7), MMP-9, p38 MAPK, or/and proinflammatory cytokines (IL-1β, IL-6, and TNF-α) may lead to novel therapies for chronic pain.

Finally, we have to point out that apart from microglia, other types of glial cells such as astrocytes are also important for inflammatory and neuropathic pain.[59–60] Our work in progress has shown that astrocytes can produce tissue plasminogen activator (tPA), a protease in the spinal cord to facilitate morphine tolerance (Liu YC and Ji RR, unpublished observation). Satellite glial cells share similar molecular features as astrocytes but are localized in the dorsal root ganglion (DRG) in the peripheral nervous system. Activation of satellite cells in the DRG after morphine treatment could antagonize morphine analgesia via releasing IL-1β (Liu YC and Ji RR, unpublished observation). It remains to be investigated how different types of glial cells control pain sensitivity under various injury and treatment conditions.

Acknowledgments

This study was partially supported by NIH grants NS54932 and DE17794 to R.R.J; NSC 97-2314-B-341-002-MY3, SKH-8302-98-DR-32 to Y.R.W; NSC 97-2314-B-214-006-MY3, EDPJ 98023, 99038, EDAHP 99030 to P.H.T; NSC 98-2314-B-195-002-MY3 to J.K.C;, and NSC 97-2918-I-006-012 to Y.C.L.

References

1. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science. 2005; 308:1314–1318. [PubMed: 15831717]
2. Suter MR, Wen YR, Decosterd I, et al. Do glial cells control pain? Neuron Glia Biol. 2007; 3:255–268. [PubMed: 18504511]
3. 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. [PubMed: 17560721]
4. Decosterd I, Woolf CJ. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain. 2000; 87:149–158. [PubMed: 10924808]
5. Tsuda M, Kohro Y, Yano T, et al. JAK-STAT3 pathway regulates spinal astrocyte proliferation and neuropathic pain maintenance in rats. Brain. 2011; 134:1127–1139. [PubMed: 21371995]
6. Tsuda M, Shigemoto-Mogami Y, Koizumi S, et al. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature. 2003; 424:778–783. [PubMed: 12917686]
7. Verge GM, Milligan ED, Maier SF, et al. Fractalkine (CX3CL1) and fractalkine receptor (CX3CR1) distribution in spinal cord and dorsal root ganglia under basal and neuropathic pain conditions. Eur J Neurosci. 2004; 20:1150–1160. [PubMed: 15341587]
8. Zhuang ZY, Kawasaki Y, Tan PH, et al. Role of the CX3CR1/p38 MAPK pathway in spinal microglia for the development of neuropathic pain following nerve injury-induced cleavage of fractalkine. Brain Behav Immun. 2007; 21:642–651. [PubMed: 17174525]
9. Abbadie C, Lindia JA, Cumiskey AM, et al. Impaired neuropathic pain responses in mice lacking the chemokine receptor CCR2. Proc Natl Acad Sci U S A. 2003; 100:7947–7952. [PubMed: 12808141]
10. Tanga FY, Nutile-McMenemy N, DeLeo JA. The CNS role of Toll-like receptor 4 in innate neuroimmunity and painful neuropathy. Proc Natl Acad Sci U S A. 2005; 102:5856–5861. [PubMed: 15809417]
11. Ji RR, Suter MR. p38 MAPK, microglial signaling, and neuropathic pain. Mol Pain. 2007; 3:33. [PubMed: 17974036]
12. Ji RR, Gereau RWt, Malcangio M, et al. MAP kinase and pain. Brain Res Rev. 2009; 60:135–148. [PubMed: 19150373]

13. Jin SX, Zhuang ZY, Woolf CJ, et al. p38 mitogen-activated protein kinase is activated after a spinal nerve ligation in spinal cord microglia and dorsal root ganglion neurons and contributes to the generation of neuropathic pain. J Neurosci. 2003; 23:4017–4022. [PubMed: 12764087]
14. Tsuda M, Mizokoshi A, Shigemoto-Mogami Y, et al. Activation of p38 mitogen-activated protein kinase in spinal hyperactive microglia contributes to pain hypersensitivity following peripheral nerve injury. GLIA. 2004; 45:89–95. [PubMed: 14648549]
15. Wen YR, Suter MR, Kawasaki Y, et al. Nerve conduction blockade in the sciatic nerve prevents but does not reverse the activation of p38 mitogen-activated protein kinase in spinal microglia in the rat spared nerve injury model. Anesthesiology. 2007; 107:312–321. [PubMed: 17667577]
16. Xu JT, Xin WJ, Wei XH, et al. p38 activation in uninjured primary afferent neurons and in spinal microglia contributes to the development of neuropathic pain induced by selective motor fiber injury. Exp Neurol. 2007; 204:355–365. [PubMed: 17258708]
17. Hains BC, Waxman SG. Activated microglia contribute to the maintenance of chronic pain after spinal cord injury. J Neurosci. 2006; 26:4308–4317. [PubMed: 16624951]
18. Zhuang ZY, Wen YR, Zhang DR, et al. A peptide c-Jun N-terminal kinase (JNK) inhibitor blocks mechanical allodynia after spinal nerve ligation: respective roles of JNK activation in primary sensory neurons and spinal astrocytes for neuropathic pain development and maintenance. J Neurosci. 2006; 26:3551–3560. [PubMed: 16571763]
19. Raghavendra V, Tanga F, DeLeo JA. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. J Pharmacol Exp Ther. 2003; 306:624–630. [PubMed: 12734393]
20. Kawasaki Y, Xu ZZ, Wang X, et al. Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. Nat Med. 2008; 14:331–336. [PubMed: 18264108]
21. Tan PH, Yang LC, Shih HC, et al. Gene knockdown with intrathecal siRNA of NMDA receptor NR2B subunit reduces formalin-induced nociception in the rat. Gene Ther. 2005; 12:59–66. [PubMed: 15470478]
22. Tan PH, Yang LC, Ji RR. Therapeutic potential of RNA interference in pain medicine. Open Pain J. 2009; 2:57–63. [PubMed: 19966919]
23. Devor M. Neuropathic pain and injured nerve: peripheral mechanisms. Br Med Bull. 1991; 47:619–630. [PubMed: 1794075]
24. Suter MR, Berta T, Gao YJ, et al. Large A-fiber activity is required for microglial proliferation and p38 MAPK activation in the spinal cord: different effects of resiniferatoxin and bupivacaine on spinal microglial changes after spared nerve injury. Mol Pain. 2009; 5:53. [PubMed: 19772627]
25. Kehlet H, Jensen TS, Woolf CJ. Persistent postsurgical pain: risk factors and prevention. Lancet. 2006; 367:1618–1625. [PubMed: 16698416]
26. Brennan TJ, Vandermeulen EP, Gebhart GF. Characterization of a rat model of incisional pain. Pain. 1996; 64:493–501. [PubMed: 8783314]
27. Wen YR, Lin CP, Tsai MD, et al. Combination of nerve blockade and intravenous alfentanil is better than single treatment in relieving postoperative pain. J Formos Med Assoc. 2011 (Accepted).
28. Wen YR, Suter MR, Ji RR, et al. Activation of p38 mitogen-activated protein kinase in spinal microglia contributes to incision-induced mechanical allodynia. Anesthesiology. 2009; 110:155–165. [PubMed: 19104183]
29. Duarte AM, Pospisilova E, Reilly E, et al. Reduction of postincisional allodynia by subcutaneous bupivacaine: findings with a new model in the hairy skin of the rat. Anesthesiology. 2005; 103:113–125. [PubMed: 15983463]
30. Kawamata M, Koshizaki M, Shimada SG, et al. Changes in response properties and receptive fields of spinal dorsal horn neurons in rats after surgical incision in hairy skin. Anesthesiology. 2005; 102:141–151. [PubMed: 15618798]
31. Buvanendran A, Kroin JS, Kerns JM, et al. Characterization of a new animal model for evaluation of persistent postthoracotomy pain. Anesth Analg. 2004; 99:1453–1460. table of contents. [PubMed: 15502048]
32. Roughan JV, Flecknell PA. Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. Eur J Pain. 2003; 7:397–406. [PubMed: 12935791]

33. Flatters SJ. Characterization of a model of persistent postoperative pain evoked by skin/muscle incision and retraction (SMIR). Pain. 2008; 135:119–130. [PubMed: 17590272]
34. Ji RR, Kohno T, Moore KA, et al. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci. 2003; 26:696–705. [PubMed: 14624855]
35. Zhu X, Conklin D, Eisenach JC. Cyclooxygenase-1 in the spinal cord plays an important role in postoperative pain. Pain. 2003; 104:15–23. [PubMed: 12855310]
36. Mao J, Price DD, Mayer DJ. Mechanisms of hyperalgesia and morphine tolerance: a current view of their possible interactions. Pain. 1995; 62:259–274. [PubMed: 8657426]
37. Ossipov MH, Lai J, King T, et al. Antinociceptive and nociceptive actions of opioids. J Neurobiol. 2004; 61:126–148. [PubMed: 15362157]
38. Horvath RJ, Romero-Sandoval EA, De Leo JA. Inhibition of microglial P2X4 receptors attenuates morphine tolerance, Iba1, GFAP and mu opioid receptor protein expression while enhancing perivascular microglial ED2. Pain. 2010; 150:401–413. [PubMed: 20573450]
39. Zhou D, Chen ML, Zhang YQ, et al. Involvement of spinal microglial P2X7 receptor in generation of tolerance to morphine analgesia in rats. J Neurosci. 2010; 30:8042–8047. [PubMed: 20534852]
40. Cui Y, Chen Y, Zhi JL, et al. Activation of p38 mitogen-activated protein kinase in spinal microglia mediates morphine antinociceptive tolerance. Brain Res. 2006; 1069:235–243. [PubMed: 16403466]
41. Cui Y, Liao XX, Liu W, et al. A novel role of minocycline: attenuating morphine antinociceptive tolerance by inhibition of p38 MAPK in the activated spinal microglia. Brain Behav Immun. 2008; 22:114–123. [PubMed: 17919885]
42. Coull JA, Beggs S, Boudreau D, et al. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature. 2005; 438:1017–1021. [PubMed: 16355225]
43. Wang Z, Ma W, Chabot JG, et al. Calcitonin gene-related peptide as a regulator of neuronal CaMKII-CREB, microglial p38-NFkappaB and astroglial ERK-Stat1/3 cascades mediating the development of tolerance to morphine-induced analgesia. Pain. 2010; 151:194–205. [PubMed: 20691540]
44. Clark AK, Staniland AA, Marchand F, et al. P2X7-dependent release of interleukin-1beta and nociception in the spinal cord following lipopolysaccharide. J Neurosci. 2010; 30:573–582. [PubMed: 20071520]
45. Ren K, Torres R. Role of interleukin-1beta during pain and inflammation. Brain Res Rev. 2009; 60:57–64. [PubMed: 19166877]
46. Kawasaki Y, Zhang L, Cheng JK, et al. Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. J Neurosci. 2008; 28:5189–5194. [PubMed: 18480275]
47. Xu ZZ, Zhang L, Liu T, et al. Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nat Med. 2010; 16:592–597. 1p following 7. [PubMed: 20383154]
48. Watkins LR, Hutchinson MR, Johnston IN, et al. Glia: novel counter-regulators of opioid analgesia. Trends Neurosci. 2005; 28:661–669. [PubMed: 16246435]
49. Watkins LR, Milligan ED, Maier SF. Glial activation: a driving force for pathological pain. Trends Neurosci. 2001; 24:450–455. [PubMed: 11476884]
50. Raghavendra V, Rutkowski MD, DeLeo JA. The role of spinal neuroimmune activation in morphine tolerance/hyperalgesia in neuropathic and sham-operated rats. J Neurosci. 2002; 22:9980–9989. [PubMed: 12427855]
51. Zhang L, Berta T, Xu ZZ, et al. TNF-alpha contributes to spinal cord synaptic plasticity and inflammatory pain: distinct role of TNF receptor subtypes 1 and 2. Pain. 2011; 152:419–427. [PubMed: 21159431]
52. Ren K, Dubner R. Interactions between the immune and nervous systems in pain. Nat Med. 2010; 16:1267–1276. [PubMed: 20948535]
53. Samad TA, Moore KA, Sapirstein A, et al. Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature. 2001; 410:471–475. [PubMed: 11260714]

54. Gustafson-Vickers SL, Lu VB, Lai AY, et al. Long-term actions of interleukin-1beta on delay and tonic firing neurons in rat superficial dorsal horn and their relevance to central sensitization. Mol Pain. 2008; 4:63. [PubMed: 19091115]

55. Zhang H, Nei H, Dougherty PM. A p38 mitogen-activated protein kinase-dependent mechanism of disinhibition in spinal synaptic transmission induced by tumor necrosis factor-alpha. J Neurosci. 2010; 30:12844–12855. [PubMed: 20861388]

56. Gao YJ, Zhang L, Samad OA, et al. JNK-induced MCP-1 production in spinal cord astrocytes contributes to central sensitization and neuropathic pain. J Neurosci. 2009; 29:4096–4108. [PubMed: 19339605]

57. Sung CS, Wen ZH, Chang WK, et al. Inhibition of p38 mitogen-activated protein kinase attenuates interleukin-1beta-induced thermal hyperalgesia and inducible nitric oxide synthase expression in the spinal cord. J Neurochem. 2005; 94:742–752. [PubMed: 16033422]

58. Lewis SS, Hutchinson MR, Rezvani N, et al. Evidence that intrathecal morphine-3-glucuronide may cause pain enhancement via toll-like receptor 4/MD-2 and interleukin-1beta. Neuroscience. 2010; 165:569–583. [PubMed: 19833175]

59. Gao YJ, Ji RR. Targeting astrocyte signaling for chronic pain. Neurotherapeutics. 2010; 7:482–493. [PubMed: 20880510]

60. Ji RR, Kawasaki Y, Zhuang ZY, et al. Possible role of spinal astrocytes in maintaining chronic pain sensitization: review of current evidence with focus on bFGF/JNK pathway. Neuron Glia Biol. 2006; 2:259–269. [PubMed: 17710215]

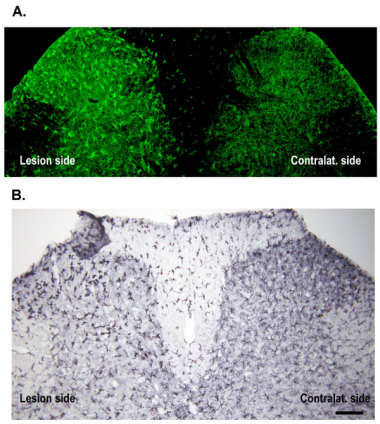

Figure 1.
Microglial reaction in the spinal dorsal horn of rats after nerve injury and paw incision. (A) OX-42 immunoflurorescence (dark field) in the dorsal horn one day after spinal nerve ligation. The lesion side shows marked microglial activation in comparison with the contralateral side. (B) Immunohistochemical staining (bright field) of Iba-1 in the dorsal horn one day after plantar incision. The reactive microglia in the injured side display a dense and amoeboid appearance in contrast to the ramified morphology of microglia in the contralateral side. Scar bar: 100 μm.

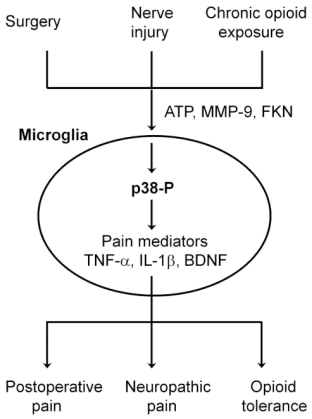

Figure 2.

Schematic illustration of microglia-evoked pain. Nerve injury, surgical procedures, and chronic opioid exposure result in activation of microglial cells in the spinal cord. This activation could be initiated by the release of ATP, matrix metalloprotease-9 (MMP-9), and the chemokine fractalkine (FKN/CX3CL1), leading to the phosphorylation of p38 MAPK in microglia. Activation of p38 induces the synthesis and release of several pain mediators including the proinflammatory cytokines (IL-1β, IL-6, and TNF-α) and neurotrophin (BDNF) from microglia. These glia-produced pain mediators can initiate and maintain postoperative pain, neuropathic pain, and antinociceptive tolerance of opioids, via inducing hyperexcitability of nociceptive neurons in the spinal cord dorsal horn.