

Review

Open Access

p38 MAPK, microglial signaling, and neuropathic pain

Ru-Rong Ji* and Marc R Suter

Address: Pain Research Center, Department of Anesthesiology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA

Email: Ru-Rong Ji* - rrji@zeus.bwh.harvard.edu; Marc R Suter - msuter1@partners.org

* Corresponding author

Published: 1 November 2007

Received: 24 August 2007

Molecular Pain 2007, **3**:33 doi:10.1186/1744-8069-3-33

Accepted: 1 November 2007

This article is available from: <http://www.molecularpain.com/content/3/1/33>

© 2007 Ji and Suter; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Accumulating evidence over last several years indicates an important role of microglial cells in the pathogenesis of neuropathic pain. Signal transduction in microglia under chronic pain states has begun to be revealed. We will review the evidence that p38 MAPK is activated in spinal microglia after nerve injury and contributes importantly to neuropathic pain development and maintenance. We will discuss the upstream mechanisms causing p38 activation in spinal microglia after nerve injury. We will also discuss the downstream mechanisms by which p38 produces inflammatory mediators. Taken together, current data suggest that p38 plays a critical role in microglial signaling under neuropathic pain conditions and represents a valuable therapeutic target for neuropathic pain management.

Background

Injuries of the nervous system, including peripheral nervous system (PNS, e.g. peripheral nerves, dorsal root ganglia, and dorsal roots) and central nervous system (CNS, e.g. spinal cord and thalamus), often result in neuropathic pain. These injuries may result from diabetic neuropathy, viral infection, major surgeries (e.g. amputation, thoracotomy), spinal cord injury, and stroke [1-3]. Spontaneous pain, described as shooting, lancinating or burning pain, and mechanical allodynia (painful responses to normally innocuous tactile stimuli) are distinct symptoms of neuropathic pain, although neuropathic pain is also characterized by heat hyperalgesia, mechanical hyperalgesia, and cold allodynia. Neuropathic pain is a consequence of neural plasticity, developed both in the PNS (peripheral sensitization) and CNS (central sensitization). After nerve injury, neuropathic pain can arise from injury discharge at

the site of axonal injury and ectopic/spontaneous activity in dorsal root ganglion (DRG) neurons [4-6]. Inflammatory mediators (e.g. cytokines) play a critical role in the generation of spontaneous activity and neuropathic pain. Peripheral nerve injury also induces marked phenotypic changes in DRG neurons [1,2]. While spontaneous activity from primary afferents drives central sensitization, central sensitization is responsible for persistent neuropathic pain. Central sensitization may also directly drive neuropathic pain in central neuropathic pain conditions caused by spinal cord injury or stroke. Central sensitization is induced by enhanced synaptic strength in the spinal cord and brain regions, due to an increase in excitatory synaptic transmission (e.g. AMPA and NMDA currents) or/and a reduction in inhibitory synaptic transmission (e.g. GABA currents) [7-9]. In addition to increased primary afferent input, enhanced descending facilitation also contributes

to spinal neuron hypersensitivity and neuropathic pain [10,11].

Despite our intensive investigation on neuronal mechanisms of neuropathic pain, current treatment has only resulted in limited success. Most analgesics are designed to block neurotransmission, but pain rapidly comes back after drug effects wear off. This is because many "inflammatory mediators (IFMs)" are still produced to activate nociceptive neurons in the PNS and CNS, causing pain hypersensitivity. These IFMs include proinflammatory cytokines [interleukin-1beta (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF α)], prostaglandin E₂ (PGE₂), nitric oxide, nerve growth factor, etc. Unlike neurotransmitters, these IFMs are mainly produced by non-neuronal cells. The IFMs are produced not only at the site of nerve injury by Schwann cells, keratinocytes and immune cells, but also by glial cells in the spinal cord. Microglia are regarded as a main source of IFMs in the CNS [12,13]. Although brain-derived neurotrophic factor (BDNF) is not regarded as an IFM, it is produced by microglia and plays important role in neuropathic pain development [14]. Thus our list of IFMs also includes BDNF. Since IFMs produced by spinal microglia are crucial to the development of central sensitization and neuropathic pain (see below), it is extremely important to know how these IFMs are produced in microglia. We will summarize the data showing that p38 MAPK is a key regulator of IFM synthesis and release in microglia and also an essential contributor to neuropathic pain sensitization.

Microglia and neuropathic pain

Although glial cells were originally regarded as supporting cells in the CNS, mounting evidence indicates that glia actively communicate with neurons and contribute importantly to the development of different types of neurodegenerative diseases. Increasing evidence also suggests that glial cells in the spinal cord play an important role in pain facilitation [13,15-17]. For example, peripheral nerve injury produces profound changes in glial cells including morphological changes of microglia and astrocytes and increased expression of glial markers, such as CD11b, Iba-1 and GFAP [18]. Glia inhibitors or glia modifying drugs such as fluorocitrate and propentofylline can alter pain sensitivity [19-21]. While these early studies are important to demonstrate an overall role of glia in regulating pain sensitivity, they did not distinguish which type of glial cells is important in pain regulation.

Among three types of glial cells in the CNS, although oligodendrocytes and astrocytes are found in close apposition to neurons, microglia have gained more attention, in part because nerve injury-induced microglial changes are much more robust than that of oligodendrocytes and astrocytes. A recent microarray study shows that the most

regulated genes following nerve injury are expressed in spinal microglia [22]. Nerve injury also induces a profound proliferation of spinal microglia [23]. Current studies on microglial regulation of pain are limited by the lack of specific markers for microglial activation. CD11b and Iba-1 are two of the most used markers for demonstrating microglial activation. However, whether CD11b and Iba-1 contribute to pain hypersensitivity remains unclear. Fortunately, nerve injury up regulates the ATP receptor P2X4 and the chemokine receptor CX3CR1 specifically in spinal microglia, and blocking these receptors results in decreased neuropathic pain [24-26]. The chemokine receptor CCR2 and Toll-like receptor-4 are also expressed in spinal microglia and contribute to neuropathic pain sensitization [27,28]. Different types of nerve injuries all induce complement cascade in spinal microglia, and mice null for C5 terminal complement component exhibit reduced neuropathic pain sensitivity [22]. Further, minocycline, a microglial inhibitor was shown to prevent or delay neuropathic pain [29,30]. In particular, intrathecal injection of ATP-activated microglia induces mechanical allodynia, indicating that microglial activation is sufficient to induce pain sensitization [24]. Activation of P2X4 receptor is likely to mediate neuropathic pain by producing BDNF in microglia [14].

Activation of p38 MAPK in spinal microglia and neuropathic pain

The mitogen-activated protein kinases (MAPKs) are a family of evolutionally conserved molecules that play a critical role in cell signaling and gene expression. MAPK family includes three major members: extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), representing three different signaling cascades. MAPKs are activated by phosphorylation and transduce a broad range of extracellular stimuli into diverse intracellular responses by both transcriptional and non-transcriptional regulation. p38 MAPK, which is activated by upstream kinase MKK3/MMK6, is regarded as a stress-induced kinase and plays a critical role in inflammatory responses. p38 inhibitor was shown to effectively alleviate rheumatoid arthritis and inflammatory pain [31-33].

We used a phosphorylated p38 antibody to examine p38 activation in the rat spinal cord after spinal nerve ligation (SNL), a commonly used neuropathic pain model [34]. While phospho-p38 (p-p38) levels are low in non-injured spinal cord, SNL induces a substantial increase in p-p38 levels in the injured side of the spinal cord (Fig 1a). The increase in p38 phosphorylation is accompanied by an increase in p38 activity, as shown by elevated phosphorylation of ATF-2, a substrate of p38 (Fig 1b). Unexpectedly, p38 is activated neither in NeuN-expressing neurons nor in GFAP-expressing astrocytes after SNL. Instead, p38 is activated in spinal cells labeled with microglial marker

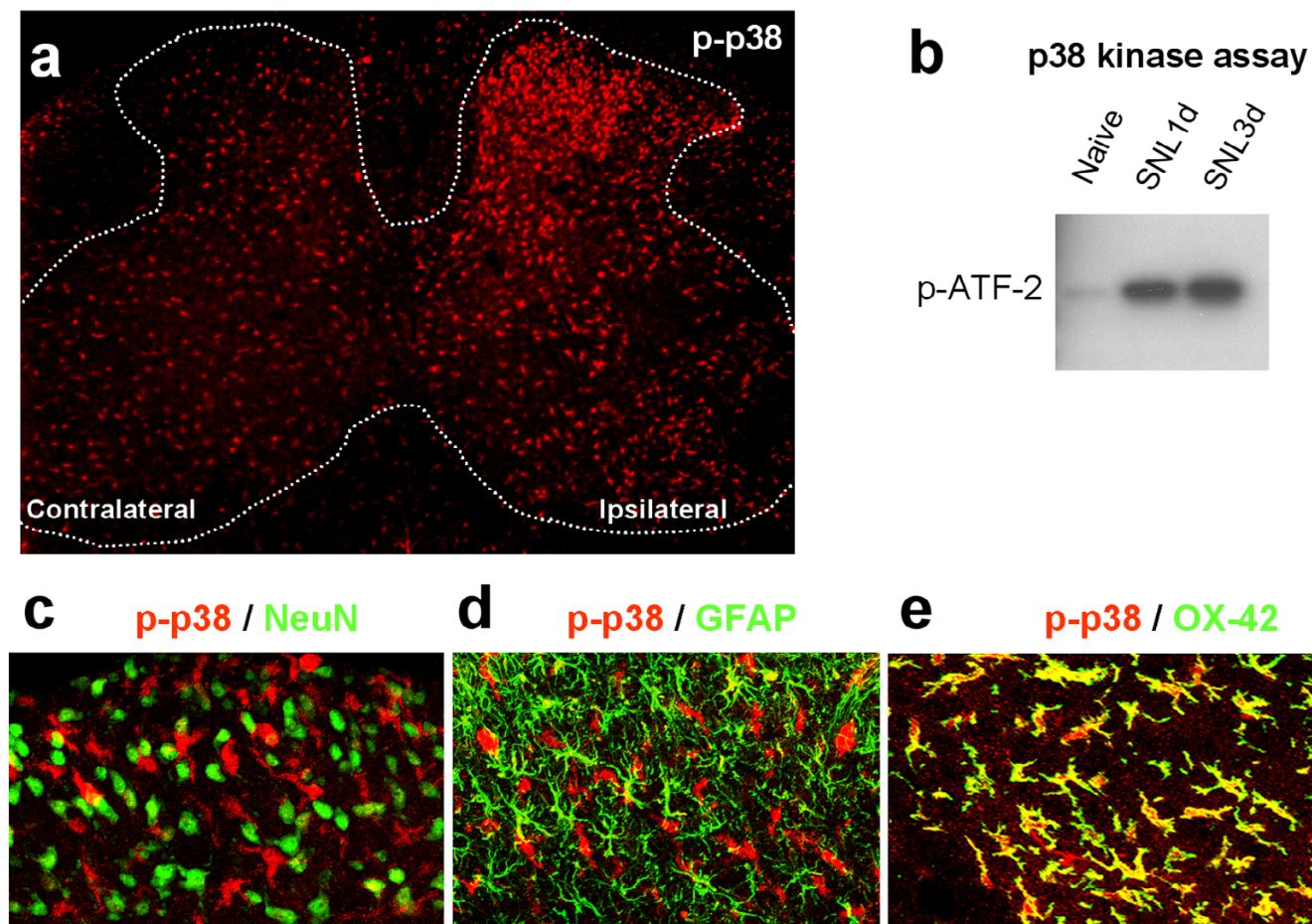


Figure 1

Ligation of L5-spinal nerve (SNL) induces p38 activation in microglial cells in the spinal cord. (a) Immunostaining of phosphorylated p38 (p-p38) in the spinal cord 3 days after SNL. Dotted line indicates the outline of the spinal cord gray matter. Modified from [34]. (b) p38 kinase assay reveals that SNL increases the phosphorylation of the p38 substrate ATF-2, suggesting an increase in p38 activity. To perform kinase assay, p-p38 was first immunoprecipitated with a p-p38 antibody, then ATF-2 fusion protein was added. Finally, pATF-2 level was detected using Western blotting with a pATF-2 antibody. (c-e) Double immunofluorescence indicates that p-p38 is not colocalized with NeuN, a neuronal marker (c) and GFAP, an astroglial marker (d), but colocalized with OX-42 (CD11b), a microglial marker (e). Modified from [34].

CD11b (recognized by OX-42 antibody, Fig 1c-e) [34]. p38 activation in spinal microglia was also reported in the SNL model by other investigators [35-37], as well as in the spared nerve injury model [37] taken away [38], after ventral root lesion [39] and spinal cord injury [40]. Apart from neuropathic pain conditions, microglial activation of p38 has also been shown in other pain conditions [41,42].

We have also examined p38 activation at different times following nerve injury. After SNL, p38 activation in spinal microglia begins on day one and peaks on day 3. It is note-

worthy that the increase in p-p38 levels is maintained in spinal microglia even after 3 weeks, despite the fact that at this late time point p-p38 levels decline from the peak levels [43]. Although most studies confirm a specific microglial activation of p38 in the spinal cord, we do not exclude the possibility that moderate p38 activation may also be observed in other spinal cell types when different species or treatment conditions are tested.

p38 inhibition and neuropathic pain

Mounting evidence indicates that p38 activation plays an important role in the development of neuropathic pain.

Daily administration of the p38 inhibitor SB203580 intrathecally (i.t.) prevents SNL-induced mechanical allodynia [34,35]. p38 inhibitors FR167653 or CNI-1493 (i.t.) also prevents the development neuropathic pain symptoms in the spared nerve injury model [38] and in a sciatic inflammatory neuropathy model [44].

Can p38 inhibitor or microglial inhibitor also reverse established neuropathic pain? This is an extremely important question regarding the role of p38 and microglia, and potential clinical treatment of neuropathic pain with p38 or microglia inhibitors. Early studies showed that SB203580 cannot reverse neuropathic pain symptoms when given after nerve injury ref36 taken away [45]. Similarly, a microglial inhibitor minocycline was shown to prevent/delay neuropathic pain, but not to reverse established neuropathic pain [29,30]. Minocycline also inhibits spinal p38 activation in a neuropathic pain condition [40]. These data suggest that p38 and microglia are only important for the induction or development of neuropathic pain, and p38 or microglia inhibitor can only be used to treat neuropathic pain at early stages, which would greatly limit the therapeutic potential of p38 and microglial inhibitors. However, further studies suggest that inhibition of p38 is also able to reverse neuropathic

pain symptoms in different animal models [34,38,40,44,46]. Our data show that a single injection of the p38 inhibitor FR167653 (50 mg/kg, i.p.), 10 days after SNL, reverses mechanical allodynia for more than 6 hours, with a complete reversal at 3 hours. Further, multiple injections (once every day) of FR167653 persistently attenuate mechanical allodynia for several days, and pain attenuation is maintained for additional 2 days after the last injection (Fig 2a), supporting a role of p38 in the maintenance of neuropathic pain. Blockade of P2X4 signaling in microglia at 2 weeks after nerve injury can also reverse neuropathic pain [25]. Recently, a glial modifying drug propentofylline, given 6 weeks after nerve injury, was shown to reverse neuropathic pain by inhibiting microglial responses [47].

Nevertheless, the peak activation of p38 and microglia in the spinal cord occurs in the first several days of nerve injury, varying from 3 days to 2 weeks due to different injury conditions [43,48]. After its peak, p38 and microglial activation starts to decrease significantly, although still maintained at elevated levels at 3 weeks. In contrast, the peak activation of astrocytes in the spinal cord persists for a longer period [48]. Therefore, microglia are probably more important for the early development of neuropathic

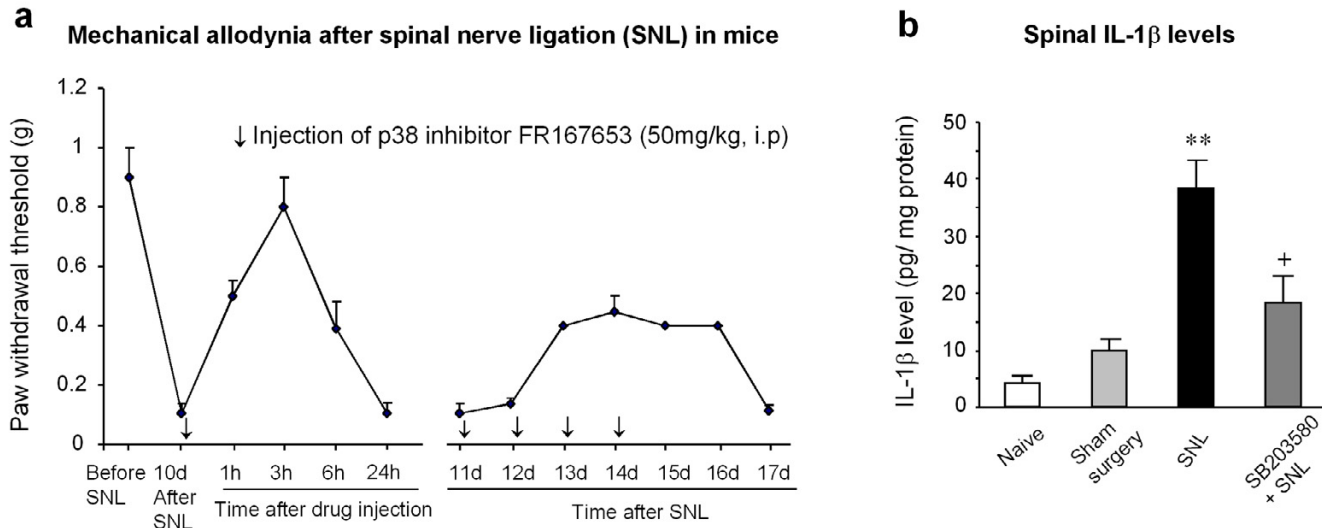


Figure 2

(a) Reversal of tactile allodynia by p38 inhibition after spinal nerve ligation (SNL) on mice. The p38 inhibitor FR167653 was injected (i.p., 50 mg/kg, in 10% DMSO) at 10 days after SNL. The same injection was repeated daily for additional 4 days (indicated with small arrows). SNL-induced mechanical allodynia was tested at 1 h, 3 h, 6 h, and 1 d after the first injection, then daily for another 6 days. Mean \pm SEM (n = 5). Note that p38 inhibitor is still effective in reversing neuropathic pain. Interestingly, the effect is maintained for 2 more days after the last injection. **(b) Inhibition of SNL-induced IL-1 β upregulation in the spinal cord by p38 inhibition.** ELISA shows that intrathecal injection of the p38 inhibitor SB203580 (10 μ g, twice a day for 3 days) reduces SNL-induced IL-1 β increase in the L5 spinal cord (dorsal part) 3 days after SNL (L5). **, P < 0.01, compared to Sham; +, P < 0.05, compared to SNL, ANOVA.

pain, although they are also involved in the maintenance of neuropathic pain.

p38 isoforms

There are four different p38 isoforms: α , β , γ and δ . α and β are two of the major isoforms in the mature nervous system [31] and their activated forms are recognized by commercial p-p38 antibodies. While p38 α is the most abundant isoform and expressed in DRG neurons (unpublished observation), p38 β appears to be expressed in spinal microglia [49]. Current p38 inhibitors are not isoform-specific due to their design to target common-ATP binding sites on the kinases and structural similarities between the p38 isoforms. To explore the distinct role of p38 isoforms in the development of neuropathic pain, it is important to specifically inhibit a particular isoform. More importantly, isoform-specific inhibition will minimize the side effects produced by blocking all p38 isoforms with a general inhibitor. Interestingly, it has been shown that knockdown of p38 β but not p38 α with antisense oligonucleotides prevents acute pain sensitization [49]. However, the respective role of p38 α and p38 β in chronic pain conditions has not been examined. Also, therapeutic enthusiasm for antisense oligonucleotides is not high due to their low efficacy and specificity. A new strategy, such as RNA interference, may be helpful to develop potent and selective knock down of a specific isoform of p38.

How does peripheral nerve injury activate p38 in spinal microglia?

Several studies began to explore the upstream mechanisms causing p38 activation in spinal microglia [26,36,50,51]. TNF α inhibitor prevents neuropathic pain development by inhibiting spinal p38 activation [36]. Spinal p38 is activated by intrathecal infusion of IL-1 β and substance P, and p38 inhibitor abolishes the hyperalgesia produced by IL-1 β and substance P [50,51]. In particular, recent studies have demonstrated that chemokines play important roles in mediating neural-glia interaction and neuropathic pain sensitization. Nerve injury induces an up-regulation of the chemokine MCP-1 in primary sensory neurons [48,52]. Interestingly, CCR2, one type of receptors for MCP-1, was expressed in spinal microglia; spinal p38 activation after nerve injury is reduced in CCR-2 null mice [27].

Unlike other chemokines that have multiple receptors, fractalkine (FKN), also called CX3CL1, only has one known receptor, CX3CR1. It is of great interest that the CX3CR1 receptor is expressed in spinal microglia and further upregulated after nerve injury [25,26]. On the other hand, FKN is primarily produced in DRG and dorsal horn neurons [25]. FKN has 2 forms, a membrane-bound form (large size) and a soluble form (small size) that mediate

cell adhesion and chemotaxis, respectively. FKN is normally membrane-bound, but this membrane-bound FKN in the DRG is cleaved after nerve injury, leading to the release of FKN [26]. A recent study shows that cysteine protease cathepsin S (CatS), which is produced in microglia after nerve injury, can enhance neuropathic pain by cleavage of FKN on the membrane of DRG neurons. CatS-induced hyperalgesia is lost in CX3CR1 null mice [53]. FKN can also be cleaved by metalloproteinases (MMPs), such as MMP-9 that is released from DRG neurons after nerve injury (Xu and Ji, unpublished observation). Intrathecal infusion of FKN not only induces mechanical allodynia but also activates p38 in spinal microglia [26]. Perfusion of spinal cord slices with FKN activates p38 in microglia [26]. Conversely, a neutralizing antibody against CX3CR1 reduces both p38 activation and neuropathic pain [26]. Thus, FKN-CX3CR1-p38 cascade is required for the development of neuropathic pain.

After peripheral nerve injury, spontaneous activity (ectopic discharge), which is crucial for the genesis of neuropathic pain, can be recorded in axons as well as in DRG soma. Is this spontaneous activity required for p38 activation in spinal microglia? To produce long-term blockade of nerve activity, we applied bupivacaine microspheres to the sciatic nerve, which produces a persistent (> 1 week) conduction blockade due to slow release of bupivacaine [38]. Using this method for long-term blockade, we found that spontaneous activity is fully required to initiate p38 activation in spinal microglia. However, nerve conduction blockade does not reverse p38 activation when applied after nerve injury [38]. It is suggested that after initiation, microglia activation could be self-maintained given that multiple mediators released by microglia can also feed back to microglia via an autocrine or paracrine fashion. Since FKN released from neurons triggers microglial activation, it remains to be tested whether spontaneous activity after nerve injury can induce the cleavage of FKN.

p38 and microglial signaling

How does p38 activation in spinal microglia result in pain hypersensitivity? As mentioned above, activated microglia produce numerous IFMs (e.g., IL-1 β , IL-6, TNF α , PGE2, NO, BDNF), which are also produced in the spinal cord after nerve injury. All of these IFMs are implicated in pain facilitation [13-17,44,54,55].

p38 is known to regulate the synthesis of numerous IFMs via transcriptional regulation [31,32]. For example, SNL-induced IL-1 β up-regulation is attenuated by spinal inhibition of p38 (Fig 2b). Especially, p38 has been shown to activate the transcription factor NF- κ B in cultured microglia [56,57], leading to the expression of the IL-1 β , IL-6, and COX-2 [58]. Interestingly, NF- κ B is upregulated in spinal microglia after nerve injury (unpublished observa-

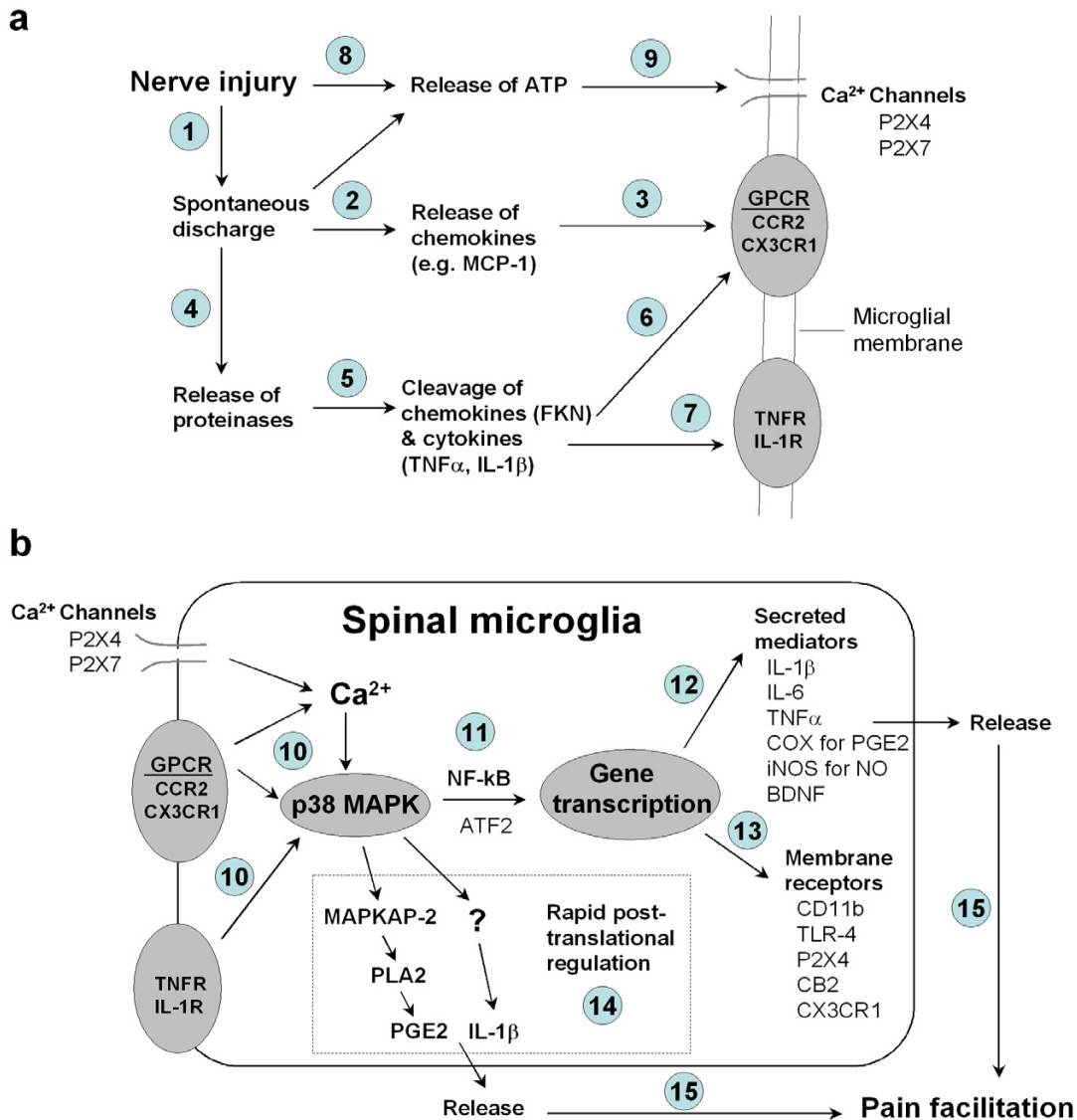


Figure 3

Schematic representation of signal transduction in spinal microglia after nerve injury. (a) Activation of microglial receptors after nerve injury. Peripheral nerve injury generates spontaneous activity (step-1) leading to the release of chemokines (e.g. MCP-1) from primary sensory DRG neurons (step-2). MCP-1 will activate CCR2 receptors on microglia (step-3). Spontaneous activity may also release the proteinases, leading to the cleavage of the chemokine FKN (step-5). After its cleavage from the membrane, FKN will be released to bind CX3CR1 receptor on microglia (step-6). Proteinases may also cleave the precursors of the cytokines TNF α and IL-1 β , leading to the activation of TNF α and IL-1 β receptors on microglia (step-7). Nerve injury will further release ATP (step-8), activating P2X4 and P2X7 receptors on microglia (step-9). **(b)** p38 activation in spinal microglia and downstream signaling of p38. Activation of GPCR, or cytokine receptors, or P2X receptors results in p38 MAPK activation in spinal microglia (step-10). p38 activation results in increased expression, through the transcription factor NF- κ B (step-11) or other transcription factors (e.g. ATF-2), of secreted inflammatory mediators/growth factors (e.g., cytokines and BDNF, step-12) or of genes encoding membrane receptors (step-13). In addition, p38 also induces release of PGE2 and IL-1 β via rapid posttranslational regulation (step-14). Upon release, these mediators will sensitize nociceptive dorsal horn neurons via presynaptic and postsynaptic mechanisms, leading to persistent pain hypersensitivity (step-15). Abbreviations used in Fig 3: BDNF, brain-derived neurotrophic factor; CatS, cysteine protease cathepsin S; DRG, dorsal root ganglion; FKN, fractalkine; GPCR, G-protein coupled receptor; IL-1 β , interleukin-1beta; MAPK, mitogen-activated protein kinase; MAPKAP2: MAPK-activated protein kinase-2; PLA2, phospholipase A2; TNF α , tumor necrosis factor-alpha

tion). Although it was traditionally believed that COX-1 is constitutively expressed while COX-2 is inducible, COX-1 was shown to be induced in spinal microglia after SNL [59] and COX-1 inhibitor can attenuate neuropathic pain [60]. It is worthy to investigate whether p38 is required for this upregulation. p38 activation after intrathecal IL-1 β is required for the synthesis of iNOS [51]. In addition to p38 activation in spinal microglia, we found that ERK, another MAPK family member, is also activated in spinal microglia at early times (first several days) of neuropathic pain development, which is required for neuropathic pain sensitization [61]. p38 and ERK appear to be activated in different populations of spinal microglia. It is likely that the ERK and p38 pathways can act together to regulate neuropathic pain. For example, a combination of MEK and p38 inhibitors has been shown to suppress endotoxin-induced expression of iNOS and TNF α in microglia with a much greater efficacy than each inhibitor alone [62].

p38 can also regulate the synthesis of IFMs by posttranslational regulation. For example, p38 activates phospholipase A2 (PLA2) via its downstream kinase MAPKAP-2 (MAPK activated protein kinase-2). PLA2 plays an important role in inflammatory responses. The activation of PLA2 results in the genesis of arachidonic acid for prostaglandin production, which can be catalyzed via COX to produce PGE2 [32]. Several PLA2 forms are expressed in spinal cord, and inhibiting spinal PLA2 induces a potent antihyperalgesia [63]. PGE2 release evoked by intrathecal dynorphin requires spinal p38 activation [64]. p38 activation also causes a rapid release of IL-1 β from spinal microglia. For example, following application of lipopolysaccharide to an ex vivo spinal slice preparation, there is a rapid activation of p38 in spinal microglia and a rapid secretion of IL-1 β . Further, IL-1 β secretion is prevented by p38 inhibitor [65].

Concluding remarks and future directions

In last several years there has been dramatically increasing interest in studying microglial regulation of neuropathic pain. We now know that many receptors such as P2X4, CX3CR1, and CCR2 are specifically expressed or up regulated in spinal microglia after nerve injury or spinal cord injury, which is essential for neuropathic pain development and maintenance. We also know that microglia enhance pain sensitivity by producing numerous inflammatory and pain mediators such as proinflammatory cytokines and BDNF. However, little is known as to how activation of these microglial receptors leads to the generation and release of inflammatory mediators. Many studies from different laboratories have shown that p38 MAPK is activated in spinal microglia and contributes to neuropathic pain development/maintenance. p38 stands in a central place in microglial signaling (Fig 3). First, activation of multiple microglial receptors can converge on p38 activation. Second, activation of p38 will lead to the syn-

thesis and release of multiple inflammatory mediators. Therefore, understanding p38 signaling in microglia will greatly improve our knowledge of microglia-mediated pain regulation.

Currently, most people use CD11b or IBA-1 as markers for microglial activation. Although these are specific markers and show profound changes after injuries, their roles in neuropathic pain have not been clearly addressed, due to the lack of specific tools to block the action of these markers. In several cases, neuropathic pain is dissociated with CD11b expression [24,66]. Thus additional markers are needed to demonstrate microglia activation. Phosphorylated MAPKs (e.g. p-p38 or pERK) can be used as extra markers to reflect the activity of microglia after nerve injury, because phosphorylation is very rapid and sensitive in response to changes in cellular environment.

Can p38 inhibitors be used to treat clinical pain? Many pharmaceutical companies have been trying to develop specific p38 inhibitors. Several p38 MAPK inhibitors including SB281838, BIRB0796, Ro320-1195, and SCIO-469 have been tested in clinical trials for inflammatory diseases such as rheumatoid arthritis as well as for cancer [67,68]. Given that p38 is a critical contributor in several chronic pain conditions, p38 inhibitors should also be tested for clinical pain. A major concern is p38 inhibitors may produce psychiatric and cardiovascular side effects or liver toxicity. Development of more specific p38 inhibitors, such as activation status-specific inhibitors and isoform-specific inhibitors is highly demanded. Local delivery of p38 inhibitors or siRNA strategy may also be considered.

Competing interests

The author(s) declare that they have no competing interests.

Acknowledgements

The work was supported in part by NIH grants NS40698, DE17794, and TW7180, and by the Swiss National Science Foundation PBLAB118504.

References

1. Ji RR, Strichartz G: **Cell signaling and the genesis of neuropathic pain.** *Sci STKE* 2004, **2004**:reE14.
2. Kehlet H, Jensen TS, Woolf CJ: **Persistent postsurgical pain: risk factors and prevention.** *Lancet* 2006, **367**:1618-1625.
3. Woolf CJ, Mannion RJ: **Neuropathic pain: aetiology, symptoms, mechanisms, and management.** *Lancet* 1999, **353**:1959-1964.
4. Devor M, Seltzer Z: **Pathophysiology of damaged nerves in relation to chronic pain.** In *Textbook of Pain Volume 5*. 4th edition. Edited by: Wall PD and Melzack R. Edinburgh, Churchill Livingstone; 1999:129-164.
5. Ma C, Shu Y, Zheng Z, Chen Y, Yao H, Greenquist KW, White FA, LaMotte RH: **Similar electrophysiological changes in axotomized and neighboring intact dorsal root ganglion neurons.** *J Neurophysiol* 2003, **89**:1588-1602.
6. 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.
7. Woolf CJ, Salter MW: **Neuronal plasticity: increasing the gain in pain.** *Science* 2000, **288**:1765-1769.
 8. Ji RR, Kohno T, Moore KA, Woolf CJ: **Central sensitization and LTP: do pain and memory share similar mechanisms?** *Trends Neurosci* 2003, **26**:696-705.
 9. Coull JA, Boudreau D, Bachand K, Prescott SA, Nault F, Sik A, De Koninck P, De Koninck Y: **Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain.** *Nature* 2003, **424**:938-942.
 10. Porreca F, Ossipov MH, Gebhart GF: **Chronic pain and medullary descending facilitation.** *Trends Neurosci* 2002, **25**:319-325.
 11. Zhuo M: **Neuronal mechanism for neuropathic pain.** *Mol Pain* 2007, **3**:14.
 12. Hanisch UK: **Microglia as a source and target of cytokines.** *Glia* 2002, **40**:140-155.
 13. Tsuda M, Inoue K, Salter MW: **Neuropathic pain and spinal microglia: a big problem from molecules in "small" glia.** *Trends Neurosci* 2005, **28**:101-107.
 14. Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter MW, De Koninck Y: **BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain.** *Nature* 2005, **438**:1017-1021.
 15. Watkins LR, Milligan ED, Maier SF: **Glial activation: a driving force for pathological pain.** *Trends Neurosci* 2001, **24**:450-455.
 16. DeLeo JA, Yezierski RP: **The role of neuroinflammation and neuroimmune activation in persistent pain.** *Pain* 2001, **90**:1-6.
 17. Marchand F, Perretti M, McMahon SB: **Role of the immune system in chronic pain.** *Nat Rev Neurosci* 2005, **6**:521-532.
 18. Garrison CJ, Dougherty PM, Kajander KC, Carlton SM: **Staining of glial fibrillary acidic protein (GFAP) in lumbar spinal cord increases following a sciatic nerve constriction injury.** *Brain Res* 1991, **565**:1-7.
 19. Watkins LR, Martin D, Ulrich P, Tracey KJ, Maier SF: **Evidence for the involvement of spinal cord glia in subcutaneous formalin induced hyperalgesia in the rat.** *Pain* 1997, **71**:225-235.
 20. Meller ST, Dykstra C, Grzybycki D, Murphy S, Gebhart GF: **The possible role of glia in nociceptive processing and hyperalgesia in the spinal cord of the rat.** *Neuropharmacology* 1994, **33**:1471-1478.
 21. Sweitzer SM, Schubert P, DeLeo JA: **Propentofylline, a glial modulating agent, exhibits antiallodynic properties in a rat model of neuropathic pain.** *J Pharmacol Exp Ther* 2001, **297**:1210-1217.
 22. Griffin RS, Costigan M, Brenner GJ, Ma CH, Scholz J, Moss A, Allchorne AJ, Stahl GL, Woolf CJ: **Complement induction in spinal cord microglia results in anaphylatoxin C5a-mediated pain hypersensitivity.** *J Neurosci* 2007, **27**:8699-8708.
 23. 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* 2007.
 24. 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.
 25. Verge GM, Milligan ED, Maier SF, Watkins LR, Naeve GS, Foster AC: **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.
 26. Zhuang ZY, Kawasaki Y, Tan PH, Wen YR, Huang J, Ji RR: **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.
 27. Abbadie C, Lindia JA, Cumiskey AM, Peterson LB, Mudgett JS, Bayne EK, DeMartino JA, MacIntyre DE, Forrest MJ: **Impaired neuropathic pain responses in mice lacking the chemokine receptor CCR2.** *Proc Natl Acad Sci U S A* 2003, **100**:7947-7952.
 28. 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.
 29. 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.
 30. Ledebner A, Sloane EM, Milligan ED, Frank MG, Mahony JH, Maier SF, Watkins LR: **Minocycline attenuates mechanical allodynia and proinflammatory cytokine expression in rat models of pain facilitation.** *Pain* 2005, **115**:71-83.
 31. Kumar S, Boehm J, Lee JC: **p38 MAP kinases: key signalling molecules as therapeutic targets for inflammatory diseases.** *Nat Rev Drug Discov* 2003, **2**:717-726.
 32. Ji RR, Woolf CJ: **Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain.** *Neurobiol Dis* 2001, **8**:1-10.
 33. Ji RR, Samad TA, Jin SX, Schmolli R, Woolf CJ: **p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia.** *Neuron* 2002, **36**:57-68.
 34. Jin SX, Zhuang ZY, Woolf CJ, Ji RR: **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.
 35. Tsuda M, Mizokoshi A, Shigemoto-Mogami Y, Koizumi S, Inoue K: **Activation of p38 mitogen-activated protein kinase in spinal hyperactive microglia contributes to pain hypersensitivity following peripheral nerve injury.** *Glia* 2004, **45**:89-95.
 36. Svensson CI, Schafers M, Jones TL, Powell H, Sorkin LS: **Spinal blockade of TNF blocks spinal nerve ligation-induced increases in spinal P-p38.** *Neurosci Lett* 2005, **379**:209-213.
 37. Katsura H, Obata K, Mizushima T, Sakurai J, Kobayashi K, Yamanaka H, Dai Y, Fukuoka T, Sakagami M, Noguchi K: **Activation of Src-family kinases in spinal microglia contributes to mechanical hypersensitivity after nerve injury.** *J Neurosci* 2006, **26**:8680-8690.
 38. Wen YR, Suter MR, Kawasaki Y, Huang J, Pertin M, Kohno T, Berde CB, Decosterd I, Ji RR: **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.
 39. Xu JT, Xin WJ, Wei XH, Wu CY, Ge YX, Liu YL, Zang Y, Zhang T, Li YY, Liu XG: **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.
 40. Hains BC, Waxman SG: **Activated microglia contribute to the maintenance of chronic pain after spinal cord injury.** *J Neurosci* 2006, **26**:4308-4317.
 41. Boyle DL, Jones TL, Hammaker D, Svensson CI, Rosengren S, Albani S, Sorkin L, Firestein GS: **Regulation of peripheral inflammation by spinal p38 MAP kinase in rats.** *PLoS Med* 2006, **3**:e338.
 42. Cui Y, Chen Y, Zhi JL, Guo RX, Feng JQ, Chen PX: **Activation of p38 mitogen-activated protein kinase in spinal microglia mediates morphine antinociceptive tolerance.** *Brain Res* 2006, **1069**:235-243.
 43. Zhuang ZY, Wen YR, Zhang DR, Borsello T, Bonny C, Strichartz GR, Decosterd I, Ji RR: **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.
 44. Milligan ED, Twining C, Chacur M, Biedenkapp J, O'Connor K, Poole S, Tracey K, Martin D, Maier SF, Watkins LR: **Spinal glia and proinflammatory cytokines mediate mirror-image neuropathic pain in rats.** *J Neurosci* 2003, **23**:1026-1040.
 45. Schafers M, Svensson CI, Sommer C, Sorkin LS: **Tumor necrosis factor-alpha induces mechanical allodynia after spinal nerve ligation by activation of p38 MAPK in primary sensory neurons.** *J Neurosci* 2003, **23**:2517-2521.
 46. Sweitzer SM, Medicherla S, Almiraz R, Dugar S, Chakravarty S, Shumilla JA, Yeomans DC, Protter AA: **Antinociceptive action of a p38alpha MAPK inhibitor, SD-282, in a diabetic neuropathy model.** *Pain* 2004, **109**:409-419.
 47. Tawfik VL, Nutile-McMenemy N, Lacroix-Fralish ML, DeLeo JA: **Efficacy of propentofylline, a glial modulating agent, on existing mechanical allodynia following peripheral nerve injury.** *Brain Behav Immun* 2007, **21**:238-246.
 48. Zhang J, De Koninck Y: **Spatial and temporal relationship between monocyte chemoattractant protein-1 expression**

- and spinal glial activation following peripheral nerve injury. *J Neurochem* 2006, **97**:772-783.
49. Svensson CI, Fitzsimmons B, Azizi S, Powell HC, Hua XY, Yaksh TL: **Spinal p38beta isoform mediates tissue injury-induced hyperalgesia and spinal sensitization.** *J Neurochem* 2005, **92**:1508-1520.
 50. Svensson CI, Marsala M, Westerlund A, Calcutt NA, Campana WM, Freshwater JD, Catalano R, Feng Y, Protter AA, Scott B, Yaksh TL: **Activation of p38 mitogen-activated protein kinase in spinal microglia is a critical link in inflammation-induced spinal pain processing.** *J Neurochem* 2003, **86**:1534-1544.
 51. Sung CS, Wen ZH, Chang WK, Chan KH, Ho ST, Tsai SK, Chang YC, Wong CS: **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.
 52. White FA, Sun J, Waters SM, Ma C, Ren D, Ripsch M, Steflik J, Cortright DN, LaMotte RH, Miller RJ: **Excitatory monocyte chemoattractant protein-1 signaling is up-regulated in sensory neurons after chronic compression of the dorsal root ganglion.** *Proc Natl Acad Sci U S A* 2005, **102**:14092-14097.
 53. Clark AK, Yip PK, Grist J, Gentry C, Staniland AA, Marchand F, Dehvari M, Wotherspoon G, Winter J, Ullah J, Bevan S, Malcangio M: **Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain.** *Proc Natl Acad Sci U S A* 2007.
 54. Winkelstein BA, Rutkowski MD, Sweitzer SM, Pahl JL, DeLeo JA: **Nerve injury proximal or distal to the DRG induces similar spinal glial activation and selective cytokine expression but differential behavioral responses to pharmacologic treatment.** *J Comp Neurol* 2001, **439**:127-139.
 55. Sweitzer S, Martin D, DeLeo JA: **Intrathecal interleukin-1 receptor antagonist in combination with soluble tumor necrosis factor receptor exhibits an anti-allodynic action in a rat model of neuropathic pain.** *Neuroscience* 2001, **103**:529-539.
 56. Jana M, Dasgupta S, Saha RN, Liu X, Pahan K: **Induction of tumor necrosis factor-alpha (TNF-alpha) by interleukin-12 p40 monomer and homodimer in microglia and macrophages.** *J Neurochem* 2003, **86**:519-528.
 57. Wilms H, Rosenstiel P, Sievers J, Deuschl G, Zecca L, Lucius R: **Activation of microglia by human neuromelanin is NF-kappaB dependent and involves p38 mitogen-activated protein kinase: implications for Parkinson's disease.** *FASEB J* 2003, **17**:500-502.
 58. Krakauer T: **Molecular therapeutic targets in inflammation: cyclooxygenase and NF-kappaB.** *Curr Drug Targets Inflamm Allergy* 2004, **3**:317-324.
 59. Zhu X, Eisenach JC: **Cyclooxygenase-1 in the spinal cord is altered after peripheral nerve injury.** *Anesthesiology* 2003, **99**:1175-1179.
 60. Hefferan MP, O'Rielly DD, Loomis CW: **Inhibition of spinal prostaglandin synthesis early after L5/L6 nerve ligation prevents the development of prostaglandin-dependent and prostaglandin-independent allodynia in the rat.** *Anesthesiology* 2003, **99**:1180-1188.
 61. 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.
 62. Bhat NR, Zhang P, Lee JC, Hogan EL: **Extracellular signal-regulated kinase and p38 subgroups of mitogen-activated protein kinases regulate inducible nitric oxide synthase and tumor necrosis factor-alpha gene expression in endotoxin-stimulated primary glial cultures.** *J Neurosci* 1998, **18**:1633-1641.
 63. Yaksh TL, Kokotos G, Svensson CI, Stephens D, Kokotos CG, Fitzsimmons B, Hadjipavlou-Litina D, Hua XY, Dennis EA: **Systemic and intrathecal effects of a novel series of phospholipase A2 inhibitors on hyperalgesia and spinal prostaglandin E2 release.** *J Pharmacol Exp Ther* 2006, **316**:466-475.
 64. Svensson CI, Hua XY, Powell HC, Lai J, Porreca F, Yaksh TL: **Prostaglandin E2 release evoked by intrathecal dynorphin is dependent on spinal p38 mitogen activated protein kinase.** *Neuropeptides* 2005, **39**:485-494.
 65. Clark AK, D'Aquisto F, Gentry C, Marchand F, McMahon SB, Malcangio M: **Rapid co-release of interleukin 1beta and caspase 1 in spinal cord inflammation.** *J Neurochem* 2006, **99**:868-880.
 66. 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.
 67. Ji RR, Kawasaki Y, Zhuang ZY, Wen YR, Zhang YQ: **Protein kinases as potential targets for the treatment of pathological pain.** *Handb Exp Pharmacol* 2007:359-389.
 68. Watkins LR, Maier SF: **Glia: a novel drug discovery target for clinical pain.** *Nat Rev Drug Discov* 2003, **2**:973-985.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

