



Published in final edited form as:

*Neuron Glia Biol.* 2006 November ; 2(4): 259–269. doi:10.1017/S1740925X07000403.

## Possible role of spinal astrocytes in maintaining chronic pain sensitization: review of current evidence with focus on bFGF/JNK pathway

RU-RONG JI<sup>1</sup>, YASUHIKO KAWASAKI<sup>1</sup>, ZHI-YE ZHUANG<sup>1</sup>, YEONG-RAY WEN<sup>1</sup>, and ISABELLE DECOSTERD<sup>2,3</sup>

<sup>1</sup> Pain Research Center, Dept of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, USA <sup>2</sup> Anesthesiology Pain Research Group, Anesthesiology Dept, Lausanne University Hospital, Lausanne, Switzerland <sup>3</sup> Dept of Cell Biology and Morphology, Faculty of Biology and Medicine, Lausanne University, Lausanne, Switzerland

### Abstract

Although pain is regarded traditionally as neuronally mediated, recent progress shows an important role of spinal glial cells in persistent pain sensitization. Mounting evidence has implicated spinal microglia in the development of chronic pain (e.g. neuropathic pain after peripheral nerve injury). Less is known about the role of astrocytes in pain regulation. However, astrocytes have very close contact with synapses and maintain homeostasis in the extracellular environment. In this review, we provide evidence to support a role of spinal astrocytes in maintaining chronic pain. In particular, c-Jun N-terminal kinase (JNK) is activated persistently in spinal astrocytes in a neuropathic pain condition produced by spinal nerve ligation. This activation is required for the maintenance of neuropathic pain because spinal infusion of JNK inhibitors can reverse mechanical allodynia, a major symptom of neuropathic pain. Further study reveals that JNK is activated strongly in astrocytes by basic fibroblast growth factor (bFGF), an astroglial activator. Intrathecal infusion of bFGF also produces persistent mechanical allodynia. After peripheral nerve injury, bFGF might be produced by primary sensory neurons and spinal astrocytes because nerve injury produces robust bFGF upregulation in both cell types. Therefore, the bFGF/JNK pathway is an important signalling pathway in spinal astrocytes for chronic pain sensitization. Investigation of signaling mechanisms in spinal astrocytes will identify new molecular targets for the management of chronic pain.

### Keywords

neuropathic pain; MAP kinases; neuron–glial interaction

### INTRODUCTION

Chronic pain, caused by disease and injury, is a major health problem worldwide. Chronic pain includes, but is not limited to, neural damage associated neuropathic pain (Woolf and Mannion, 1999), arthritis associated inflammatory pain (Dubner and Ruda, 1992), and tumor growth associated cancer pain (Mantyh *et al.*, 2002). These chronic conditions can outlive the initial

injuries and damage, and are considered disorders in their own right. Analgesic drugs that are useful for treating acute pain are only partially effective for chronic pain. The reasons for this lack of success in pain treatment include a complex etiology of pain and an incomplete understanding of the mechanisms underlying the induction and maintenance of chronic pain. However, it is generally believed that chronic pain results from neural plasticity in the pain pathway (Dubner and Ruda, 1992; Ji and Woolf, 2001).

Previously, it was thought that only neurons and neural circuits mediated pain, and glial cells served only as a structural support for neurons. However, recent evidence indicates that glial cells are active and that they respond to environmental changes and interact with neurons. This neural–glial interaction is bidirectional. On the one hand, glia express different types of neurotransmitter receptors, which enables them to respond to neural signals. On the other hand, glial cells produce numerous mediators (e.g. proinflammatory cytokines and growth factors) that are neuroactive. There are three types of glial cells in the CNS: microglia, astrocytes and oligodendrocytes. The microglia are derived from bone marrow precursors and were believed to be quiescent under normal conditions. However, a recent study shows that resting microglia are highly dynamic surveillants of brain function (Nimmerjahn *et al.*, 2005). Oligodendrocytes and astrocytes are derived from ectodermal precursors and occur in close apposition to neurons. The oligodendrocytes produce myelin, which ensheathes neuronal axons and allows fast nerve conduction. The astrocytes form networks with themselves and are closely associated with neurons and blood vessels. Their activity is generally thought to mirror the metabolic activity of neurons (Haydon and Carmignoto, 2006). In contrast to the rapid propagation of nerve impulses on the order of  $\text{m sec}^{-1}$ , glial cell signaling is much slower with rates of propagation measured in  $\mu\text{m second}^{-1}$ .

Early evidence for the involvement of spinal glial cells in pain regulation came from studies using metabolic inhibitors specific to glial cells such as fluorocitrate (Meller *et al.*, 1994; Watkins *et al.*, 1997). Also, it was believed that proinflammatory cytokines such as interleukin  $1\beta$  (IL- $1\beta$ ), IL-6 and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) are produced in glial cells. These cytokines are upregulated in the spinal cord in chronic pain conditions. Functional inhibition of these cytokines can attenuate persistent pain and enhance opioid analgesia (Sweitzer *et al.*, 2001; Watkins *et al.*, 2001; Milligan *et al.*, 2003; Watkins *et al.*, 2005). However, the distinct roles of specific glial subtypes in pain sensitization were not assessed in these studies.

Recently, evidence has accumulated that supports a role of spinal microglia in chronic pain, especially the facilitation of neuropathic pain. Nerve injury induces the expression of microglial markers (e.g. CD11b, TLR4 and CD14) within several hours (DeLeo *et al.*, 2004). Initially, microglia appear to be activated, which activates astrocytes (Aldskogius and Kozlova, 1998; DeLeo *et al.*, 2004). Specifically, nerve injury upregulates several receptors, such as the chemokine receptor CX3CR1 and ATP receptor P2X4 in spinal microglia. Either blocking or deleting these receptors results in decreased neuropathic pain (Tsuda *et al.*, 2003; Milligan *et al.*, 2004; Verge *et al.*, 2004; Zhuang *et al.*, 2006b). Intrathecal injection of ATP-activated microglia induces mechanical allodynia (a nociceptive response to normally innocuous mechanical stimulation) that requires microglial production of BDNF (Tsuda *et al.*, 2003; Coull *et al.*, 2005). A non-specific microglial inhibitor minocycline prevents/delays pain development (Raghavendra *et al.*, 2003; Ledebner *et al.*, 2005; Hua *et al.*, 2005). Notably, studies from different laboratories have demonstrated that p38 mitogen-activated protein kinase (MAPK) is activated in spinal microglia under different chronic pain conditions, and that blocking this kinase attenuates pain hypersensitivity (Table 1 (Jin *et al.*, 2003; Schafers *et al.*, 2003; Tsuda *et al.*, 2004; Boyle *et al.*, 2006; Hains and Waxman, 2006). A recent study shows that nerve injury-induced cleavage of the chemokine fractalkine results in activation of p38 in spinal microglia via CX3CR1 receptors (Zhuang *et al.*, 2006b).

Compared with the ample evidence for microglial regulation of pain, less is known about the importance of spinal astrocytes in chronic pain, largely because of the lack of specific pharmacological tools. However, there are more astrocytes in the CNS than other cells, and they have strong structural inter-relationship with neurons by enwrapping synaptic terminals, enabling signaling between glia and neurons. It is becoming evident that astrocytes are involved intimately in neuronal signaling by releasing glutamate and ATP (Haydon, 2001). Astrocytes are thought to signal each other through gap junctions in waves of  $\text{Ca}^{2+}$  release (Haydon, 2001). Evidence is accumulating that spinal astrocytes have a role in maintaining chronic pain sensitization.

### Involvement of spinal astrocytes in chronic pain

**Persistent changes in spinal astrocytes in chronic pain states**—Glial fibrillary acidic protein (GFAP), vimentin and S-100 $\beta$  are the markers that are used most often to identify astrocytes (Ridet *et al.*, 1997). Although astrocytes are not as homogenous as previously thought, GFAP appears to label most astrocytes in the spinal cord. The change in staining density of GFAP was analyzed initially in the chronic constriction injury model of neuropathic pain. In this study, increased density of GFAP staining was attributed primarily to hypertrophy of astrocytes rather than either their proliferation or their migration. The magnitude of the increase in GFAP staining appears to correlate with the degree of hyperalgesia (Garrison *et al.*, 1991). A subsequent study shows that the NMDA receptor is, in part, responsible for GFAP expression (Garrison *et al.*, 1994). A correlation between GFAP expression and chronic pain has also been identified in other studies (Colburn *et al.*, 1997; Colburn *et al.*, 1999). However, chronic pain can also be suppressed without inhibition of GFAP expression (Zhuang *et al.*, 2006a). It is unclear whether upregulation of GFAP is required or/and sufficient for chronic pain sensitization, but mounting evidence indicates that persistent activation (e.g. GFAP upregulation) of spinal astrocytes is a unique feature of chronic pain in different animal models following bone cancer (Honore *et al.*, 2000; Mantyh *et al.*, 2002), spinal nerve ligation (SNL) (Tanga *et al.*, 2004; Zhuang *et al.*, 2006a), partial sciatic-nerve ligation (Zhang *et al.*, 2006), spinal cord injury (Nesic *et al.*, 2006) and adjuvant-induced inflammation (Raghavendra *et al.*, 2004). In addition, S100 $\beta$  is also upregulated in the spinal cord after nerve injury. Whereas S100 $\beta$ -deficient mice have reduced mechanical allodynia after nerve injury, allodynia is enhanced in mice that overexpress S100 $\beta$ , supporting a role of this astroglial protein in the pathophysiology of neuropathic pain (Tanga *et al.*, 2006).

**Are astrocytes sufficient and required for chronic pain sensitization?**—Spinal astrocytes appear to be sufficient to produce persistent pain. Implantation of neural stem cells into the injured spinal cord improves motor recovery and causes allodynia-like hypersensitivity of the forepaws (Hofstetter *et al.*, 2005; Macias *et al.*, 2006). Because most of the stem cells that are implanted in the spinal cord become astrocytes, implantation-induced allodynia is likely to be attributed to the action of astrocytes. This allodynia is associated with aberrant sprouting of pain-mediating calcitonin gene-related peptide (CGRP)-positive fibers in the dorsal horn due to the release of growth factors (e.g. nerve growth factor) from astrocytes (Hofstetter *et al.*, 2005). In contrast, sprouting and allodynia are prevented if neural stem cells are transfected with neurogenin-2 before transplantation to suppress the generation of astrocytes (Hofstetter *et al.*, 2005).

Spinal astrocytes might also be required for the maintenance of chronic pain. L-alpha-amino adipate (L- $\alpha$ -AA) is a cytotoxin that is relatively specific for astrocytes. Ultrastructural studies indicate that cell degeneration is confined to astrocytes following the injection of this toxin into the striatum (Huck *et al.*, 1984; Khurgel *et al.*, 1996; Rodriguez *et al.*, 2004). Intrathecal injection of L- $\alpha$ -AA to spinal nerve-ligated rats produces a dose-dependent attenuation of mechanical allodynia, which is a major symptom of chronic pain (Fig. 1). In

agreement with previous studies (Khurgel *et al.*, 1996; Rodriguez *et al.*, 2004), L- $\alpha$ -AA produces a marked reduction of GFAP-positive astrocytes in the dorsal horn but has no effect on NeuN-positive spinal neurons (Zhuang *et al.*, 2006a). The anti-allodynic effect of the toxin is reversible, recovering after 3 days (Fig. 1). This reversible effect might be attributed to migration of neighboring astrocytes. Furthermore, intrathecal injection of this astroglial toxin does not alter the basal sensitivity to pain, which indicates that astrocytes might not be essential for mediating basal pain sensitivity (Zhuang *et al.*, 2006a).

**Gap junctions and astroglial networks**—Astrocytes are characterized by the formation of astroglial networks (gap junctions) (Giaume and McCarthy, 1996; Giaume and Venance, 1998; Nagy *et al.*, 2004). Gap junctions exist at apposing plasma membranes of many cell types, and contribute to local metabolic homeostasis and synchronization of cellular activities by allowing direct intercellular movements of ions, metabolites and second messenger molecules up to 1000 Daltons. These junctions are composed of hemi-channels called connexons. Each connexon is composed of six gap-junction proteins, termed connexins (Giaume and Venance, 1998; Nagy *et al.*, 2004). In the adult dorsal horn, gap junctions form predominantly between astrocytes. Connexin-43 (Cx43) is regarded as the main functional connexin in astrocytes (Giaume and Venance, 1998; Nagy *et al.*, 2004). Facial nerve lesion induces a rapid upregulation of Cx43 in the facial nucleus (Rohlmann *et al.*, 1993). Cx43 is also upregulated in the spinal cord after spinal cord injury (Lee *et al.*, 2005). It is noteworthy that the gap-junction blocker carbenoxolone suppresses the spread of pain (Spataro *et al.*, 2004). In a model of nerve inflammation, high concentrations of zymosan delivered to the sciatic nerve produces a ‘mirror pain’ in the contralateral paw (mechanical allodynia), which is suppressed by intrathecal carbenoxolone (Spataro *et al.*, 2004).

**Signaling molecules in spinal astrocytes and their roles in pain regulation**—

Astrocytes express the glutamate transporters GLT-1 and GLAST. These transporters are believed to provide principal route for glutamate removal from synaptic clefts and the extracellular space (Huang and Bergles, 2004; Tawfik *et al.* 2006). Nerve injury produces a persistent downregulation of the transporters, after an initial rise. Downregulation might result in a decrease in glutamate uptake and a subsequent increase in excitatory synaptic transmission. Thus, neuropathic pain is attenuated by riluzole, a glutamate-transporter activator given intrathecally (Sung *et al.* 2003). However, GLT-1 appears to have an opposite role in acute pain conditions. For example, intrathecal injection of a GLT-1 inhibitor inhibits rather than enhance long-term potentiation (LTP) of spinal neurons following tetanic stimulation of the sciatic nerve (Wang *et al.*, 2006). Spinal LTP has been implicated in pain sensitization (Sandkuhler, 2000). Also GLT-1 is upregulated transiently in the spinal cord after injection of formalin into the hindpaw, and either inhibition or knockdown of GLT-1 suppresses formalin-induced pain behavior (Niederberger *et al.*, 2003). These studies indicate that glutamate transporters might have different roles in acute and chronic pain conditions.

Spinal cord injury induces an immediate increase in plasma endothelin (ET) levels and a sustained increase in tissue ET levels. ET-1 also induces hypertrophy of astrocytes (Rogers *et al.*, 2003). Interestingly, ET receptor-B (ETB) is induced in spinal astrocytes after spinal cord injury (Table 2) (Peters *et al.*, 2003). Thus, strategies that block ET receptors following spinal cord injury might reduce ischemia and also suppress astrogliosis and chronic pain.

Although cells normally produce L-type amino acids, astrocytes can produce D-serine. Astrocytes also contain the major enzyme for the biosynthesis of D-serine, serine racemase. D-serine increases the sensitivity of NMDA receptors in hippocampal neurons by binding to the glycine site of the receptor and facilitating the induction of LTP (Wolosker *et al.*, 2006). Intrathecal injection of D-serine facilitates pain via NMDA receptors (Kolhekar *et al.*, 1994).

Conversely, injection of an inhibitor of the enzyme that degrades D-serine into the anterior cingulate cortex attenuates affective pain (Ren *et al.* 2006).

MAPKs are a family of protein kinases that play important role in intracellular signal transduction. This family includes three major members: extracellular signal-regulated kinase (ERK), p38 and c-Jun-N-terminal kinase (JNK). As mentioned above, p38 is activated in spinal microglia (Jin *et al.*, 2003), and therefore is not the focus of this review. ERK is the most studied member of the MAPK family. In acute and inflammatory pain conditions, ERK is activated in dorsal horn neurons, which contributes to the induction and maintenance of dorsal horn neuron sensitization and pain hypersensitivity (Ji *et al.*, 1999; Ji *et al.*, 2002). Notably, ERK is activated in spinal glial cells after nerve injury. Phosphorylated ERK (pERK), which is the active form, is induced in spinal astrocytes at late times after SNL (Zhuang *et al.* 2005). pERK was found in microglia on day 2, in both microglia and astrocytes on day 10, but in astrocytes only on day 21 (Table 1) (Zhuang *et al.*, 2005). pERK is also present in spinal astrocytes 3 weeks after partial sciatic nerve injury (Ma and Quirion, 2002). Spinal inhibition of this late-phase activation of ERK by intrathecal MEK inhibitor reverses mechanical allodynia, supporting a role of astrocytic ERK in the maintenance of neuropathic pain (Zhuang *et al.*, 2005).

Zerari *et al.* show that the neurokinin-2 receptor, which is activated by extrasynaptic neurokinin A, is expressed exclusively in spinal astrocytes (Zerari *et al.*, 1998). An NK2 receptor agonist activates ERK and causes behavioral sensitization of pain that is prevented by a MEK inhibitor. Conversely, an NK2 receptor antagonist suppresses ERK activation and neuropathic pain following nerve injury (Garry *et al.*, 2005).

IL-1 $\beta$  is upregulated in the spinal cord in different chronic pain conditions and plays an important role in pain facilitation (Sweitzer *et al.*, 2001; Milligan *et al.*, 2003). In a recent model of bone cancer pain in rats, which results from inoculation of the tibia with prostate cancer cells, IL-1 $\beta$  is induced in spinal astrocytes at certain times (Zhang *et al.*, 2005). However, IL-1 $\beta$  is also found in neurons in the spinal cord (DeLeo *et al.*, 1997; Fu *et al.*, 2006). It is likely that IL-1 $\beta$  is induced in different types of spinal cells either at different times of pain development or in different chronic pain models.

### Activation of the JNK cascade in spinal astrocytes and neuropathic pain

JNK is the signaling molecule that is the focus of this review. JNK is the least studied member of the MAPK family. Although all three MAPKs are activated in spinal glial cells after nerve injury, they have different patterns: ERK is activated sequentially in microglia and astrocytes, p38 is activated in microglia, whereas JNK is activated persistently in astrocytes (Table 1) (Jin *et al.*, 2003; Zhuang *et al.*, 2005; Zhuang *et al.*, 2006a). Thus, SNL induces a marked increase in pJNK-immunoreactive (IR) cells in the dorsal horn of the injured side (Fig. 2a,b). Furthermore, although pJNK colocalizes with the astroglial marker GFAP (Fig. 2c,d), pJNK is only expressed in a portion of spinal astrocytes (Zhuang *et al.*, 2006a). JNK activation also occurs in spinal astrocytes 3 weeks after partial sciatic nerve injury (Ma and Quirion, 2002) and in amyotrophic lateral sclerosis (Migheli *et al.*, 1997). JNK is also activated in spinal astrocytes in another chronic pain condition following adjuvant-induced inflammation (Zhuang and Ji, unpublished observation). However, it is noteworthy that pJNK occurs in spinal neurons after traumatic spinal cord injury, a condition that produces robust neuronal apoptosis in the spinal cord. It is known that JNK has a role in stress-induced apoptosis in the nervous system (Borsello *et al.*, 2003). However, following peripheral nerve injury, neuronal apoptosis in the spinal cord is not prominent (Polgar *et al.*, 2005; Scholz *et al.*, 2005). There is no evidence to indicate that the pJNK-positive spinal astrocytes undergo apoptosis after nerve injury.

The transcription factor c-Jun is the best-known substrate of JNK. JNK activates c-Jun by phosphorylation to form p-c-Jun. SNL also upregulates p-c-Jun in the ipsilateral spinal cord. P-c-Jun also localizes to GFAP-expressing astrocytes, predominantly in the nucleus (Zhuang *et al.*, 2006a). Of the three isoforms of JNK (JNK1, JNK2 and JNK3), JNK3 is expressed in neurons and JNK1 is expressed in non-neuronal cells (e.g. immune cells) (Ip and Davis 1998; Borsello *et al.*, 2003; Kuan *et al.*, 2003). JNK1 is also expressed in spinal astrocytes and only this isoform is hyperphosphorylated in the spinal cord after SNL (Zhuang *et al.*, 2006a). Thus, the whole JNK cascade is localized preferentially in spinal astrocytes after nerve injury (Table 2).

Is activation of JNK in spinal astrocytes after nerve injury essential for chronic pain sensitization? Answering this question requires a potent, specific inhibition of JNK. Recently, a peptide inhibitor of JNK, which is derived from the JNK-binding domain of JNK-interacting protein-1 (JIP-1), was designed to block selectively the access of JNK to c-Jun and other substrates by a competitive mechanism (Borsello and Bonny, 2004). A TAT sequence (transporter sequence) is linked to the peptide to render it membrane permeable. A convert to D-form amino acids further makes the peptide proteinase-resistant. This highly-specific peptide inhibitor, called D-JNKI-1 is a potent neuroprotectant against excitotoxicity of cortical neurons (Borsello *et al.*, 2003; Borsello and Bonny, 2004). Spinal infusion of this inhibitor intrathecally does not change basal pain thresholds, but it prevents mechanical allodynia, a major neuropathic pain symptom, for >10 days (Zhuang *et al.*, 2006a). Because JNK is also activated transiently (<3 days) in primary sensory neurons, the preventive effect of D-JNKI-1 in the first several days might be mediated by JNK in dorsal root ganglia (DRG). However, the maintenance of neuropathic pain (reversal) is predominantly, if not exclusively, mediated by spinal JNK (Zhuang *et al.*, 2006a).

It is important to investigate whether inhibition of JNK also reverses established neuropathic pain, a treatment mode that is more relevant to clinical situation. Infusing D-JNKI-1 intrathecally via osmotic pump effectively reverses SNL-induced allodynia for several days (Fig. 3a). A single bolus injection of D-JNKI-1 inhibitors also effectively reverses mechanical allodynia for >12 hours (Fig. 3b). D-JNKI-1 is more potent than the small molecule inhibitor SP600125, which is used currently, with an ED50 that is 50-times less (Zhuang *et al.*, 2006a). D-JNKI-1 also suppresses nerve injury-induced activation of c-Jun in astrocytes, which is a major downstream target of JNK (Zhuang *et al.*, 2006a).

### **bFGF/JNK pathway in astrocytes and persistent pain**

bFGF (OR FGF-2) is a well-known activator of astrocytes. bFGF is produced by astrocytes and strongly induces their mitosis, growth, differentiation and gliosis (Ferrara *et al.*, 1988; Eclancher *et al.*, 1990). bFGF is induced in the CNS in many injury conditions. For example, bFGF is induced in injured brain regions (mainly in astrocytes) after trauma and in disease pathology such as Alzheimer's where astrogliosis is very active (Gomez-Pinilla, *et al.*, 1990). After spinal cord injury, bFGF is upregulated in the spinal cord, which promotes functional recovery (Koshinaga *et al.*, 1993; Lee *et al.*, 1999; Rabchevsky *et al.*, 2000). bFGF is also upregulated in the spinal cord after sciatic cryoneurolysis but not after chronic constriction injury (DeLeo *et al.*, 1997), which indicates that upregulation might be associated with the severity of the injury. In particular, after SNL, Madias *et al.* show that bFGF immunoreactivity increases in reactive astrocytes in the ipsilateral dorsal horn at either 1 week or 3 weeks after nerve ligation (Table 2) (Madias *et al.*, 2003). The release of bFGF from astrocytes might act in an autocrine manner to further augment astroglial activation (e.g. astrogliosis and proliferation).

As a pleiotropic cytokine, bFGF is synthesized and secreted by both astrocytes and neurons. bFGF is expressed in primary sensory neurons in the DRG. Normally, mRNA that encodes

bFGF is expressed in 5% of small neurons in DRG. Nerve injury (e.g. transection of the sciatic nerve and axotomy) induces a dramatic, rapid upregulation of bFGF mRNA; almost all injured DRG neurons contain bFGF mRNA 3 days after axotomy (Fig. 4). An increase in bFGF protein is also evident in DRG neurons after nerve injury (Ji *et al.*, 1995). Although release of bFGF from central terminals of primary sensory neurons after nerve injury has not been shown, bFGF immunoreactivity is present in vesicle-like structures in the cytoplasm, which indicates the possibility that bFGF is released after nerve injury (Ji *et al.*, 1995). bFGF upregulation in the DRG has also been shown in the SNL model (Madias *et al.*, 2003).

As discussed above, after peripheral nerve injury, bFGF is produced both in spinal astrocytes and DRG primary sensory neurons. Is endogenous bFGF in the spinal cord important for producing chronic pain? An antagonist of the bFGF receptor is not available, so Madias *et al.* tested the role of bFGF in neuropathic pain using a neutralizing antibody to bFGF, delivered intrathecally after SNL. This antibody reduces SNL-induced expression of GFAP in the spinal cord and reverses SNL-induced tactile allodynia, which indicates that endogenous bFGF contributes to maintaining neuropathic tactile allodynia (Madias *et al.*, 2005).

To examine whether exogenous bFGF is sufficient to induce pain hypersensitivity, we infused bFGF into spinal cord for 1 week through an osmotic pump. Mechanical allodynia develops slowly after infusion of bFGF: mechanical thresholds to Von Frey hair stimuli do not decrease until 4 days after the infusion. Interestingly, allodynia is maintained even after the termination of the infusion (Fig. 5). This slow, persistent development of allodynia is indicative of a role of spinal astrocytes. Infusion of bFGF also increases expression of GFAP in the spinal cord. Notably, spinal injection of adenovirus that encodes bFGF causes overexpression of bFGF in dorsal horn astrocytes and produces persistent hyperalgesia (Romero *et al.*, 2000). Together, these results indicate that bFGF is both sufficient and required for producing chronic pain.

bFGF is a primary 'activator' of astrocytes and JNK is an important signaling molecule in spinal astrocytes, so it is reasonable to ask whether bFGF activates JNK in spinal astrocytes. Intrathecal infusion of bFGF induces a marked activation of JNK in the spinal cord (Fig. 6a,b). JNK activation has also been examined in astroglial cultures. bFGF is a powerful activator of JNK in these cultures (Fig. 6b). However, two additional potential activators of astrocytes, plasminogen (Liu *et al.*, 2000) and ciliary neurotrophic factor (CNTF) (Escartin *et al.*, 2006) do not cause obvious activation of JNK (Fig. 6b). bFGF also induces marked activation of ERK/MAPK in astrocytes, which is evident in the spinal cord at late times of nerve injury (Zhuang *et al.*, 2005). However, activation of p38 MAPK is not evident in astroglial cultures (Fig. 6b), in support of the observation *in vivo* that p38 is activated in spinal microglia (Jin *et al.*, 2003).

It is noteworthy that bFGF induces the release of NGF from astrocytes (Fukumoto *et al.*, 1991; Yoshida and Gage, 1991). NGF is a crucial signalling molecule for regulating the phenotype of nociceptive primary sensory neurons. NGF released from spinal astrocytes can be taken up by spinal axonal terminals, causing sprouting of substance P- and CGRP-positive axons and hyperalgesia (Romero *et al.*, 2000). The NGF that is taken up might also be transported retrogradely to DRG neurons, where it induces the expression of pronociceptive genes encoding, for example, the capsaicin receptor transient receptor potential V1 subtype (TRPV1), and the neuropeptides substance P and CGRP.

How does activation of JNK in spinal astroglia regulate chronic pain? Because inhibition of JNK suppresses SNL-induced phosphorylation of c-Jun in spinal astrocytes (Zhuang *et al.*, 2006a), JNK activation is likely to regulate gene transcription in spinal astrocytes via activation of the transcription factor c-Jun and other transcription factors such as ATF-2. After nerve injury, there is increased synthesis in the spinal cord of inflammatory mediators such as

cytokines (IL-1 $\beta$ , TNF- $\alpha$  and IL-6), nitric oxide (NO), which is produced by inducible NO synthase (iNOS), and prostaglandin E2 (PGE2) (produced by either COX-1 or COX-2); all these mediators and enzymes are implicated in pain sensitization (Samad *et al.*, 2001; Watkins *et al.*, 2001; DeLeo *et al.*, 2004; Ji and Strichartz, 2004). Mixed lineage kinases (MLK) are specific JNK kinases. Falsig *et al.* have shown that MLK inhibitors such as CEP-1347 are potent astrocyte immune modulators (Falsig *et al.*, 2004). In astrocytes in culture, CEP-1347 blocks activation of JNK, expression of COX-2 and iNOS, and release of nitric oxide, PGE2 and IL-6 following challenge with a mixture of cytokines (Falsig *et al.*, 2004). JNK might also regulate chronic pain by modulating the activity of gap junctions that form mainly between astrocytes (Petrich *et al.*, 2004).

### Concluding remarks and future directions

Accumulating evidence shows that persistent changes in spinal astrocytes in different chronic pain conditions often outlast microglial changes. This feature of spinal astrocytes implies a role of these cells in the maintenance of chronic pain. Consistent with this, several drugs that affect astroglial function (e.g. JNK inhibitors, MEK inhibitors and propentofylline) reverse persistent, chronic pain. Spinal astrocytes appear to be both sufficient and required for chronic pain sensitization. Although GFAP upregulation and gliosis are generally regarded as the markers of 'astroglial activation', studies reviewed here indicate that additional markers are needed that go beyond the vaguely defined state of 'activation' and are associated with cellular function related to pain regulation. Signaling molecules such as JNK are not only activated in spinal astrocytes, but also contribute to chronic pain. The bFGF/JNK cascade is an important signaling pathway in spinal astrocytes that promotes chronic pain, especially after nerve and spinal cord injury.

Although current evidence supports a pronociceptive role of spinal astrocytes, presumably by enhancing spinal synaptic transmission via the release of neuroactive substances and inflammatory mediators (e.g. cytokines, NGF and PGE2), studies that establish a causative link between inhibiting spinal cord astrocytic activation and physiological function of spinal neurons are lacking and should be considered in future. Studies in the hippocampus have shown that astroglial activation might have the dual role of enhancing synaptic transmission by releasing glutamate and suppressing synaptic transmission by releasing ATP, which is hydrolyzed to adenosine (Haydon and Carmignoto, 2006). This discrepancy might be attributable to different roles of astrocytes in physiological and pathological conditions. Recently, 2-photon laser scanning microscopy has been used to image astrocytes in intact brains (Tian *et al.*, 2006). This imaging method will be helpful in demonstrating the activation of astroglial circuits in the intact spinal cord following peripheral noxious and innocuous stimuli. Functional understanding of the cellular and molecular alterations of astroglia-dependent synaptic transmission will help to clarify the role of astrocytes in pain regulation and lead to the identification of novel therapeutic targets for chronic pain.

### Acknowledgments

This study is supported by NIH grants DE17794, NS54932, and TW7180 (JRR) and a collaboration grant from International Association for the Study of Pain (JRR and ID).

### References

- Aldskogius H, Kozlova EN. Central neuron-glia and glial-glia interactions following axon injury. *Progress in Neurobiology* 1998;55:1–26.
- Borsello T, Bonny C. Use of cell-permeable peptides to prevent neuronal degeneration. *Trends in Molecular Medicine* 2004;10:239–244. [PubMed: 15121051]



- Borsello T, Clarke PG, Hirt L, Vercelli A, Repici M, Schorderet DF, et al. A peptide inhibitor of c-Jun N-terminal kinase protects against excitotoxicity and cerebral ischemia. *Molecular Medicine* 2003;9:1180–1186.
- Boyle DL, Jones TL, Hammaker D, Svensson CI, Rosengren S, Albani S, et al. Regulation of Peripheral Inflammation by Spinal p38 MAP Kinase in Rats. *PLoS Medicine* 2006;3:e338. [PubMed: 16953659]
- 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. *Journal of Neuroimmunology* 1997;79:163–175. [PubMed: 9394789]
- Colburn RW, Rickman AJ, DeLeo JA. The effect of site and type of nerve injury on spinal glial activation and neuropathic pain behavior. *Experimental Neurology* 1999;157:289–304. [PubMed: 10364441]
- Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, et al. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 2005;438:1017–1021. [PubMed: 16355225]
- DeLeo JA, Colburn RW, Rickman AJ. Cytokine and growth factor immunohistochemical spinal profiles in two animal models of mononeuropathy. *Brain Research* 1997;759:50–57. [PubMed: 9219862]
- DeLeo JA, Tanga FY, Tawfik VL. Neuroimmune activation and neuroinflammation in chronic pain and opioid tolerance/hyperalgesia. *Neuroscientist* 2004;10:40–52. [PubMed: 14987447]
- Dubner R, Ruda MA. Activity-dependent neuronal plasticity following tissue injury and inflammation. *Trends in Neurosciences* 1992;15:96–103. [PubMed: 1373925]
- Eclancher F, Perraud F, Faltin J, Labourdette G, Sensenbrenner M. Reactive astrogliosis after basic fibroblast growth factor (bFGF) injection in injured neonatal rat brain. *Glia* 1990;3:502–509. [PubMed: 2148552]
- Escartin C, Brouillet E, Gubellini P, Trioulier Y, Jacquard C, Smadja C, et al. Ciliary neurotrophic factor activates astrocytes, redistributes their glutamate transporters GLAST and GLT-1 to raft microdomains, and improves glutamate handling in vivo. *Journal of Neuroscience* 2006;26:5978–5989. [PubMed: 16738240]
- Falsig J, Porzgen P, Lotharius J, Leist M. Specific modulation of astrocyte inflammation by inhibition of mixed lineage kinases with CEP-1347. *Journal of Immunology* 2004;173:2762–2770.
- Ferrara N, Ousley F, Gospodarowicz D. Bovine brain astrocytes express basic fibroblast growth factor, a neurotropic and angiogenic mitogen. *Brain Research* 1988;462:223–232. [PubMed: 3191384]
- Fu D, Guo Q, Ai Y, Cai H, Yan J, Dai R. Glial activation and segmental upregulation of interleukin-1beta (IL-1beta) in the rat spinal cord after surgical incision. *Neurochemistry Research* 2006;31:333–340.
- Fukumoto H, Kakihana M, Suno M. Recombinant human basic fibroblast growth factor (rhbFGF) induces secretion of nerve growth factor (NGF) in cultured rat astroglial cells. *Neuroscience Letters* 1991;122:221–224. [PubMed: 2027522]
- Garrison CJ, Dougherty PM, Carlton SM. GFAP expression in lumbar spinal cord of naive and neuropathic rats treated with MK-801. *Experimental Neurology* 1994;129:237–243. [PubMed: 7957738]
- 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 Research* 1991;565:1–7. [PubMed: 1723019]
- Garry EM, Delaney A, Blackburn-Munro G, Dickinson T, Moss A, Nakalembe I, et al. Activation of p38 and p42/44 MAP kinase in neuropathic pain: involvement of VPAC2 and NK2 receptors and mediation by spinal glia. *Molecular and Cellular Neuroscience* 2005;30:523–537. [PubMed: 16202621]
- Giaume C, McCarthy KD. Control of gap-junctional communication in astrocytic networks. *Trends in Neurosciences* 1996;19:319–325. [PubMed: 8843600]
- Giaume C, Venance L. Intercellular calcium signaling and gap junctional communication in astrocytes. *Glia* 1998;24:50–64. [PubMed: 9700489]
- Gomez-Pinilla F, Cummings BJ, Cotman CW. Induction of basic fibroblast growth factor in Alzheimer's disease pathology. *Neuroreport* 1990;1:211–214. [PubMed: 1717039]
- Hains BC, Waxman SG. Activated microglia contribute to the maintenance of chronic pain after spinal cord injury. *Journal of Neuroscience* 2006;26:4308–4317. [PubMed: 16624951]
- Haydon PG. GLIA: listening and talking to the synapse. *Nature Reviews Neuroscience* 2001;2:185–193.

- Haydon PG, Carmignoto G. Astrocyte control of synaptic transmission and neurovascular coupling. *Physiological Reviews* 2006;86:1009–1031. [PubMed: 16816144]
- Hofstetter CP, Holmstrom NA, Lilja JA, Schweinhardt P, Hao J, Spenger C, et al. Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome. *Nature Neuroscience* 2005;8:346–353.
- Honore P, Rogers SD, Schwei MJ, Salak-Johnson JL, Luger NM, Sabino MC, et al. Murine models of inflammatory, neuropathic and cancer pain each generates a unique set of neurochemical changes in the spinal cord and sensory neurons. *Neuroscience* 2000;98:585–598. [PubMed: 10869852]
- Hua XY, Svensson CI, Matsui T, Fitzsimmons B, Yaksh TL, Webb M. Intrathecal minocycline attenuates peripheral inflammation-induced hyperalgesia by inhibiting p38 MAPK in spinal microglia. *European Journal of Neuroscience* 2005;22:2431–2440.
- Huang YH, Bergles DE. Glutamate transporters bring competition to the synapse. *Current Opinions in Neurobiology* 2004;14:346–352.
- Huck S, Grass F, Hortnagl H. The glutamate analogue alpha-aminoadipic acid is taken up by astrocytes before exerting its gliotoxic effect in vitro. *Journal of Neuroscience* 1984;4:2650–2657. [PubMed: 6491728]
- Ip YT, Davis RJ. Signal transduction by the c-Jun N-terminal kinase (JNK)--from inflammation to development. *Current Opinions in Cell Biology* 1998;10:205–219.
- Ji RR, Baba H, Brenner GJ, Woolf CJ. Nociceptive-specific activation of ERK in spinal neurons contributes to pain hypersensitivity. *Nature Neuroscience* 1999;2:1114–1119.
- 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. *Journal of Neuroscience* 2002;22:478–485. [PubMed: 11784793]
- Ji RR, Strichartz G. Cell signaling and the genesis of neuropathic pain. *Science STKE* 2004;reE14.
- Ji RR, Woolf CJ. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. *Neurobiology of Disease* 2001;8:1–10. [PubMed: 11162235]
- Ji RR, Zhang Q, Zhang X, Piehl F, Reilly T, Pettersson RF, et al. Prominent expression of bFGF in dorsal root ganglia after axotomy. *European Journal of Neuroscience* 1995;7:2458–2468. [PubMed: 8845951]
- 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. *Journal of Neuroscience* 2003;23:4017–4022. [PubMed: 12764087]
- Khurgel M, Koo AC, Ivy GO. Selective ablation of astrocytes by intracerebral injections of alpha-aminoadipate. *Glia* 1996;16:351–358. [PubMed: 8721675]
- Kolhekar R, Meller ST, Gebhart GF. N-methyl-D-aspartate receptor-mediated changes in thermal nociception: allosteric modulation at glycine and polyamine recognition sites. *Neuroscience* 1994;63:925–936. [PubMed: 7535397]
- Koshinaga M, Sanon HR, Whitemore SR. Altered acidic and basic fibroblast growth factor expression following spinal cord injury. *Experimental Neurology* 1993;120:32–48. [PubMed: 7682969]
- Kuan CY, Whitmarsh AJ, Yang DD, Liao G, Schloemer AJ, Dong C, et al. A critical role of neural-specific JNK3 for ischemic apoptosis. *Proceedings of the National Academy of Sciences of the USA* 2003;100:15184–15189. [PubMed: 14657393]
- Ledeboer A, Sloane EM, Milligan ED, Frank MG, Mahony JH, Maier SF, et al. Minocycline attenuates mechanical allodynia and proinflammatory cytokine expression in rat models of pain facilitation. *Pain* 2005;115:71–83. [PubMed: 15836971]
- Lee IH, Lindqvist E, Kiehn O, Widenfalk J, Olson L. Glial and neuronal connexin expression patterns in the rat spinal cord during development and following injury. *Journal of Comparative Neurology* 2005;489:1–10. [PubMed: 15977163]
- Lee TT, Green BA, Dietrich WD, Yeziarski RP. Neuroprotective effects of basic fibroblast growth factor following spinal cord contusion injury in the rat. *Journal of Neurotrauma* 1999;16:347–356. [PubMed: 10369555]

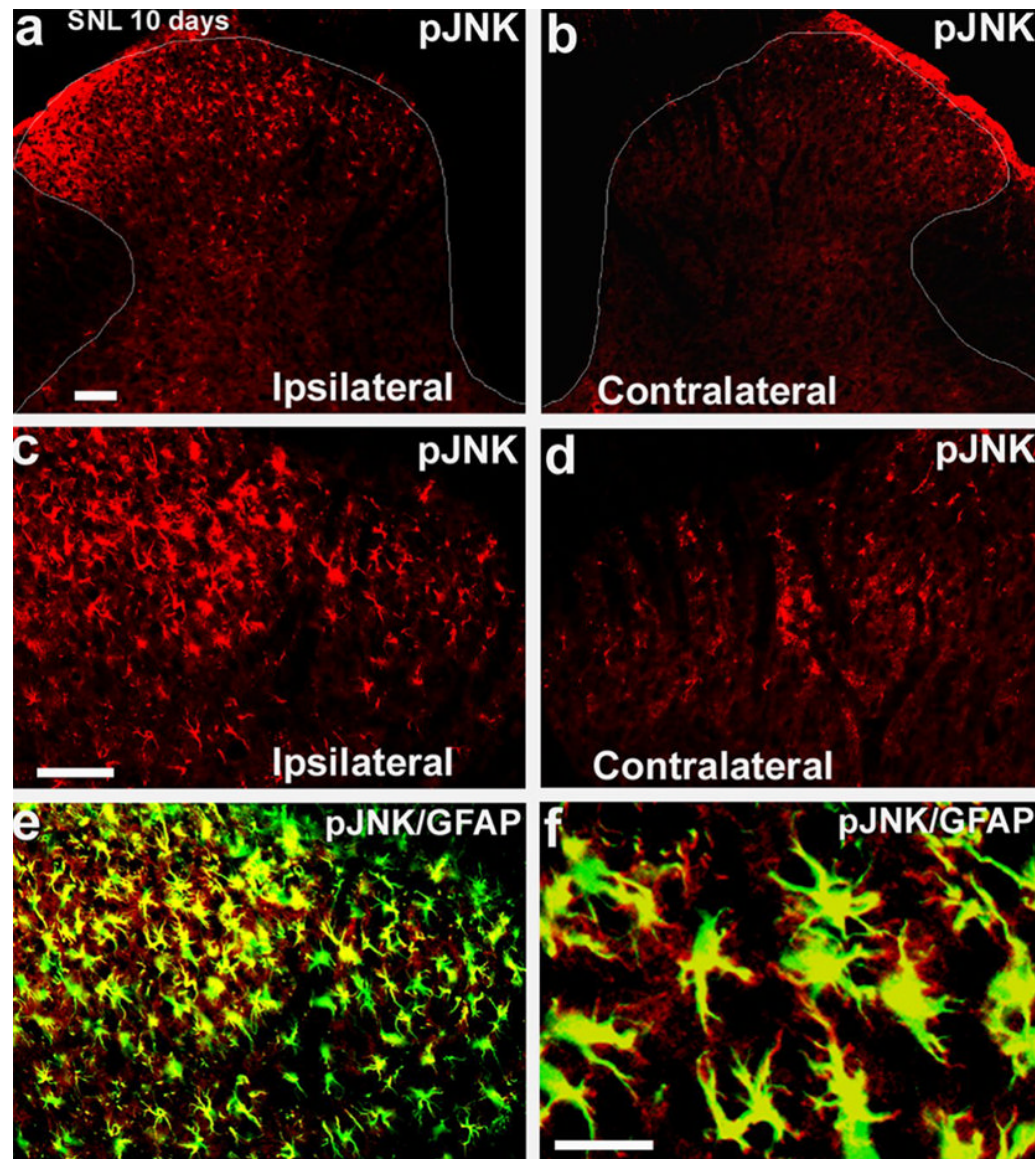
- Liu Y, Honda S, Kohsaka S, Nakajima K. Plasminogen enhances the secretion of plasminogen activator inhibitor-1 from cultured rat astrocytes. *Neuroscience Letters* 2000;282:137–140. [PubMed: 10717410]
- Ma W, Quirion R. Partial sciatic nerve ligation induces increase in the phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) in astrocytes in the lumbar spinal dorsal horn and the gracile nucleus. *Pain* 2002;99:175–184. [PubMed: 12237195]
- Macias MY, Syring MB, Pizzi MA, Crowe MJ, Alexanian AR, Kurpad SN. Pain with no gain: allodynia following neural stem cell transplantation in spinal cord injury. *Experimental Neurology* 2006;201:335–348. [PubMed: 16839548]
- Madiat F, Goettl VM, Hussain SR, Clairmont AR, Stephens RL Jr, Hackshaw KV. Anti-fibroblast growth factor-2 antibodies attenuate mechanical allodynia in a rat model of neuropathic pain. *Journal of Molecular Neuroscience* 2005;27:315–324. [PubMed: 16280602]
- Madiat F, Hussain SR, Goettl VM, Burry RW, Stephens RL Jr, Hackshaw KV. Upregulation of FGF-2 in reactive spinal cord astrocytes following unilateral lumbar spinal nerve ligation. *Experimental Brain Research* 2003;148:366–376.
- Mantyh PW, Clohisey DR, Koltzenburg M, Hunt SP. Molecular mechanisms of cancer pain. *Nature Reviews Cancer* 2002;2:201–209.
- 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. [PubMed: 7532831]
- Migheli A, Piva R, Atzori C, Troost D, Schiffer D. c-Jun, JNK/SAPK kinases and transcription factor NF-kappa B are selectively activated in astrocytes, but not motor neurons, in amyotrophic lateral sclerosis. *Journal of Neuro pathology and Experimental Neurology* 1997;56:1314–1322. [PubMed: 9413280]
- Milligan ED, Twining C, Chacur M, Biedenkapp J, O'Connor K, Poole S, et al. Spinal glia and proinflammatory cytokines mediate mirror-image neuropathic pain in rats. *Journal of Neuroscience* 2003;23:1026–1040. [PubMed: 12574433]
- Milligan ED, Zapata V, Chacur M, Schoeniger D, Biedenkapp J, O'Connor KA, Verge GM, et al. Evidence that exogenous and endogenous fractalkine can induce spinal nociceptive facilitation in rats. *European Journal of Neuroscience* 2004;20:2294–2302. [PubMed: 15525271]
- Nagy JI, Dudek FE, Rash JE. Update on connexins and gap junctions in neurons and glia in the mammalian nervous system. *Brain Research. Brain Research Review* 2004;47:191–215.
- Nesic O, Lee J, Johnson KM, Ye Z, Xu GY, Unabia GC, Wood TG, et al. Transcriptional profiling of spinal cord injury-induced central neuropathic pain. *Journal of Neurochemistry* 2005;95:998–1014. [PubMed: 16219025]
- Niederberger E, Schmidt A, Rothstein JD, Geisslinger G, Tegeder I. Modulation of spinal nociceptive processing through the glutamate transporter GLT-1. *Neuroscience* 2003;116:81–87. [PubMed: 12535941]
- 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]
- Peters CM, Rogers SD, Pomonis JD, Egnaczyk GF, Keyser CP, Schmidt JA, et al. Endothelin receptor expression in the normal and injured spinal cord: potential involvement in injury-induced ischemia and gliosis. *Experimental Neurology* 2003;180:1–13. [PubMed: 12668144]
- Petrich BG, Eloff BC, Lerner DL, Kovacs A, Saffitz JE, Rosenbaum DS, et al. Targeted activation of c-Jun N-terminal kinase in vivo induces restrictive cardiomyopathy and conduction defects. *Journal of Biological Chemistry* 2004;279:15330–15338. [PubMed: 14742426]
- Polgar E, Hughes DI, Arham AZ, Todd AJ. Loss of neurons from laminae I–III of the spinal dorsal horn is not required for development of tactile allodynia in the spared nerve injury model of neuropathic pain. *Journal of Neuroscience* 2005;25:6658–6666. [PubMed: 16014727]
- Rabchevsky AG, Fugaccia I, Turner AF, Blades DA, Mattson MP, Scheff SW. Basic fibroblast growth factor (bFGF) enhances functional recovery following severe spinal cord injury to the rat. *Experimental Neurology* 2000;164:280–291. [PubMed: 10915567]

- Raghavendra V, Tanga F, DeLeo JA. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. *Journal of Pharmacology and Experimental Therapeutics* 2003;306:624–630. [PubMed: 12734393]
- Raghavendra V, Tanga FY, DeLeo JA. Complete Freund's adjuvant-induced peripheral inflammation evokes glial activation and proinflammatory cytokine expression in the CNS. *European Journal of Neuroscience* 2004;20:467–473. [PubMed: 15233755]
- Ren K, Hylden JL, Williams GM, Ruda MA, Dubner R. The effects of a non-competitive NMDA receptor antagonist, MK-801, on behavioral hyperalgesia and dorsal horn neuronal activity in rats with unilateral inflammation. *Pain* 1992;50:331–344. [PubMed: 1454389]
- Ridet JL, Malhotra SK, Privat A, Gage FH. Reactive astrocytes: cellular and molecular cues to biological function. *Trends in Neurosciences* 1997;20:570–577. [PubMed: 9416670]
- Rodriguez MJ, Martinez-Sanchez M, Bernal F, Mahy N. Heterogeneity between hippocampal and septal astroglia as a contributing factor to differential in vivo AMPA excitotoxicity. *Journal of Neuroscience Research* 2004;77:344–353. [PubMed: 15248290]
- Romero MI, Rangappa N, Li L, Lightfoot E, Garry MG, Smith GM. Extensive sprouting of sensory afferents and hyperalgesia induced by conditional expression of nerve growth factor in the adult spinal cord. *Journal of Neuroscience* 2000;20:4435–4445. [PubMed: 10844012]
- Rogers SD, Peters CM, Pomonis JD, Hagiwara H, Ghilardi JR, Mantyh PW. Endothelin B receptors are expressed by astrocytes and regulate astrocyte hypertrophy in the normal and injured CNS. *Glia* 2003;41:180–190. [PubMed: 12509808]
- Rohlmann A, Laskawi R, Hofer A, Dobo E, Dermietzel R, Wolff JR. Facial nerve lesions lead to increased immunostaining of the astrocytic gap junction protein (connexin 43) in the corresponding facial nucleus of rats. *Neuroscience Letters* 1993;154:206–208. [PubMed: 8395666]
- Samad TA, Moore KA, Sapirstein A, Billet S, Allchorne A, Poole S, et al. Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. *Nature* 2001;410:471–475. [PubMed: 11260714]
- Sandkuhler J. Learning and memory in pain pathways. *Pain* 2000;88:113–118. [PubMed: 11050365]
- 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. *Journal of Neuroscience* 2003;23:2517–2521. [PubMed: 12684435]
- Schol J, Broom DC, Youn DH, Mills CD, Kohno T, Suter MR, et al. Blocking caspase activity prevents transsynaptic neuronal apoptosis and the loss of spinal inhibition following peripheral nerve injury. *Journal of Neuroscience* 2005;25:7317–7323. [PubMed: 16093381]
- Spataro LE, Sloane EM, Milligan ED, Wieseler-Frank J, Schoeniger D, Jekich BM, et al. Spinal gap junctions: potential involvement in pain facilitation. *Journal of Pain* 2004;5:392–405. [PubMed: 15501197]
- Sung B, Lim G, Mao J. Altered expression and uptake activity of spinal glutamate transporters after nerve injury contribute to the pathogenesis of neuropathic pain in rats. *Journal of Neuroscience* 2003;23:2899–2910. [PubMed: 12684477]
- 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. [PubMed: 11246166]
- Tanga FY, Raghavendra V, DeLeo JA. Quantitative real-time RT-PCR assessment of spinal microglial and astrocytic activation markers in a rat model of neuropathic pain. *Neurochemistry International* 2004;45:397–407. [PubMed: 15145554]
- Tanga FY, Raghavendra V, Nutile-McMenemy N, Marks A, DeLeo JA. Role of astrocytic S100beta in behavioral hypersensitivity in rodent models of neuropathic pain. *Neuroscience* 2006;140:1003–1010. [PubMed: 16600520]
- Tawfik VL, Lacroix-Fralish ML, Bercury KK, Nutile-McMenemy N, Harris BT, Deleo JA. Induction of astrocyte differentiation by propentofylline increases glutamate transporter expression in vitro: heterogeneity of the quiescent phenotype. *Glia* 2006;54:193–203. [PubMed: 16819765]
- Tian GF, Takano T, Lin JH, Wang X, Bekar L, Nedergaard M. Imaging of cortical astrocytes using 2-photon laser scanning microscopy in the intact mouse brain. *Advanced Drug Delivery Reviews* 2006;58:773–787. [PubMed: 17045697]

- 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. [PubMed: 14648549]
- Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, et al. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* 2003;424:778–783. [PubMed: 12917686]
- 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. *European Journal of Neuroscience* 2004;20:1150–1160. [PubMed: 15341587]
- Wang ZY, Zhang YQ, Zhao ZQ. Inhibition of tetanically sciatic stimulation-induced LTP of spinal neurons and Fos expression by disrupting glutamate transporter GLT-1. *Neuropharmacology* 2006;51:764–772. [PubMed: 16815482]
- Watkins LR, Hutchinson MR, Johnston IN, Maier SF. Glia: novel counter-regulators of opioid analgesia. *Trends in Neurosciences* 2005;28:661–669. [PubMed: 16246435]
- 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. [PubMed: 9231865]
- Watkins LR, Milligan ED, Maier SF. Glial activation: a driving force for pathological pain. *Trends in Neurosciences* 2001;24:450–455. [PubMed: 11476884]
- Wolosker H. D-serine regulation of NMDA receptor activity. *Science STKE* 2006:e41.
- Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. *Lancet* 1999;353:1959–1964. [PubMed: 10371588]
- Yoshida K, Gage FH. Fibroblast growth factors stimulate nerve growth factor synthesis and secretion by astrocytes. *Brain Research* 1991;538:118–126. [PubMed: 1708303]
- Zerari F, Karpitskiy V, Krause J, Descarries L, Couture R. Astroglial distribution of neurokinin-2 receptor immunoreactivity in the rat spinal cord. *Neuroscience* 1998;84:1233–1246. [PubMed: 9578409]
- Zhang J, De Koninck Y. Spatial and temporal relationship between monocyte chemoattractant protein-1 expression and spinal glial activation following peripheral nerve injury. *Journal of Neurochemistry* 2006;97:772–783. [PubMed: 16524371]
- Zhang RX, Liu B, Wang L, Ren K, Qiao JT, Berman BM, Lao L. Spinal glial activation in a new rat model of bone cancer pain produced by prostate cancer cell inoculation of the tibia. *Pain* 2005;118:125–136. [PubMed: 16154703]
- 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 Behavior and Immunity*. 2006b in press.
- 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. [PubMed: 15733640]
- Zhuang ZY, Wen YR, Zhang DR, Borsello T, Bonny C, Strichartz GR, 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. *Journal of Neuroscience* 2006a;26:3551–3560. [PubMed: 16571763]

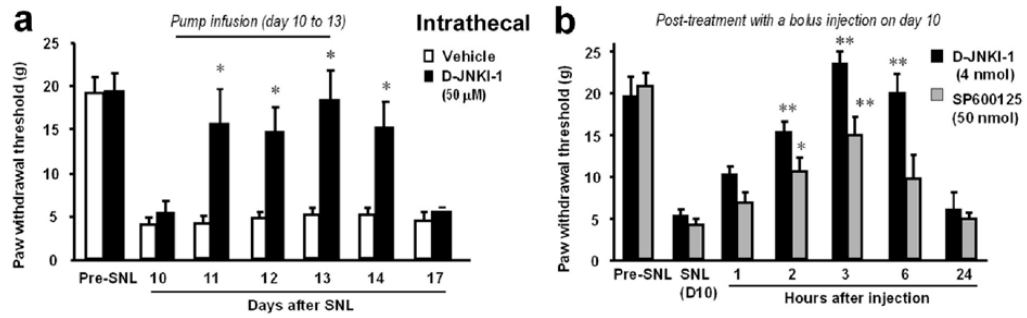


**Fig. 1. Spinal infusion of astroglial toxin alpha-aminoadipate (L-α-AA) blocks neuropathic pain** L-α-AA (10, 50, 150 nmol) was injected intrathecally 10 days after SNL. Mechanical allodynia, a major feature of chronic pain, is dose-dependently suppressed by the toxin. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ , compared to vehicle (saline) control; ANOVA;  $n = 6$ . Mechanical allodynia was tested using von Frey hairs. Reproduced, with permission, from (Zhuang *et al.*, 2006a).



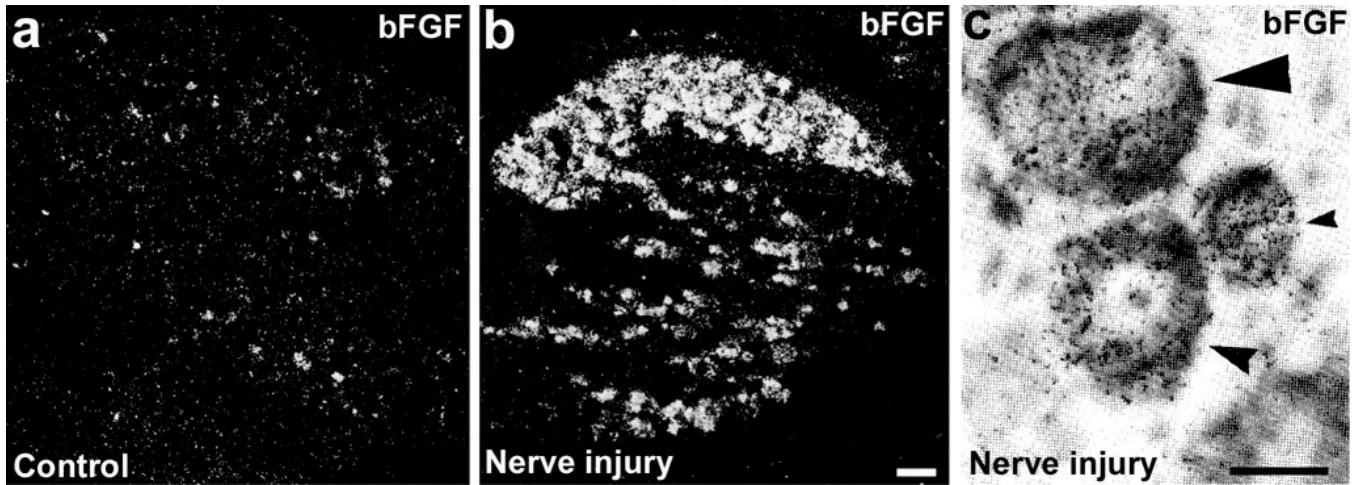
**Fig. 2. SNL induces persistent JNK activation in spinal astroglia**

(a,b) Immunohistochemistry reveals an increase in pJNK in the ipsilateral spinal dorsal horn (L5) 10 days after SNL. White lines indicate the border of the dorsal horn. Scale, 50  $\mu$ m. (c,d) High-magnification images of (a) and (b), respectively, showing pJNK staining in the medial superficial dorsal horn. Scale, 50  $\mu$ m. (e) Double immunofluorescence shows that pJNK (red) colocalizes with the astroglial marker GFAP (green) in the medial superficial dorsal horn. Two single-stained images are merged. c, d, e have the same magnification. (f) High-magnification image of (e) demonstrates colocalization of pJNK and GFAP. Note that some fine processes of astrocytes are labeled by pJNK but not by GFAP antibody. Scale bar, 25  $\mu$ m. Reproduced, with permission, from (Zhuang *et al.*, 2006a).

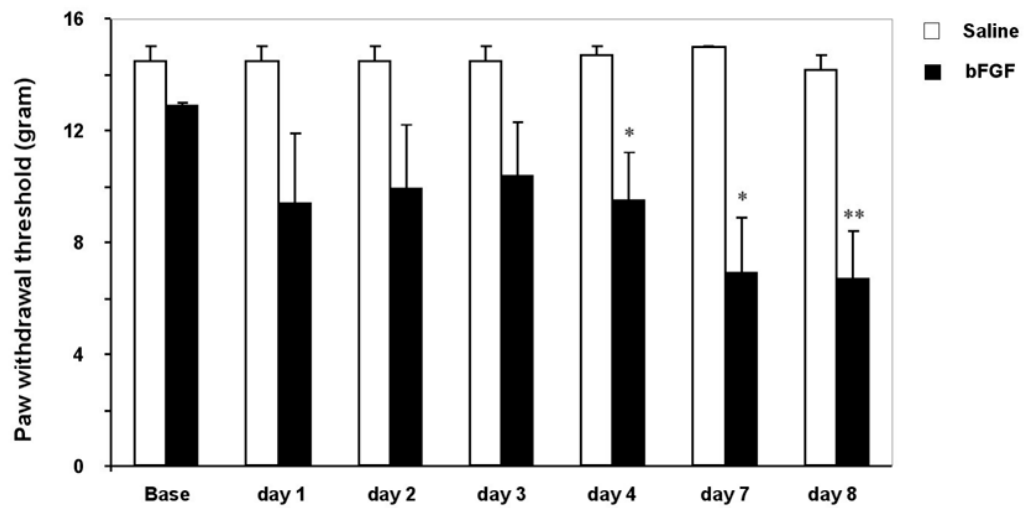


**Fig. 3. Spinal infusion of the JNK inhibitors reverses neuropathic pain after SNL**  
**(a)** Reversal of SNL-induced mechanical allodynia by intrathecal infusion of the peptide inhibitor of JNK D-JNKI-1 (50 μM) via an osmotic pump (0.5 μl hr<sup>-1</sup> for 3 days) starting 10 days after SNL. \*, *P*<0.05, compared to corresponding saline controls; *t*-test; *n*=5. **(b)** Reversal of SNL-induced mechanical allodynia by a bolus intrathecal injection of D-JNKI-1 (4 nmol) and SP600125 (50 nmol) 10 days after SNL. \*, *P*<0.05; \*\*, *P*<0.01, compared to corresponding pre-injection baseline; ANOVA; *n*=6. Note that the peptide inhibitor D-JNKI-1 is more potent than the small molecule inhibitor SP600125 in reversing allodynia. Reproduced, with permission, from (Zhuang *et al.*, 2006a).

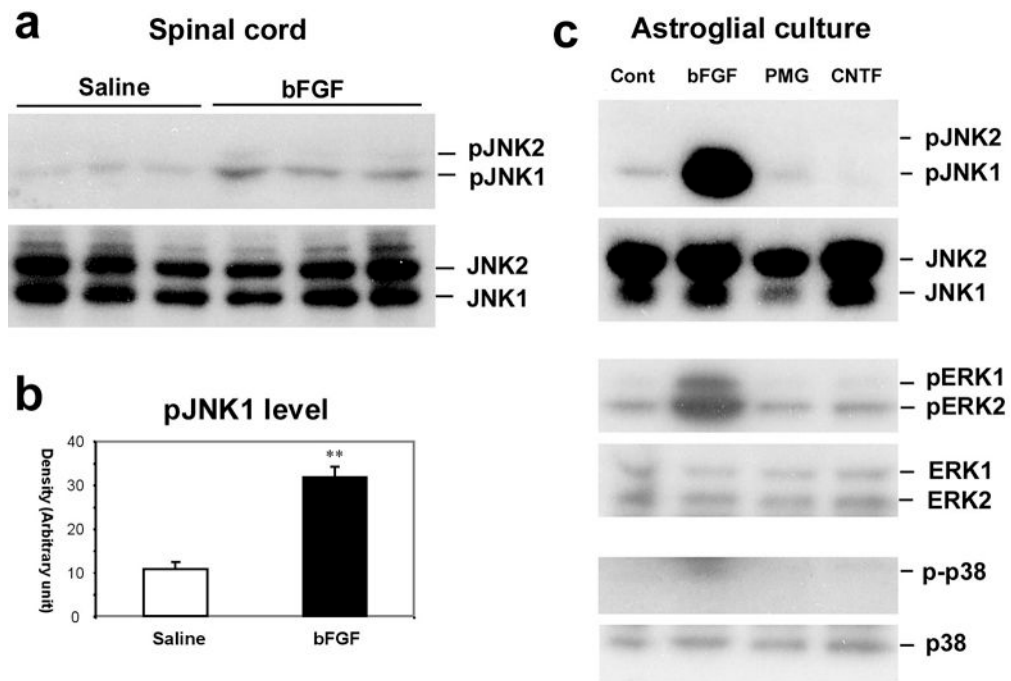




**Fig. 4. Nerve injury induces expression of bFGF mRNA in the dorsal root ganglion (DRG)** (a,b) Dark-field images following *in situ* hybridization show expression of bFGF mRNA in the control, noninjured DRG (a) and 3-day axotomized DRG (b). There are much fewer bFGF-positive cells in the control DRG than in the DRG after nerve injury. Scale bar, 100  $\mu$ m. (c) Bright-field image following *in situ* hybridization shows expression of bFGF mRNA in the injured DRG neurons. The section is counterstained with toluidine blue. Small, medium and large arrowheads indicate small, medium and large neurons, respectively. bFGF-positive cells are labeled with silver grains. The oligodeoxynucleotide probe for bFGF mRNA is labeled with  $^{35}$ S-dATP. Scale bar, 25  $\mu$ m. Reproduced, with permission, from (Ji *et al.*, 1995).



**Fig. 5. Spinal infusion of bFGF induces delayed but persistent mechanical allodynia**  
Either bFGF or saline was infused intrathecally via an osmotic pump ( $10 \text{ ng } \mu\text{l}^{-1} \text{ h}^{-1}$ ) for one week. \*\*,  $P < 0.01$  compared to saline control; unpaired  $t$ -test;  $n=6$ . Mechanical allodynia does not develop until day 4 and is maintained on day 8.



**Fig. 6. Activation of JNK by bFGF in astrocytes**

(a) Upper panel: Western blotting in the upper panel reveals an increase in pJNK1 levels in the spinal dorsal horn after bFGF infusion intrathecally via an osmotic pump ( $10 \text{ ng } \mu\text{l}^{-1}\text{h}^{-1}$ ) for one week. The spinal tissues were collected on day 8 after final behavioral testing (see Fig. 5). Note that only pJNK1 is expressed in the spinal cord. Lower panel: total concentration of JNK does not change after bFGF infusion. (b) Density of pJNK1 bands in (a), normalized to JNK1 loading control. \*\*,  $P < 0.01$  compared to saline control. (c) Western blotting shows that bFGF induces pJNK1, pERK1 and pERK2 in astrocytic cultures. By contrast, plasminogen (PMG) and ciliary neurotrophic factor (CNTF) do not activate JNK. p-p38 levels are barely detected in astroglial cultures following all the reagents. Astroglial cultures were prepared from brains of neonatal rats and maintained for 2–3 weeks. Cultures were stimulated with bFGF, PMG and CNTF (each at  $100 \text{ ng ml}^{-1}$ ) for 2 hours.

**Table I**Activation of MAPKs in spinal glial cells after SNL<sup>a</sup>

MAPKs	Early phase (3 days)	Mid phase (10 days)	Late phase (21 days)
ERK	Microglia	Microglia/Astrocytes	Astrocytes
p38	Microglia	Microglia	Microglia
JNK	Astrocytes	Astrocytes	Astrocytes

<sup>a</sup>Neuropathic pain is induced within 3 days (early phase), fully established at 10 days (mid phase) and maintained at 21 days (late phase).

**Table 2**  
**Signaling molecules in spinal astrocytes**

Most of them are regulated in chronic pain conditions and have a role in modulating chronic pain.

Signaling molecule	Change in expression in chronic pain	Role in chronic pain	Refs
pERK	Upregulation	Maintains neuropathic pain	Zhuang <i>et al.</i> , 2005
pJNK	Upregulation	Maintains neuropathic pain	Zhuang <i>et al.</i> , 2006a
p-c-Jun	Upregulation	Not tested	Zhuang <i>et al.</i> , 2006a
JNK1	Not tested	Not tested	Zhuang <i>et al.</i> , 2006a
Neurokinin-2 receptor	Not tested	Maintains neuropathic pain	Garry <i>et al.</i> , 2005
Interleukin-1 $\beta$	Upregulation	Maintains neuropathic pain	Zhang <i>et al.</i> , 2005
Glutamate transporter-I	Downregulation	Suppresses neuropathic pain	Sung <i>et al.</i> , 2003
Endothelin receptor-B	Upregulation	Not tested	Peters <i>et al.</i> , 2003
Connexin-43	Upregulation	Not tested	Lee <i>et al.</i> , 2005
bFGF	Upregulation	Maintains neuropathic pain	Madiai <i>et al.</i> , 2005