BRAIN RESEARCH REVIEWS 67 (2011) 282–310

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainresrev

BRAIN RESEARCH REVIEWS

Review

Cellular and molecular insights into neuropathy-induced pain hypersensitivity for mechanism-based treatment approaches

Julie V. Berger[a,b,1], *Liesbeth Knaepen*[a,1], *Sofie P.M. Janssen*[a], *Robby J.P. Jaken*[a], *Marco A.E. Marcus*[a], *Elbert A.J. Joosten*[a], *Ronald Deumens*[a,*]

[a]*Department of Anesthesiology, Maastricht University Medical Centre, Maastricht, The Netherlands*
[b]*Neuropharmacology, Institute of Neuroscience, Université catholique de Louvain, Brussels, Belgium*

ARTICLE INFO

Article history:
Accepted 18 March 2011
Available online 24 March 2011

Keywords:
Neuropathic pain
Chronic pain
Glia
Neuroplasticity
Mechanism
Nociceptive network
Sprouting
Inflammation
Microglia
Astrocyte
GABA
Disinhibition
Ectopic firing
PKC
Glutamate

ABSTRACT

Neuropathic pain is currently being treated by a range of therapeutic interventions that above all act to lower neuronal activity in the somatosensory system (e.g. using local anesthetics, calcium channel blockers, and opioids). The present review highlights novel and often still largely experimental treatment approaches based on insights into pathological mechanisms, which impact on the spinal nociceptive network, thereby opening the 'gate' to higher brain centers involved in the perception of pain. Cellular and molecular mechanisms such as ectopia, sensitization of nociceptors, phenotypic switching, structural plasticity, disinhibition, and neuroinflammation are discussed in relation to their involvement in pain hypersensitivity following either peripheral neuropathies or spinal cord injury. A mechanism-based treatment approach may prove to be successful in effective treatment of neuropathic pain, but requires more detailed insights into the persistence of cellular and molecular pain mechanisms which renders neuropathic pain unremitting. Subsequently, identification of the therapeutic window-of-opportunities for each specific intervention in the particular peripheral and/or central neuropathy is essential for successful clinical trials. Most of the cellular and molecular pain mechanisms described in the present review suggest pharmacological interference for neuropathic pain management. However, also more invasive treatment approaches belong to current and/or future options such as neuromodulatory interventions (including spinal cord stimulation) and cell or gene therapies, respectively.

Contents

* *Corresponding author*. Fax: +31 43 367 1096.
E-mail address: r.deumens@maastrichtuniversity.nl (R. Deumens).
[1] Both authors contributed equally to this work and are therefore joint first authors.

0165-0173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainresrev.2011.03.003

1. Introduction

Neuropathic pain following injury and/or dysfunction of the somatosensory system, either peripherally or centrally represents one of the most debilitating disorders of mankind (Treede et al., 2008). The prevalence of neuropathic pain depends on the type of related trauma/dysfunction. The majority of patients with peripheral neuropathies such as radiculopathies and postherpetic neuralgia (but not diabetic polyneuropathy) suffer from neuropathic pain, while spinal cord injury results in neuropathic pain in about 30–50% of cases (Baron et al., 2009; Radhakrishnan et al., 1994; Siddall et al., 2003; Tarulli and Raynor, 2007; Veves et al., 2008). Neuropathic pain can manifest itself as spontaneous and/or evoked pain. Although neuropathic pain is a common cause of chronic pain, treatment effects are still unsatisfactory. There are many pharmacological treatments for neuropathic pain, most of which predominantly act on neuronal signal transduction and include ion channel blockers, antidepressants, anticonvulsants, and opioids. These drugs give limited, if any, therapeutic benefit and patients may show tolerance and/or unwanted side effects (see Attal et al., 2009; Baron et al., 2010; Dworkin et al., 2007; Finnerup et al., 2010; Teasell et al., 2010), which depending on the drug can include nausea, sedation, constipation, tolerance, and dependence to name but a few. Therefore, neuropathic pain is still regarded as a chronic and intractable condition, which requires better management. Improved pain management relies on an interdisciplinary approach to understand better what is going on at a cellular and molecular basis following neuropathies. As such, mechanism-based treatment approaches are thought to result in more effective treatment of neuropathic pain (Baron et al., 2010). A surge of investigations using animal models of peripheral and central neuropathic pain has unveiled neuronal and non-neuronal mechanisms which act, most likely in synergy, to trigger and/or maintain neuropathic pain states. A crucial question to be answered relates to the persistence of such mechanisms, thus, rendering neuropathic pain unremitting. This review highlights mechanistic pathways which trigger and/or maintain pain hypersensitivity and sheds light on novel treatment approaches for more effective management of neuropathic pain.

2. Modeling neuropathic pain in rodents

A variety of rodent models exists for investigations of neuropathic pain. In most of these models trauma is induced to either the peripheral nervous system (PNS) or central nervous system (CNS). Injury to the PNS mostly involves mononeuropathies by ligation and/or transection of (branches of) peripheral nerves, spinal nerves, spinal roots or dorsal root ganglia (DRG). The most frequently used models include ligation/transection of the L5 and/or L6 spinal nerve (Chung model) (Chung et al., 2004; Kim and Chung, 1992), spared nerve injury

(ligation/transection of the tibial and common-peroneal nerve branches of the sciatic nerve, leaving the sural nerve branch intact) (Decosterd and Woolf, 2000), partial sciatic nerve ligation (Seltzer model) (Seltzer et al., 1990), and chronic constriction injury (CCI/Bennett model) (Bennett et al., 2000; Bennett and Xie, 1988). The common factor of these models is that only part of the sciatic nerve is injured. In addition, models of complete sciatic nerve injury (transection) are used to study neuropathic pain (Dowdall et al., 2005; Wall et al., 1979). Furthermore, injury to spinal roots has been described as well as injury to DRG for modeling radicular pain conditions (Hu and Xing, 1998; Li et al., 2002). Non-traumatic models of peripheral polyneuropathies include drug-induced toxic neuropathy (Peltier and Russell, 2002; Russell et al., 2001; Wallace et al., 2007) and metabolic neuropathy (Ahlgren and Levine, 1993; Calcutt et al., 1996). For the investigation of neuropathic pain after injury to the CNS, a range of animal models is available (also see Hulsebosch, 2005). Among these experimental models are so-called 'open injuries' (the spinal cord is penetrated resulting in partial or complete laceration/transection) and 'closed injuries' (the spinal cord is not penetrated, but rather compressed or contused). For the study of neuropathic pain after experimental spinal cord injury, merely incomplete spinal cord injuries are chosen. Spinal cord hemi-laceration/transection (Christensen et al., 1996) is the most frequently used 'open injury' model. For 'closed injuries' a wide range of models are used including spinal cord contusion (Gruner, 1992; Hulsebosch et al., 2000), spinal cord ischemia (Hao et al., 1991; Xu et al., 1992), excitotoxic spinal cord injury (Yezierski et al., 1998), and spinal cord compression (Bruce et al., 2002).

Pain hypersensitivity to mechanical and/or thermal stimuli is observed in most of the abovementioned animal models and used as signs and symptoms to study neuropathic pain. While this hypersensitivity is typically detected in the dermatomes which are/were innervated by the affected nerve(s) in peripheral neuropathies, its presence in spinal cord injury is rather different. In spinal cord injury, three subdivisions can be made, which are based on the location of the pain in relation to the level of the spinal injury: above-level, at-level, and below-level pain (Hulsebosch, 2005; Siddall et al., 2003). Pain hypersensitivity following peripheral or central neuropathies can be characterized by a decrease in the threshold required to elicit withdrawal responses to potentially harmful or noxious stimuli (i.e. hyperalgesia) and by withdrawal responses to innocuous stimuli, which do not normally elicit a withdrawal response (i.e. allodynia). In animal studies it is very difficult to discriminate between these two hypersensitivity symptoms. Interestingly, it has been claimed that the term 'mechanical allodynia' should only be used when it is known that the stimulus does not elicit nociceptor activity, and hence is mediated via low-threshold afferent fibers (Sandkuhler, 2009). For abovementioned reasons, we select the term 'hypersensitivity' for this review in the context of enhanced (withdrawal) responses evoked by peripheral tissue stimulation.

3. The spinal gate for pain signals to the brain

Investigations of the spinal cord are of particular relevance to studies on neuropathic pain because the spinal cord is regarded as the 'gateway' for the relay of nociceptive signals to higher brain centers where information about location, affective and motivational aspects of these signals is integrated and related to the stimulus. Noxious and innocuous stimuli are transmitted to the spinal cord via high-threshold fibers (non-myelinated C-fibers and thinly-myelinated Aδ-fibers from small-sized and medium-sized DRG neurons or also called 'nociceptors') and low-threshold fibers (highly-myelinated Aβ-fibers from large-sized DRG neurons), respectively. The termination patterns of these fibers in the spinal dorsal horn are highly organized as reviewed elsewhere (Wu et al., 2010). Nociceptors (peptidergic and non-peptidergic fibers) specifically innervate the superficial laminae (lamina I and the inner and outer lamina II) and deeper laminae (lamina V and VI), while Aβ-fibers selectively innervate deeper dorsal horn laminae (innermost lamina II and lower) or ascend to dorsal column nuclei in the brainstem immediately upon entering the spinal cord (Fig. 1). The superficial dorsal horn contains many nociception-specific (NS) neurons projecting to supraspinal areas involved in processing of spatial and emotional/affective aspects of nociception. In addition, inhibitory and excitatory interneurons, which are thought to modulate nociceptive signaling, reside in the superficial dorsal horn, particularly in lamina II: the substantia gelatinosa (Fig. 1). The deeper dorsal horn (lamina V) contains wide-dynamic-range (WDR) projection neurons which respond to both noxious and innocuous peripheral stimuli. At present we are beginning to understand characteristics of the various dorsal horn neurons, functional connections and nociceptive circuits within the dorsal horn. This information will be vital for our understanding of nociception, and may shed more light on the processes underlying neuropathic pain symptoms such as pain hypersensitivity.

The spinal dorsal horn contains a 'silent' circuit between low-threshold afferent fibers and NS projection neurons, which when activated, is suggested to 'turn touch into pain'. Up to now, the composition of this circuit has been only partly described. Of vital importance to the functioning of this circuit are putatively excitatory interneurons in the innermost part of lamina II, which express the γ-isoform of protein kinase C (PKC-γ) (Fig. 1). These interneurons receive innervations of low-threshold primary afferents such as Aβ fiber and C mechanoceptors (Neumann et al., 2008; Seal et al., 2009), and innocuous stimuli are thus able to activate PKC-γ interneurons via these fibers (Neumann et al., 2008). This information is not gated to NS projection neurons in the more superficial dorsal horn because PKC-γ interneurons are under inhibition of glycinergic and gamma-amino-butyric acid (GABA)ergic interneurons (Miraucourt et al., 2009). When glycine receptors were blocked under experimental conditions in naïve animals (using intrathecal strychnine), innocuous stimuli were found to induce early activation (cFos expression) in NS projection neurons, and more importantly, mechanical hypersensitivity was observed (Miraucourt et al., 2007). Notably, inhibition of PKC-γ as well as blockade of glutamate receptors of the N-methyl-D-aspartate (NMDA)-type prevented the activation of NS neurons following innocuous stimulation, showing the vital role of PKC-γ and NMDA receptors in the gating of Aβ fiber input to NS projection neurons (Miraucourt et al., 2007). Hence, 'touch can be turned

into pain' by means of activation and/or sensitization of a normally silent dorsal horn circuit containing PKC-γ interneurons, thereby gating Aβ fiber input to NS projection neurons. PKC-γ has been shown of specific importance to neuropathic pain as mice lacking this particular enzyme have an intact acute pain phenotype (i.e. respond normally to acute pain stimuli), but are strongly impaired in the development of hypersensitivity after peripheral nerve injury (Malmberg et al., 1997).

4. Neuropathy-induced modulation of the spinal pain gate

Nerve injury dramatically changes the way in which incoming signals are processed in the spinal cord. This is evidenced by a strong increase in expression of the early neuronal activation marker c-Fos in the superficial and deeper dorsal horn upon low-threshold mechanical stimulation of the hindpaw ipsilateral to a nerve injury (Zhang et al., 2007a). As mentioned above, the dorsal horn contains two different types of projection neurons: NS and WDR. In models of neuropathic pain (both peripheral and central neuropathies), dorsal horn neurons have been found to increase their firing patterns following innocuous and noxious stimulation of the skin (Carlton et al., 2009; Hains et al., 2004; Hains and Waxman, 2006; Keller et al., 2007). Also, a higher number of neurons with WDR characteristics have been noted (Hains et al., 2003b; Keller et al., 2007). The former phenomenon may relate to an altered input to the spinal neurons and/or intrinsic changes in the spinal neurons themselves (giving them a 'hyperexcitable' character). The latter phenomenon suggests that NS neurons undergo a functional switch from NS to WDR by acquiring characteristics of WDR neurons (i.e. they become responsive to innocuous stimuli).

The spinal dorsal horn is the gate for pain signals and its enhanced activity is, thus, of vital importance to the understanding of neuropathic pain. In this review, we particularly focus on possible alterations in the input to dorsal horn neurons following peripheral nerve injury and spinal cord injury. This information is then used to elaborate on novel treatment options based on such pathological mechanisms. Altered input into dorsal horn 'gate' neurons can (1) derive from changes in peripheral afferents (e.g. by enhanced

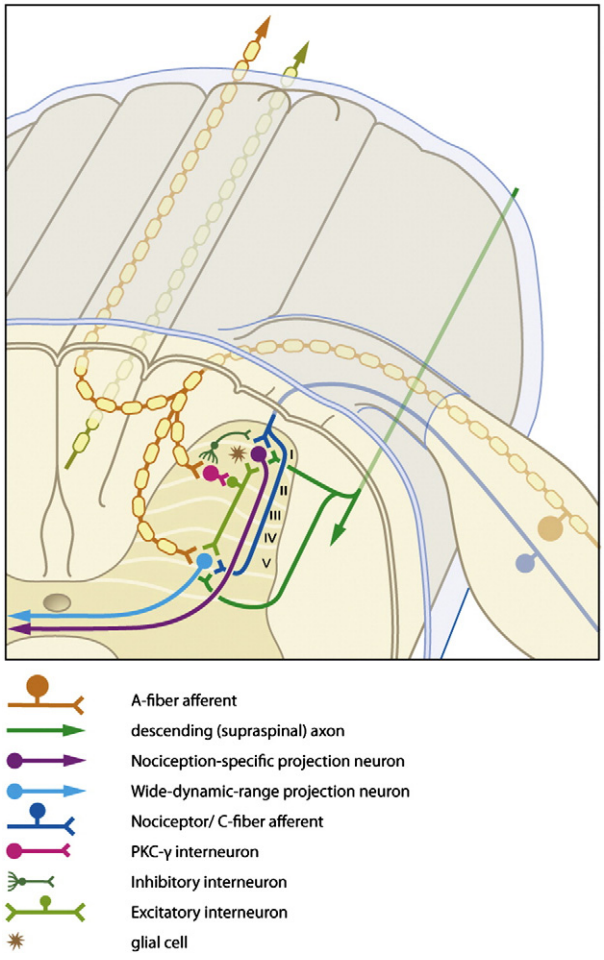

Fig. 1 – The spinal nociceptive network. Two main types of projection neurons reside in the dorsal horn: NS in the superficial dorsal horn and WDR in the deeper dorsal horn. These projection neurons receive input from primary afferents, descending (aminergic) pathways, and spinal interneurons. Primary afferents are either low-threshold such as highly-myelinated Aβ fibers from large-sized DRG neurons and unmyelinated mechanoceptive C fibers from small-to-medium-sized DRG neurons or high-threshold such as thinly-myelinated Aδ fibers from medium-sized DRG neurons and unmyelinated C fibers from small-sized DRG neurons. Descending (aminergic) fibers are mostly serotonergic and noradrenergic, derive from supraspinal structures (such as the nucleus raphe and locus coeruleus), and predominantly modulate (via inhibitory and/or excitatory effects) the processing of nociceptive signals in the spinal nociceptive network. The spinal nociceptive network also contains numerous interneurons, both of excitatory and inhibitory nature, which largely complicate the processing of nociceptive signals at the 'gate to the brain'. The spinal nociceptive network also contains a silent circuit between low-threshold primary afferents and NS projection neurons. This circuit, which contains interneurons expressing PKC-γ is normally inactive, but is thought to be activated under neuropathic conditions and, as such, 'turns touch into pain'. Finally, it is now generally accepted that glial cells, which are found near neuronal synapses can actively participate in communications between neurons as they do not only express receptors for neurotransmitters but also contain synaptic vesicles with neurotransmitters. In conditions of nerve injury, these glial cells (microglia and astrocytes) release a wide range of neuroactive (immune-related) modulators, which have substantial effects on neuronal signal transmission. NS, nociception-specific; WDR, wide-dynamic-range; DRG, dorsal root ganglion; A-fiber afferent, Aβ-large-sized DRG neuron; Aδ, Aδ-medium-sized neuron; C-fiber afferent, C-small-sized DRG neuron; Nociceptor, C-fiber afferent and Aδ-fiber afferent; PKC-γ, protein kinase Cγ.
Art-design: Rogier Trompert, medical illustrator.

excitatory input), (2) be due to the phenomenon of 'disinhibition', and/or (3) be related to the action of neuromodulatory substances (which can modulate excitatory and/or inhibitory input or have direct effects on the activity of spinal nociceptive neurons).

4.1. *Neuropathy-induced changes in peripheral afferents and hypersensitivity*

Enhanced peripheral input can derive from an increase in the activity of peripheral afferents due to spontaneous electrical activity referred to as *ectopia* or due to their sensitization (i.e. peripheral sensitization leading to hyperexcitability to an incoming stimulus). Ectopia and (peripheral) hyperexcitability are different as the latter requires a stimulus, while the former does not. The generation of spontaneous electrical discharges which follows both peripheral and central neuropathies typically originates at ectopic places along the nociceptive pathway, including the DRG, the spinal cord and the thalamus as well as at the nerve injury site (Bedi et al., 2010; Carlton et al., 2009; Govrin-Lippmann and Devor, 1978; Hains et al., 2004; Zhao et al., 2007b). Ectopia is thought to originate from a blockade in the trafficking of voltage-operated ion channels upon axon damage (Devor, 2009), although this may not be the sole explanation because uninjured fibers have also been reported to display ectopia (Wu et al., 2001). Membrane insertion of ion channels following axotomy renders high channel densities at the site of nerve injury and/or DRG (Devor, 2006b; Devor, 2009). As a result, the electrogenicity of the primary afferent neuron is altered, leading to pacemaker actions and this may relate to some extent to the spontaneous oscillations in membrane potentials below the threshold for generating action potentials, which are thought to underlie neuropathy-induced ectopia (Amir et al., 1999, 2002a, 2002b; Kovalsky et al., 2008, 2009; Liu et al., 2000, 2002). With respect to peripheral sensitization, several processes have been described which lead to hyperexcitability of peripherally located primary afferent neurons projecting to the spinal cord. These processes occur either at the peripheral nerve ending or at the level of the DRG. Many, but not all, of these changes involve alterations in ion channels (Cummins and Waxman, 1997) and receptors, and have been reviewed elsewhere (Cregg et al., 2010). Such alterations include both the expression and the phosphorylation-status of nociceptor receptor proteins (e.g. transient-receptor-potential-vanniloid-1 (TRPV1)).

Additionally, peripheral nerve injury may trigger 'phenotypic switching' of primary afferent fibers by *de novo* expression of proteins. Such molecular changes may occur at the peripheral and/or central nerve endings of nociceptors, but also at the somata located within the DRG. These neuronal somata are surrounded by satellite glial cells, and as such form distinct functional units (Hanani, 2005). Molecular changes within the DRG may form the basis of a first level of (neuro) pathological change in the modulation of innocuous and noxious signaling (Zimmermann, 2001). Of particular relevance is the phenotypic switching of primary afferents with *de novo* expression of neuromodulators such as substance P, calcitonin gene-related peptide (CGRP), and brain-derived neurotrophic factor (BDNF) in large-sized neurons following peripheral nerve injury (Ma et al., 1999; Malcangio et al., 2000; Marchand et al., 1994; Michael et al., 1999; Weissner et al., 2006). These neuromodulators are of particular relevance to sensitization of dorsal horn neurons (see also Latremoliere and Woolf, 2009).

Beside ectopia, peripheral sensitization, and phenotypic switching of primary afferents, also structural plasticity of primary afferent fibers has been reported under neuropathic conditions. Reported phenomena include (1) collateral sprouting of afferents in the skin (and other tissues) in the event of partial peripheral nerve injury (Diamond et al., 1992; Kingery and Vallin, 1989; Ro et al., 1999), (2) sprouting of sympathetic fibers within the DRG (triggering spontaneous electrical activity in DRG, see 'ectopia' below) (Deng et al., 2000; Jones et al., 1999; Ramer et al., 1999), and (3) sprouting of primary afferents in the spinal cord following both peripheral (Doubell and Woolf, 1997) and central neuropathies (Christensen and Hulsebosch, 1997a; Christensen and Hulsebosch, 1997b).

The enhanced excitability of peripheral afferent neurons, their spontaneous activity, and structural plasticity of these neurons can have radical consequences on the projection territories of these neurons within the CNS. Central sensitization is an inevitable and cardinal consequence in the light of neuropathy-induced pain hypersensitivity.

4.2. *Neuropathy-induced disinhibition at the spinal gate*

The spinal nociceptive network receives inhibitory input from two main sources: non-segmental inhibitory systems (such as descending pathways originating from supraspinal loci) and segmental inhibitory systems (including inhibitory interneurons). Alterations to any of these systems may render a condition of disinhibition (Saade and Jabbur, 2008; Suzuki et al., 2004). These alterations can relate to reduced activity of inhibitory neurons and/or to a different response of spinal neurons to neurotransmitters which are classically regarded as inhibitory neurotransmitters.

4.3. *Neuropathy-induced neuromodulation: central role of neuroinflammation*

The view on neuropathic pain has seen a revolutionary shift when it was discovered that non-neuronal cells are not simple by-standers in the processing of sensory neurotransmission, but play an active role or even exacerbate the enhanced interneuronal signaling in pathological conditions such as neuropathies (Austin and Moalem-Taylor, 2010; Scholz and Woolf, 2007; Watkins and Maier, 2002). Neuroinflammatory responses are highly complex and are mediated by neuronal and non-neuronal cells which act in synergy following pathological cues (Scholz and Woolf, 2007). Oligodendroglia, astroglia, and microglia constitute the major non-neuronal cell types in the CNS, but also bone marrow-derived cells including lymphocytes can invade the CNS, even after injury to peripheral nerves (Cao and DeLeo, 2008; Costigan et al., 2009; Zhang et al., 2007b). Immune cells such as endogenous glia are considered to largely influence the signal conduction between neurons (De Leo et al., 2006). The intimate association between astroglia and synapses (giving rise to the so-called tripartite synapse (Haydon and Carmignoto, 2006; Perea et al., 2009) enables astroglia to handle

neurotransmission, e.g. by buffering glutamate at glutamatergic synapses. Microglia, on the other hand, are found around blood vessels (perivascular microglia) and within the CNS parenchyma. Microglia have a main role in immune surveillance as they continuously screen the CNS environment and can rapidly respond to disturbances in homeostasis (Davalos et al., 2005; Nimmerjahn et al., 2005).

Inflammatory communications between neurons, local glial cells and blood-borne inflammatory cells are largely orchestrated by a family of cell-signaling proteins known as cytokines and chemotactic cytokines (chemokines). Cytokines/chemokines and other neuromodulators are up-regulated in peripheral nerves and also in the spinal cord within hours to days following peripheral neuropathies (Jeon et al., 2009; Mika et al., 2008; Okamoto et al., 2001; Rothman et al., 2009; Schafers et al., 2002) and injury to the CNS (Donnelly and Popovich, 2008; Streit et al., 1998). Several lines of evidence suggest that these neuromodulators contribute to pain hypersensitivity. First, cytokines/chemokines can increase the expression and release of neurotransmitters by primary afferent fibers (Qin et al., 2005; Skoff et al., 2009). Second, these mediators can induce electrical discharges in primary afferent fibers, especially following injury (Schafers et al., 2003a), or sensitize such fibers (Jung et al., 2008). Third, cytokines and chemokines have been shown to trigger spontaneous excitatory post-synaptic potentials and to modulate currents induced by excitatory and inhibitory neurotransmitters in spinal cord neurons (Gao et al., 2009; Kawasaki et al., 2008b). Fourth, intrathecal delivery of interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α) increases WDR activation in naïve animals (Reeve et al., 2000). Fifth, pain hypersensitivity has been observed following intrathecal injection of exogenous fractalkine (CX3CL1), CCL-2, IL-1β and TNF-α (Dansereau et al., 2008; Milligan et al., 2004; Reeve et al., 2000; Xu et al., 2010; Youn et al., 2008). Hence, these and other neuromodulators such as prostaglandins, nitric oxide and excitatory amino acids, released in the CNS during a neuropathy-induced inflammatory response can all contribute to pain hypersensitivity (Hains et al., 2001b; Zhao et al., 2007a).

5. Management of neuropathic pain: which way to go?

Neuropathy-induced pain hypersensitivity is, thus, mediated by an imbalance between excitatory and inhibitory input into the nociceptive circuit in the spinal dorsal horn. Dorsal horn neurons show an increased activity to incoming signals. This increased activity might (1) directly relate to altered input, but can (2) also be derived from intrinsic molecular changes in the spinal neurons themselves (i.e. the neurons have become hyperexcitable) (Devor, 2006a). In respect to the molecular basis for dorsal horn neuron hyperexcitability (central sensitization), the reader is referred to other reviews (Latremoliere and Woolf, 2009; Woolf, 2011) as this topic is only briefly addressed here. The main neurotransmitter system involved in signaling between primary afferent fibers and dorsal horn neurons is the glutamatergic system. Upon binding its ionotropic receptors (such as the NMDA, AMPA and kainate receptors), glutamate induces membrane depolarization. Hyperexcitability of dorsal horn neurons is thought to be at least partly based on an increase in the expression or phosphorylation of these glutamate-operated ion channels (Gao et al., 2005; Ultenius et al., 2006). Especially the NR2B subunit of the NMDA receptor is suggested to largely affect pain hypersensitivity, a role which it probably owes to its preferential expression in the superficial dorsal horn (Nagy et al., 2004; Qu et al., 2009; Shiokawa et al., 2010. As such, the NR2B subunit or its phosphorylation may be antagonized for effective attenuation of neuropathic pain (De Vry et al., 2004). An exciting novel mechanism that has been suggested as being implicated in sensitization processes and pain hypersensitivity after nerve injury involved scaffolding proteins. Because this mechanism is still relatively unexplored, it will be only briefly addressed here. Scaffolding proteins function to assemble synaptic intracellular signaling complexes and are thus essential for synaptic transmission. Protein interacting with C kinase 1 (PICK1) is a scaffolding protein which is expressed in about 30% of DRG neurons (primarily peptidergic and non-peptidergic nociceptors) and in pre- and postsynaptic structures within the superficial dorsal horn (Wang et al., 2011). Mechanical and thermal pain hypersensitivity following spinal nerve injury is strongly impaired in genetically modified mice lacking PICK1 and in rats which are intrathecally treated with antisense oligonucleotides against PICK1 (Wang et al., 2011), but mechanisms remain unclear to date. Nevertheless, interference with scaffolding proteins in the nociceptive system may hold great promise as an innovative treatment approach for neuropathic pain management.

This review focuses on the cellular and/or molecular mechanisms driving (peripheral and/or central) neuropathy-induced alterations of the input to spinal projection neurons, and possibly also hyperexcitability of these neurons. A deeper understanding of such mechanisms will aid in the development of effective therapeutic approaches for management of neuropathic pain. Insights into the persistence of such mechanisms are further important for the identification of therapeutic windows-of-opportunity in relation to such mechanisms. Many of the currently used pain killers preferentially target neuronal signaling *per se*, but most likely do not affect many other pathological processes which affect pain hypersensitivity following neuropathies.

Nerve trauma or disease elicits a wide range of cellular and molecular changes at several levels along the neuraxis as discussed in this review. This holds true for both peripheral and central neuropathies, although the exact nature of changes may differ between these two categories of neuropathies and heterogeneity even exists within such categories. The initial and trivial cause of neuropathic hypersensitivity is the nerve injury itself (Klusakova and Dubovy, 2009). As such, one may argue that neuropathic pain is maintained as long as the neurotrauma and/or the putative neurotrauma-induced cell loss (e.g. loss of inhibitory interneurons) remain present. 'Curing' of neuropathic pain might, then, require (1) neurorepair or (2) replacement of lost neurons such as inhibitory interneurons. Indeed, when primary afferent fibers fully regenerate (as in nerve crush injuries), pain hypersensitivity resolves (Decosterd and Woolf, 2000). As such, repair-intervention for peripheral nerve injury holds great promise in management of peripheral neuropathic pain. Today,

autologous nerve grafting is still the gold-standard treatment for peripheral nerve defects, but alternatives are increasingly explored (Deumens et al., 2010). Such alternatives mostly involve nerve guidance tubes or scaffolds with or without seeding of growth promoting cells (Gu et al., 2010). For obvious reasons such non-conventional and invasive treatment options do not belong to first-line options. Nevertheless, there are concrete ideas on managing neuropathic pain by such invasive techniques (as discussed below). Alternatively, one may turn to more conventional treatments such as pharmacotherapies, but with better therapeutic targets at appropriate locations and at appropriate timing. Effective management of neuropathic pain is dependent on at least three interrelated issues: targeting the appropriate cellular/molecular cue(s), targeting at appropriate location(s), and targeting at appropriate time(s).

6. Therapeutic approaches in painful peripheral neuropathies

An excellent and updated review article has been recently published describing the currently available drugs, such as antidepressants, calcium channel α2-δ blockers (including gabapentin and pregabalin), and opioids to treat painful peripheral neuropathies (Baron et al., 2010). Also novel drugs in these and other categories are currently being tested or have already completed clinical trials (see http://clinicaltrials.gov/). From the perspective of cellular and molecular mechanisms which have been claimed to underlie neuropathic pain, we here focus on novel, often still experimental pharmacological approaches to improve management of peripheral neuropathic pain.

6.1. *Treating ectopia*

Ectopia has been shown to be rather persistent in nature as it was observed as early as a few hours after peripheral nerve injury and up to more than 7 months (Govrin-Lippmann and Devor, 1978). Following peripheral nerve injury, ectopia is carried mostly by Aβ fibers, although C fibers also show ectopia (Devor, 2009; Wu et al., 2001). Although ectopia is thought to result from changes in the expression of voltage-gated ion channels, it is unlikely that a pathological change relating to only a single ion channel can account for neuropathy-induced hypersensitivity. Indeed animals lacking $Na_v1.3$, $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ voltage-gated sodium channels (Abrahamsen et al., 2008; Nassar et al., 2004, 2005, 2006; Priest et al., 2005) show normal development of pain hypersensitivity following peripheral nerve injury and, if studied, intact ectopia. However, it cannot be excluded that ablation of single ion channels induces compensatory changes in the expression of other ion channels. Remarkably, intrathecal treatment with anti-sense oligonucleotides against $Na_v1.3$ was reported to partly reverse established pain hypersensitivity following CCI, an effect which was lost when treatment was ceased (Hains et al., 2004). WDR neurons in the spinal dorsal horn were furthermore found to show reduced hyperactivity to noxious and innocuous stimuli during $Na_v1.3$-anti-sense treatment (Hains et al., 2004).

Treatment of the injured nerve with local anesthetics, which interfere with voltage-gated ion channels and attenuate or even abolish ectopia, has been shown to attenuate or even eliminate mechanical hypersensitivity following peripheral nerve injury (Jang et al., 2007; Sukhotinsky et al., 2004). This treatment paradigm was proven to be only effective when given immediately after injury and if the nerve was effectively blocked for several days (Xie et al., 2005). A recent study, however, showed that daily intrathecal delivery of the local anesthetic ropivacaine was effective in reversing pain hypersensitivity when given after the first week post-CCI (Toda et al., 2011). An interesting observation in studies on the efficacy of local anesthetics in peripheral neuropathic pain management is that the relief of pain hypersensitivity induced by local anesthetics often exceeds the duration in which local anesthetics are predicted to affect voltage-gated ion channels according to pharmacokinetics (Arner et al., 1990; Chaplan et al., 1995; Tian et al., 2009). These findings suggest that treatment of ectopia using local anesthetics may additionally affect cellular and/or molecular processes contributing to pain hypersensitivity, which are to a certain extent driven by ectopia (see also section 'Novel approaches to achieve anti-inflammatory effects') (Toda et al., 2011; Wen et al., 2007). Nevertheless, the duration of pain relief is only temporary when treatment is ceased. Hence, treatment of ectopia may only be efficacious with development of durable drug delivery systems, such as implantable systems which can act as drug reservoirs and can be activated 'on demand' by external cues (Keurentjes et al., 2009).

At present, a phase IV clinical trial is recruiting participants for studying predictors of response to topical lidocaine patches, which act to block sodium channels, in patients with peripheral nerve injury. A further phase IV clinical trial has recently been completed in which lidocaine patches, gabapentin or their combination was tested for pain relief in patients with diverse peripheral neuropathic conditions (see http://clinicaltrials.gov/).

6.2. *Peripheral sensitization as a therapeutic target*

At the level of sensory nerve endings, nociceptor proteins mediate the response to peripheral stimuli. The transient receptor potential vanilloid 1 (TRPV1), a non-selective cation channel activated by the active ingredient of hot chili peppers, capsaicin, but also by noxious heat, protons (low pH) and endogenous lipids, is one of the best-known nociceptor proteins. Although it may be reasoned that sensitization of nociceptor proteins (e.g. by phosphorylation) may affect pain hypersensitivity after nerve injury, mice lacking functional TRPV1 receptors show preserved mechanical and thermal hypersensitivity following partial sciatic nerve ligation (Caterina et al., 2000). Nevertheless, a phase II randomized, double-blind, placebo-controlled trial of topical capsaicin in treatment of painful diabetic neuropathy is presently recruiting participants. Additionally, a phase IV multi-center, open-label study of repeated administration of capsaicin (Qutenza) patches in treatment of peripheral neuropathic pain is recruiting participants (see http://clinicaltrials.gov/). This therapeutic approach aims to deplete TRPV1-expressing peripheral afferents from substance P, hence interfering with nociceptive

signals to the CNS. A further hurdle to tackle when interfering with particular fiber populations in management of neuropathic pain is the fact that there are only few investigations which provide insight in the nature of fiber populations which are needed for (the modality of) neuropathy-induced pain hypersensitivity. A recent report showed that a small subset of C-fibers responding to low-threshold mechanical stimuli (low-threshold mechanoreceptors; LTMR) are required for the development of mechanical, but not thermal hypersensitivity following peripheral nerve injury (Seal et al., 2009). Further insights into the molecular profile of these fibers may open possibilities to selectively target them for neuropathic pain management. Alternatively, another recent investigation demonstrated that primary afferent fibers expressing vesicular glutamate transporter 2 (VGLUT2) are required to develop thermal, but not mechanical hypersensitivity following CCI (Scherrer et al., 2010).

Growth factors in primary afferent fibers are considered to facilitate sensitization in animal models of neuropathic pain. Particularly in animal models of diabetic polyneuropathy, nerve growth factor (NGF) has been found to be up-regulated in the sciatic nerve and DRG (Cheng et al., 2009; Fernyhough et al., 1995). NGF is known to enhance expression of neuropeptides such as CGRP and substance P (Schmidt et al., 1995), and may thereby enhance nociceptive signal processing. Intraperitoneal delivery of an anti-NGF antibody could attenuate the up-regulation of substance P in the DRG as well as decrease pain hypersensitivity in a mouse model of diabetic polyneuropathy (Cheng et al., 2009). However, long-term treatment with anti-NGF antibodies is clinically not feasible as it may trigger adverse side-effects and may strongly interfere with beneficial effects of NGF, including trophic effects on axons.

6.3. *Phenotypic switching as a therapeutic target*

The *de novo* expression of neuromodulators in large-sized primary afferents may strongly affect the processing of noxious and innocuous signals, particularly at the level of the DRG. It, nevertheless, remains largely unknown as to how persistent such phenotypic switches are and whether such changes are imperative to neuropathy-induced pain hypersensitivity (Hughes et al., 2007), although increases in the expression of neuropeptides within the DRG have been reported for weeks after peripheral nerve injury (Kim et al., 2009). Nevertheless, expression of BDNF in primary afferents was found not to be required for pain hypersensitivity following peripheral nerve injury (Zhao et al., 2006). Hence, many more studies are needed to prove the role of phenotypic switching of neuronal cell populations in pain hypersensitivity before putative therapeutic targets can be described.

6.4. *Structural plasticity as a therapeutic target*

Structural plasticity in primary afferent fibers (in peripheral tissues, within the DRG and/or in the spinal cord) may have an imperative impact on the processing of noxious and innocuous information, and hence, on pain hypersensitivity following nerve injury. Structural plasticity is most likely persistent in nature, and more research may thus be needed to explore the molecular and/or cellular cues which trigger structural plasticity. This kind of knowledge may open avenues in preventive medicine rather than therapeutic medicine. About thirty years ago, Clifford Woolf discovered that peripheral nerve injury strongly induced the protein expression of growth-associated-protein-43 (GAP-43) in both the DRG and superficial dorsal horn of the spinal cord (Woolf et al., 1990). GAP-43 is traditionally regarded as a marker of sprouting fibers, but can also be indicative of regeneration or neuronal plasticity (Oestreicher et al., 1997). A relation between structural plasticity and pain hypersensitivity was claimed when peripheral nerve injury was suspected to trigger a growth response ('sprouting') of Aβ fibers into the superficial spinal cord, which is normally exclusively innervated by nociceptive primary afferent fibers (Doubell et al., 1997; Lekan et al., 1996; Nakamura and Myers, 1999; Shortland et al., 1997; Woolf et al., 1992, 1995). It is now known that bias in fiber tracing can account for this phenomenon (Bao et al., 2002), and single-Aβ-fiber tracing studies have now shown that Aβ-fibers never extend beyond the ventral most portion of lamina II which is occupied by PKC-γ neurons (Hughes et al., 2003). More recent experiments have now pointed to the regulation of spinal GAP-43 protein expression in peptidergic primary afferents (CGRP-positive fibers; Jaken et al., unpublished data). These data may be particularly relevant to pain hypersensitivity as CGRP is a neuromodulator which has been reported to increase the release of neurotransmitters from primary afferent fibers and to regulate the expression of receptors on spinal neurons including the NK1 receptor for substance P (Oku et al., 1987; Seybold et al., 2003; Seybold, 2009). Structural neuroplasticity of CGRP fibers within the spinal nociceptive network may form an unremitting source of central sensitization. Also, structural plasticity in peripheral tissues and DRG may contribute to persistent changes in the input to the spinal nociceptive network, but these issues are not further discussed in the present review. Prevention of plastic changes may aid in avoiding chronification of neuropathic pain symptoms.

6.5. *Spinal disinhibition as a therapeutic target*

Several neuronal systems have been claimed to be functionally compromised in conditions of painful peripheral neuropathies and may therefore present novel valuable therapeutic opportunities. As a non-segmental (supraspinal) system, the serotonergic pathway has been shown to be compromised following peripheral neuropathies. The major spinal projection of serotonin (5-hydroxytryptamine, 5-HT)-producing axons originates from the nucleus raphe magnus and the adjacent nucleus reticularis gigantocellularis at the rostroventral medulla (RVM) of the midbrain (Millan, 2002). Although this projection is thought to mainly modulate spinal nociceptive networks via exerting inhibitory effects, also excitatory effects have been claimed to exist (Suzuki et al., 2004). A recent study showed that molecular depletion of descending 5-HT reversed established mechanical and thermal hypersensitivity following peripheral nerve injury in the rat (Wei et al., 2010). These data imply that 5-HT has a predominant excitatory effect on spinal nociceptive networks in conditions of peripheral neuropathy and, hence, should be

antagonized in treatment of neuropathic pain. This knowledge is particularly relevant bearing in mind that first-line therapies for neuropathic pain management (such as tricyclic antidepressants (TCA), and selective serotonin-reuptake inhibitors (SSRI) or serotonin-norepinephrine-reuptake inhibitors (SNRI)) aim at increasing 5-HT levels.

The segmental (spinal) GABAergic system was reported to be affected following peripheral nerve injury. GABA-mediated inhibitory post-synaptic currents (IPSCs) of dorsal horn neurons triggered by primary afferent stimulation have been shown to decrease following partial but not complete peripheral nerve injury (Moore et al., 2002; Scholz et al., 2005). These reduced GABA-IPSCs may result from (1) excitatory rather than inhibitory GABA-induced currents (Price et al., 2005) and/or (2) attenuated release of GABA.

With respect to the former, the anion gradient of dorsal horn neurons was found to be altered under neuropathic conditions. This shift in anion gradient has dramatic effects for GABA signaling. While normally GABA signaling induces a hyperpolarization of postsynaptic membranes by acting upon $GABA_A$ receptors, the shift in anion gradients following nerve injury causes GABA-induced depolarizing currents in lamina I neurons (Coull et al., 2003). This depolarizing shift of GABA-induced membrane currents has been explained by a down-regulation of the potassium-chloride co-transporter KCC2 (Coull et al., 2003), which is also observed in other neuropathic models including an animal model of diabetic neuropathy (Jolivalt et al., 2008). Chloride ions are pumped into and out of cells by NKCC1 and KCC2 transporter proteins, respectively. Down-regulation of KCC2, thus, leads to increased intracellular chloride levels. A clear insight into the spatio-temporal characteristics of KCC2 down-regulation after nerve injury is, however, still lacking.

With respect to the latter, primary afferent-evoked GABA release is indeed attenuated following peripheral nerve injury (Lever et al., 2003), although the electrophysiological properties of GABAergic cells are unaltered in peripheral neuropathic conditions (Schoffnegger et al., 2006). Also, basal extracellular GABA levels have shown to be decreased under neuropathic conditions (Stiller et al., 1996), which, in light of potentially dysfunctional GABA release, may explain findings of increased intracellular levels of GABA (Janssen et al., unpublished data). Changes in GABA receptor protein expression are largely uncertain (Engle et al., 2006; Moore et al., 2002; Polgar and Todd, 2008) but, if present, are most likely based on a cellular response to altered (i.e. decreased) extracellular GABA levels and therefore not considered in detail here. It has been argued that GABAergic cells are prone to apoptosis under conditions of neuropathy, but the evidence is not extensive (Meisner et al., 2010; Scholz et al., 2005). In even further contrast has it been argued that there is no change in the number of GABAergic cells in the spinal dorsal horn following nerve injury (Polgar et al., 2003, 2005). Hence, loss of GABAergic cells is unlikely to account for the evident reduction in GABA release following peripheral nerve injury. Alternatively, reduced GABA production as well as compromised release of the synthesized GABA may be responsible for the reduced GABA levels. Indeed, a reduction in the spinal protein expression of the rate-limiting enzyme in GABA synthesis, glutamate decarboxylase (GAD), present in isoform-65 and isoform-67, was reported up to two weeks following peripheral nerve injury (Moore et al., 2002). The 'causes' of reduced GAD protein expression remain unclear, although cytokines have been reported to influence the protein expression of GAD in non-neural tissue (Schmidli et al., 1996). Exogenous administration of GABA by intrathecal injection of either GABA itself or bio-engineered GABA-producing neuronal cells has been found to largely prevent pain hypersensitivity but also reverses established pain hypersensitivity in an animal model of peripheral nerve injury (Eaton et al., 1999a, 1999b). Optimal treatment effects were claimed to occur when GABAergic cell grafts were transplanted within two weeks after onset of the peripheral neuropathy (Stubley et al., 2001). Also human neural precursor cells that were expanded and differentiated into a GABAergic phenotype *in vitro* and subsequently transplanted intraparenchymally into the lumbar spinal cord at 10 days after spinal nerve ligation induced a significant alleviation of pain hypersensitivity that was maintained for at least six weeks (Mukhida et al., 2007). Despite of these promising findings, the clinical feasibility of cell transplantation remains unclear as it is highly invasive and potential adverse side-effects are only poorly described. Increased spinal levels of extracellular GABA have also been achieved experimentally using spinal cord stimulation (SCS) (Cui et al., 1997; Stiller et al., 1996). SCS has been proven effective in management of neuropathic pain, where patients with complex regional pain syndrome were found to benefit from SCS for up to many years (Kemler et al., 2000; Kemler et al., 2008).

6.6. *Modulation of the neuroinflammatory response*

Neuropathy-induced glial responses are typically very persistent in nature as they have been observed for many months after injury (Coyle, 1998; Deumens et al., 2009; Zhang and De Koninck, 2006). Recent work has indicated that the chronicity of microglial responses to peripheral nerve injury may be related to a substantial and long-term down-regulation in the protein expression of G-protein-coupled-receptor kinase-2 (GRK-2) which normally acts to restrict neuroinflammatory processes (Eijkelkamp et al., 2010). Not only was GRK-2 down-regulation observed in an animal model of (chronic) neuropathic pain (i.e. spinal nerve injury), but pain hypersensitivity following inflammatory pain (including intraplantar injections with carrageenan or with IL-1β) was substantially prolonged in mice with an approximate 50% deficiency of GRK-2 in microglial cells (Eijkelkamp et al., 2010; Willemen et al., 2010) (Fig. 2).

In response to nerve injury spinal glial cells undergo morphological transformations which are typically characterized by hypertrophy and an associated up-regulation of complement receptor-3, CD11b (Ling et al., 1990) or the ionized calcium-binding adaptor molecule 1 (Iba1) for microglia (Ito et al., 1998) and glial fibrillary acidic protein (GFAP) for astroglia (Eng et al., 2000). Rapid proliferation, retraction of cellular processes, and acquisition of an amoeboid phenotype are all indicative of microglial responses to nerve injury (Davalos et al., 2005; Kreutzberg, 1996; Nimmerjahn et al., 2005). Interestingly, this type of response is not only accounted for by resident microglia, but also by bone marrow-derived cells which invade the spinal cord after nerve injury and

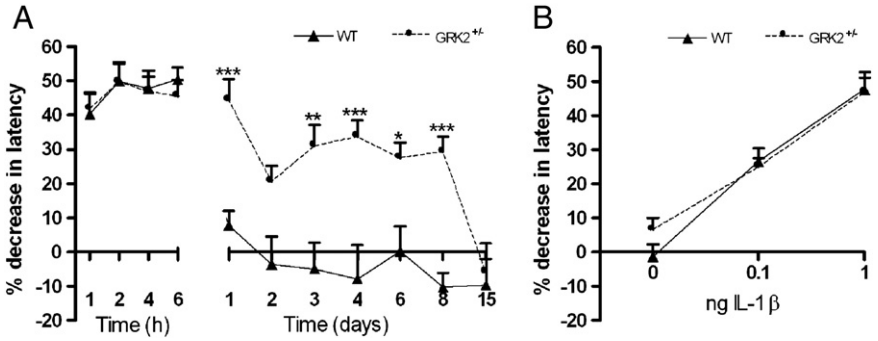

Fig. 2 – GRK2 regulates duration of peripheral IL-1β-induced hyperalgesia. Percentage decrease in heat withdrawal latency in WT and GRK2+/– mice (A) after intraplantar IL-1β at a dose of 1 ng ($n=8$), (B) 4 h after intraplantar injection of 0 (saline), 0.1 or 1 ng IL-1 β ($n=8$). Data are expressed as means ± SEM. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.
Adapted from Willemen et al. (2010) with permission.

differentiate into microglia (Zhang et al., 2007b). Morphological changes are first observed in microglia, then in astroglia, although the exact timing seems to depend on the model used. Spinal nerve injuries (ligation and/or transection) elicit microglial responses within a day, which is then followed by astrocyte responses a few days later (Romero-Sandoval et al., 2008). Also, microglial responses precede astrocyte responses following injuries of the sciatic nerve or its branches (Coyle, 1998; Hu et al., 2007; Zhang and De Koninck, 2006). Although the morphological hallmarks of glial responses as well as increased proliferation are usually used to define an 'activated state', it does not tell us anything about the functional properties of glia. In addition, it is clear that there is no 'single' activation state, but many different states can exist depending on the context the glia are in (Ransohoff and Perry, 2009). For a better understanding of the role of activated glial states in pathological pain, it has recently been suggested to use the term 'pain-related enhanced response states' if it is clear that the molecular profile of a glial cell influences pain processing (McMahon and Malcangio, 2009).

6.6.1. *Toll-like receptors*

A wide repertoire of receptor proteins on their cell membranes allows microglia to react almost immediately to changes in the homeostasis of the micro-environment. Among these receptor proteins are Toll-like receptors (TLR). Expression of TLR2, 3, or 4 (and the co-receptor for TLR4, CD14) proteins was found to be required for full-blown microglial responses to peripheral nerve injury since mice lacking any of these TLR showed attenuated neuropathy-induced up-regulation of microglial genes and proteins such as CD11b and Iba1 (Cao et al., 2009; Kim et al., 2007; Obata et al., 2008; Tanga et al., 2005). More importantly, deletion of these TLR was found sufficient to attenuate the development of pain hypersensitivity. TLR are thought to contribute to pain hypersensitivity by affecting the release of pro-inflammatory cytokines such as IL-1β (Clark et al., 2009). TLR are receptors for sensing pathogen-associated molecular patterns (PAMP) or damage-associated molecular patterns (DAMP). Indeed, TLR were suggested to bind and be activated by 'own' substances such as heat-shock proteins (Hutchinson et al., 2009). Heat shock proteins are particularly up-regulated by glia and neurons in response to stress or injury (Krueger-Naug et al., 2000), most likely for the purpose of neuroprotection (Benn et al., 2002). Although neither the exact role nor the persistence in changes of TLR ligands after nerve injury are clear to date, TLR represent an exciting, but largely experimental therapeutic target in neuropathic pain management. Meanwhile, a novel receptor antagonist for TLR4 has been developed and shown effective in treatment of CCI-induced pain hypersensitivity when given daily during the first week after nerve injury (Bettoni et al., 2008).

6.6.2. *Chemokine receptors*

Chemokines have not only been suggested to be important mediators of orchestrating the inflammatory response by attraction of immune competent cells (Zhang et al., 2007b), but also to act as chemical signals for neuronal–glial communication. Particularly monocyte chemotactic protein-1 (MCP-1 or CCL-2), acting through the CCR2 receptor, and fractalkine, acting through the fractalkine receptor (CX3CR1) have been studied in this respect. Mice lacking CCR2 failed to develop full-blown pain hypersensitivity following peripheral nerve injury (Abbadie et al., 2003) and a novel CCR2-antagonist was recently found effective in reducing WDR neuron activity and reversing established pain hypersensitivity induced by peripheral nerve injury (Serrano et al., 2010). CCL-2 is up-regulated in the DRG for several weeks following injury (White et al., 2005). While this chemokine does not normally excite neurons, it was found to induce membrane depolarization of DRG neurons after peripheral nerve injury (Sun et al., 2006). This effect may be explained by an induction of CCR2 in a small subpopulation of DRG neurons upon injury (White et al., 2005). CCL-2 was furthermore found to be packaged into synaptic vesicles which possibly also contain the neuropeptide CGRP and could be released in a calcium-dependent manner (Jung et al., 2008). *In vitro* experiments have also shown that CCL-2 can be released in a calcium-dependent manner from DRG preparations upon challenge with either potassium chloride or capsaicin (Dansereau et al., 2008). Subsequent *in vivo* experiments showed that CCL-2 is predominantly produced in small-sized DRG neurons, transported to the central terminals and released upon high intensity nerve stimulation (Thacker et al., 2009). Additionally,

astroglia have recently been proven to be a spinal source of CCL-2 following peripheral nerve injury (Gao et al., 2009). CCL-2 production by astroglia depends on the activity of the mitogen-activated protein kinase (MAPK) c-Jun-N-terminal kinase (JNK). Indeed, TNF-α was found to trigger JNK activation (by facilitating its phosphorylation) (Gao et al., 2009) and p-JNK was found to be specifically restricted to a subset of (about 30% of) astroglia (although DRG neurons also showed enhanced JNK phosphorylation) after spinal nerve ligation (Zhuang et al., 2006). Importantly, inhibition of JNK phosphorylation in the spinal cord, but not in the DRG, strongly attenuated pain hypersensitivity (Zhuang et al., 2006). Subsequent investigations convincingly showed that JNK phosphorylation in astroglia results in up-regulation of CCL-2, which can potentiate excitatory neurotransmitter signaling in dorsal horn neurons (Gao et al., 2009). Blood-derived macrophages and microglial cells resident in the spinal cord display constitutive expression of CCR2 (Abbadie et al., 2003; Zhang et al., 2007b). Nerve injured CCR2-deficient mice were indeed found to be impaired in the activation/phosphorylation of p38 MAPK (Abbadie et al., 2003), which is restricted to microglial cells after nerve injury (Ji et al., 2006). Phosphorylation of p38 itself is an essential process in the development of neuropathic hypersensitivity (Tsuda et al., 2004). In conclusion, CCL-2/CCR2 signaling may modulate the processing of noxious and innocuous stimuli at the level of both DRG and spinal cord, resulting in pain hypersensitivity.

The fractalkine receptor (CX3CR1) also plays a critical role in pain hypersensitivity following peripheral nerve injury. Daily intrathecal administration of antibodies against CX3CR1 could largely prevent, but also reverse pain hypersensitivity in a CCI model of neuropathic pain (Milligan et al., 2004). Moreover, mice lacking CX3CR1 were largely impaired in the development of pain hypersensitivity following partial sciatic nerve injury (Staniland et al., 2010). CX3CR1 is primarily expressed by spinal microglial cells, while its ligand fractalkine (CX3CL1) is expressed in a membrane-bound form by dorsal horn neurons (Clark et al., 2009), but peripheral nerve injury also induces CX3CL1 expression in spinal astrocytes (Lindia et al., 2005). Spinal levels of soluble CX3CL1 is strongly increased at 7 days after partial sciatic nerve ligation, but a detailed temporal profile of this increase is unknown to date (Clark et al., 2009). Cleavage of membrane-bound CX3CL1 requires the microglial lysosomal cysteine protease cathepsin-S (Clark et al., 2009). Pharmacological blockade of this protease could partly reverse pain hypersensitivity after partial sciatic nerve ligation (Clark et al., 2007). CX3CL1 has been reported to induce hyperactivity of WDR neurons to mechanical stimuli (Owolabi and Saab, 2006), although this phenomenon does not seem to be a direct effect of CX3CL1 on spinal neurons (Zhuang et al., 2007). Possibly, CX3CL1 induces intracellular processes in microglial cells expressing the CX3CR1, which triggers the release of neuromodulatory substances.

6.6.3. *Purinoceptors*

Spinal glia furthermore express purinoceptors, which can be divided into a group of ionotropic receptors (P2X receptors) and a group of metabotropic receptors (P1 and P2Y receptors) activated by adenosine, ATP or other nucleotides. The expression of P2Y12 (sensitive to nucleotides) on microglia was found to be required for microglial motility (Haynes et al., 2006; Honda et al., 2001; Wu et al., 2007), which is a vital characteristic of microglia when screening the local environment (Davalos et al., 2005). Interference with P2Y12 expression or activation was found to decrease microglial motility and at the same time impair mechanical hypersensitivity following spinal nerve injury (Kobayashi et al., 2008; Tozaki-Saitoh et al., 2008).

Of the P2X receptors, P2X3, P2X4, and P2X7 were shown to be of particular relevance to painful neuropathies. The P2X3 receptor is an ATP-sensitive cation-permeable ion channel and it is expressed predominantly in the DRG by satellite cells and DRG neurons, particularly small-sized neurons express P2X3 (Novakovic et al., 1999; Wang et al., 2003). Spinal P2X3 expression originates from central terminals of primary afferents innervating the superficial dorsal horn (Novakovic et al., 1999). Pharmacological interference with P2X3 receptors (using intrathecal antisense oligonucleotide treatment) was reported to largely prevent the development of pain hypersensitivity following partial sciatic nerve injury, while established pain hypersensitivity could be partly reversed (Barclay et al., 2002; Honore et al., 2002). The mode-of-action in which P2X3 receptor activation influences the processing of noxious and/or innocuous stimuli remains unclear to date.

Also, the P2X7 receptor has been linked to neuropathic pain. Indeed, mice lacking the P2X7 receptor do not develop pain hypersensitivity following partial sciatic nerve ligation (Chessell et al., 2005) and established pain hypersensitivity could be reversed by systemic delivery of a P2X7 receptor antagonist (A-438079) in several models of peripheral nerve injury (McGaraughty et al., 2007). The P2X7 receptor is expressed primarily on microglial cells in the spinal cord and its activation is known to affect the processing and release of the pro-inflammatory cytokine IL-1β (Ferrari et al., 2006). Indeed, a recent investigation showed that P2X7 was required for microglial release of IL-1β upon stimulation of TLR4 (Clark et al., 2010). Moreover, microglial cathepsin-S release (as discussed in the previous section) was recently reported to require P2X7 receptors (Clark et al., 2011). Hence, P2X7 receptors may be rooted in multiple ways within the pathological mechanisms underlying pain hypersensitivity.

The P2X4 receptor is an ATP-sensitive cation-permeable ion channel (Kawate et al., 2009) and is strongly up-regulated in microglia following peripheral nerve injury (Tsuda et al., 2003). Several lines of evidence show its relevance to pain hypersensitivity: (1) intrathecal delivery of ATP-treated P2X4-expressing microglial cells induces a transition of NS neurons into WDR neurons in the spinal dorsal horn (Keller et al., 2007) and pain hypersensitivity in naïve rats (Tsuda et al., 2003), (2) pharmacological interference with P2X4 impairs the development of nerve-injury-induced mechanical hypersensitivity (Tsuda et al., 2003), and (3) pain hypersensitivity is markedly blunted following peripheral nerve injury in mice lacking P2X4 (Tsuda et al., 2009). It is now known that P2X4 receptor signaling and subsequent activation of p38 (by its phosphorylation) is required for BDNF release by microglial cells (Trang et al., 2009; Ullrich, 2007). BDNF signaling (via its receptor tyrosine-kinase type B (trkB)), in turn, was reported to trigger a shift in the anion-gradient leading to GABA-mediated

disinhibition at the level of the spinal cord (Coull et al., 2005) (as described in the section 'Spinal disinhibition as a therapeutic target'). In line with these data, pain hypersensitivity following peripheral nerve injury is not only attenuated by inhibition of P2X4 (Tsuda et al., 2003), but also by interference with p-p38 (Tsuda et al., 2004) and trkB (Coull et al., 2005). Spinal P2X4 up-regulation is present for at least two weeks after peripheral nerve injury. A few studies have focused on the elucidation of further molecular pathways upstream of P2X4 expression. The extracellular matrix (ECM) protein fibronectin, acting through its integrin receptor, triggers a src kinase called Lyn (Tsuda et al., 2008b), which is important for nerve injury-induced up-regulation of P2X4 receptors in microglia (Tsuda et al., 2008a). Fibronectin levels have furthermore been found to increase in the spinal dorsal horn following nerve injury (Nasu-Tada et al., 2006). It has been suggested that the increased spinal fibronectin levels originate from blood plasma via a leaking blood-spinal-cord-barrier (BSCB). This suggestion gains significant importance with respect to the chronic nature of neuropathic pain bearing in mind that a compromised BSCB can be present up to weeks or months following nerve injury (Beggs et al., 2010; Gordh et al., 2006). Although it remains largely unclear why the BSCB is leaking following peripheral nerve injury, recent investigations show that experimental stimulation of C fibers can induce a leaking BSCB (Beggs et al., 2010). Hence, local anesthetics which decrease neuronal activity might attenuate pain hypersensitivity following nerve injury by a wide range of actions including beneficial effects on the integrity of the BSCB.

A recent investigation showed that ATP-induced calcium currents via rodent and human P2X4 receptors could be strongly inhibited using antidepressants such as the SSRI paroxetine (Nagata et al., 2009). As such, the anti-pain effect of antidepressants in neuropathic pain may be partly explained by their effect on purinoceptor function. Although purinergic receptors may represent an interesting novel target for management of neuropathic pain, antagonist drugs for purinergic receptors are still largely unspecific and are rapidly degraded (Jarvis, 2010).

6.6.4. *Glutamate transporters*

Excessive glutamatergic signaling is considered fundamental to many neuropathological processes following peripheral nerve injury, including central sensitization. Impaired glutamate homeostasis through decreased protein expression and activity of glutamate transporters (GT) was already evidenced in several models of peripheral nerve injury, such as CCI (Cavaliere et al., 2007; Sung et al., 2003), spared nerve injury (Mao and Yang, 2010; Mirzaei et al., 2010) or partial sciatic nerve ligation (Nie et al., 2010; Xin et al., 2009). GT indeed play a key role in controlling excitatory transmission through active clearance of extracellular glutamate. This role is mainly ensured by astrocytes, strategically surrounding synapses of communicating neurons, via the expression of 2 types of functional GT: GLAST (glutamate-aspartate transporter) and GLT-1 (glutamate transporter-1). Both were found to be down-regulated in the spinal cord for at least two weeks after an initial up-regulation induced by a peripheral nerve injury (Mirzaei et al., 2010; Nie et al., 2010; Sung et al., 2003). Treatment with riluzole, an activator of GT activity, or with ceftriaxone, a drug which specifically up-regulates GLT-1 protein expression and function, could largely prevent the development of pain hypersensitivity following CCI and partly reversed established CCI-induced pain hypersensitivity (Hu et al., 2010; Sung et al., 2003). It should be noted however, that riluzole is a broad-spectrum drug which does not only affect GT, but possibly the NMDA receptor and voltage-gated sodium channels (Doble, 1996). Although nerve injury causes GT down-regulation, a recent investigation showed that GLAST and GLT-1 protein expression, which is normally restricted to astroglial cells in the spinal cord (Rothstein et al., 1996) is actually induced in microglial cells following partial sciatic nerve ligation (Xin et al., 2009). However, GT expression and glutamate uptake by microglial cells does not seem to play an important role in regulating glutamate handling, but rather aims at allowing microglia to synthesize glutathione, the main CNS antioxidant. In order to synthesize glutathione, microglia take up cystine in exchange of glutamate using the X_c^- antiporter system (Persson et al., 2006). As glutamate is released extracellularly in this process, microglial cells do not serve as real glutamate buffers.

6.7. *Broad-spectrum immunomodulatory drugs*

The complex nature of the neuroinflammatory response may provide plentiful therapeutic targets for neuropathic pain management. Indeed, a variety of non-specific anti-inflammatory substances has been proven to be therapeutically effective in animal models of neuropathic pain. Some of these substances have been reported to both attenuate the development of pain hypersensitivity and reverse established pain hypersensitivity (e.g. propentofylline) (Raghavendra et al., 2003b; Sweitzer et al., 2001b). Other substances were only found to attenuate the development of pain hypersensitivity (e.g. minocycline) (Raghavendra et al., 2003a). Propentofylline (glial cell modulator SLC022, Solace Pharmaceuticals) is about to enter a randomized, double-blind, placebo-controlled clinical trial in which its oral administration will be tested in treatment of post-herpetic neuralgia patients. Also, the glial modulator AV411 (ibudilast), which has many mechanisms-of-action, was found to attenuate pain hypersensitivity after CCI in the rat (Ledeboer et al., 2006). A phase I and phase II double-blind, randomized, placebo-controlled, clinical trial on the safety, tolerability, pharmacokinetics and preliminary efficacy of AV411 in diabetic polyneuropathy patients has been completed, but results are not yet published. Besides glial inhibition also other immunomodulatory drugs are under investigation. For instance, inhibition of cyclooxygenase (COX) has been reported to have beneficial effects on pain hypersensitivity following CCI (De Vry et al., 2004). Soon, the COX-inhibitor etoricoxib will be tested for its efficacy in patients with neuropathic pain with and without peripheral hyperalgesia in a randomized, placebo-controlled, double-blind phase II clinical trial (see http://clinicaltrials.gov/).

6.8. *Novel approaches to achieve anti-inflammatory effects*

Anti-inflammatory effects may also be achieved using drugs which interfere with cellular and/or molecular drives of

neuroinflammation, and in particular glial responses to nerve injury. A first drive of glial responses to peripheral nerve injury involves neuronal activity such as ectopia. Blocking of ectopia by applying the local anesthetic bupivacaine or tetrodotoxin, a blocker of many sodium-channels to the injured nerve or DRG was found to prevent glial responses to peripheral nerve injury in DRG and/or spinal cord (Wen et al., 2007; Xie et al., 2009). Once established, glial responses could be also be reversed by repetitive intrathecal treatment with the local anesthetic ropivacaine (Toda et al., 2011). Especially A fiber activity showed to be a trigger of microglial responses (Suter et al., 2009). However, not only peripheral fiber activity, but also dorsal horn neuronal activity influences glial responses to nerve injury. Treatment with MK-801, an NMDA receptor blocker, has been found to prevent increased GFAP protein expression in the spinal cord after CCI (Garrison et al., 1994). Additionally, treatment with anti-sense oligonucleotides against the early neuronal activation protein c-fos suppressed GFAP up-regulation following spinal nerve ligation (Wang et al., 2009). Although the mechanisms in which neuronal activity can trigger glial responses are mostly unexplored, a recent investigation using a CCI model of neuropathic pain showed that repetitive treatment with local anesthetics blocked microglial and astroglial responses by an NGF-dependent and an NGF-independent mechanism, respectively (Toda et al., 2011). Also, an activity-dependent CCL-2/CCR2-mechanism of microglial responses to nerve injury has been proposed, based on findings that (1) microglial cells require the CCR2 receptor for a full-blown response to peripheral nerve injury and (2) calcium-dependent CCL-2 release by primary afferent fibers can be triggered after nerve injury.

A second drive of glial responses to nerve injury involves inflammatory processes themselves. Indeed, astroglial responses, which are typically associated with maintenance of neuropathic pain (Ji et al., 2006), are considered to be at least partly triggered by microglial responses. Indeed, astroglial responses (e.g. GFAP up-regulation) to nerve injury could also be attenuated when microglial responses were pharmacologically inhibited using minocycline (Raghavendra et al., 2003a). Moreover, altered protein expression of glutamate receptors and glutamate transporters in astroglial cultures can be triggered by microglial 'response states' (Tilleux et al., 2007, 2009; Tilleux and Hermans, 2008), possibly via the action of cytokines. Microglial expression of IL-18 has been identified as an important trigger of the astroglial response to peripheral nerve injury (Miyoshi et al., 2008). Interference with signaling between microglial IL-18 and IL-18 receptor on astroglia largely prevented GFAP up-regulation and mechanical hypersensitivity following peripheral nerve injury (Miyoshi et al., 2008). However, it is known that astroglial responses after nerve injury do not necessarily require microglial responses. Root avulsion typically results in astroglial responses in the absence of clear microglial responses (Colburn et al., 1999; Scholz et al., 2008).

Many pain-related inflammatory mechanisms following nerve injury converge on the same intracellular signaling cascades. MAPK pathways are heavily implicated in neuropathic pain (Ji et al., 2009). Upon nerve injury, the three main families of MAPK; JNK, p38, and ERK are differentially activated by phosphorylation in various cell types. While, JNK and p38 are selectively phosphorylated in astroglial cells and microglial cells, respectively, phosphorylation of ERK is time-and-cell specific (Ji et al., 2006). Within hours after spinal nerve ligation, phospho-ERK is detected in neurons present in the superficial dorsal horn; followed by a predominant microglial expression in the first few days and a gradual shift in this expression to astroglial cells (Zhuang et al., 2005). Pharmacological interference with the several MAPK is effective in preventing the onset but also reversing already established pain hypersensitivity (Tsuda et al., 2004; Zhuang et al., 2005, 2006) (Fig. 3). Of note, these anti-pain effects may not be exclusively mediated by inhibition with spinal MAPK phosphorylation. Indeed, DRG neurons also regulate MAPK

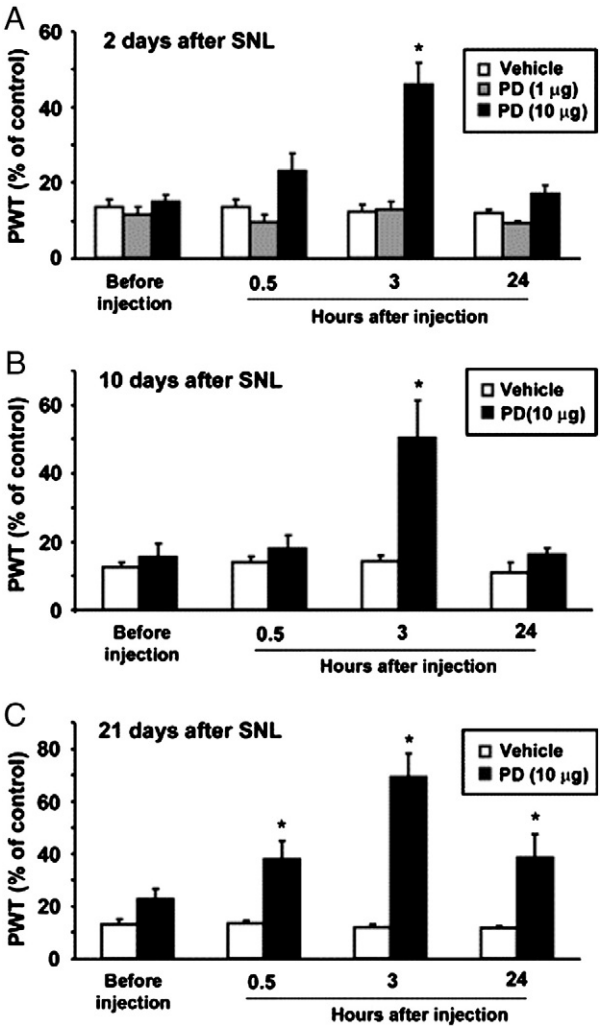

Fig. 3 – ERK activation contributes to neuropathic pain. A single injection of MEK (ERK kinase) inhibitor PD98059 (10 μg) into lumbar CSF space decreases spinal nerve ligation-induced mechanical allodynia at Day 2 (A), 10 (B), and 21 (C). The data are expressed as % of pre-SNL baseline mechanical threshold (10.3 ± 0.6 g, $n = 26$). The mechanical allodynia was tested at 0.5, 3, and 24 h after the drug injection. Low dose of PD98059 (1 μg) has no effect. *$p < 0.01$, compared to corresponding vehicle controls (20% DMSO), $n = 6–8$.
Adapted from Zhuang et al. (2005) with permission.

following nerve injury and drugs interfering with MAPK are often administered intrathecally, thereby having free access to the DRG (Jin et al., 2003; Schafers et al., 2003b; Xu et al., 2007). Three p38 MAPK inhibitors are currently in clinical trials. First, a double-blind, placebo controlled phase II clinical trial has recently been completed for a p38 MAPK inhibitor (SB-681323; GlaxoSmithKline) in patients with peripheral neuropathic pain. Second, a multi-center, randomized, double-blind, placebo-controlled phase IIA clinical trial for the p38 inhibitor PH-797804 (Pfizer) in post-herpetic neuralgia has been completed. Third, a randomized, double-blind phase II study to evaluate the safety and efficacy of a p38 MAPK inhibitor (GW856553; GlaxoSmithKline) in subjects with painful lumbosacral radiculopathy has recently been completed. This same drug is currently being tested in a second randomized, double-blind phase II trial to evaluate its efficacy in subjects with peripheral neuropathic pain (see http://clinicaltrials.gov/).

Pro-inflammatory cytokines are a direct cause of pain hypersensitivity, but can only contribute to this phenomenon when being activated. Here, cleavage of a pro-peptide, which activates the cytokine, is essentially mediated by proteolytic enzymes. An extracellular protease called tissue type plasminogen activator (tPA) was found to be expressed by astrocytes in the spinal dorsal horn for at least two weeks after spinal root ligation (Kozai et al., 2007). Intrathecal delivery of a specific tPA inhibitor fully blocked the development of mechanical hypersensitivity, while it also reversed established pain behavior when treatment was started at five but not at ten days after injury (Kozai et al., 2007). Of note, it cannot be ruled out that tPA activity in the DRG is (partly) involved in these reported anti-pain effects. Targeting proteases as a treatment of neuropathic pain requires detailed knowledge about the time-window in which such enzymes are responsible for activating cytokines. Indeed, it was shown that proteolytic cleavage of pro-IL-1β is mediated by different matrix-metalloproteases (MMP) over time after spinal nerve ligation (Kawasaki et al., 2008a). While MMP-9, primarily expressed by DRG neurons, mediates pro-IL-1β cleavage within the first days after spinal nerve ligation, MMP-2, expressed by satellite glial cells in the DRG and by astrocytes in the spinal dorsal horn, mediates this process from about one week after injury onwards (Kawasaki et al., 2008a). Besides prevention of the activation of pro-inflammatory cytokines, the cytokines themselves can be blocked. Blocking can be achieved by several pharmacological approaches of which the (soluble) TNF decoy receptor etanercept (which traps TNF-α) and IL-1β receptor antagonists (Kato et al., 2009; Schafers et al., 2003b; Sweitzer et al., 2001a) are most described and (alone or in combination) are effective in experimental treatment of neuropathy-induced pain hypersensitivity. Also a clinical phase I and phase II trial have recently been completed on the use of epidural etanercept in treatment of peripheral neuropathic pain (sciatica) (see http://clinicaltrials.gov/).

7. Therapeutic approaches for spinal cord injury pain

Several excellent reviews have previously addressed the currently used medicinal approaches to treat spinal cord injury pain (Finnerup and Jensen, 2004; Finnerup et al., 2010; Teasell et al., 2010). Evidence shows that local anesthetics, ketamine, and opioids as well as anti-epileptics such as gabapentin and pregabalin benefit spinal cord injury patients with pain, and more clinical trials are currently ongoing or planned (see http://clinicaltrials.gov/). However, an increasing knowledge about the cellular and molecular mechanisms involved in pain hypersensitivity following spinal cord injury (Hulsebosch et al., 2009), allows us here to focus on novel, often still experimental, mechanism-based interventional approaches to treat spinal cord injury-induced neuropathic pain. Many of the mechanisms contributing to an increase in the input to dorsal horn neurons following spinal cord injury are similar to those discussed in the sections on peripheral neuropathic pain.

7.1. *Treating hyperactivity of central neurons and ectopia*

Spontaneous (non-evoked) neuronal activity has been reported to occur within the human dorsal horn after spinal cord injury (Loeser et al., 1968). Moreover, rodent studies have shown that WDR neurons in the spinal cord as well as thalamic neurons show hyperactivity to noxious and innocuous input, a phenomenon which depends on the voltage-operated sodium channel $Na_v1.3$ (Hains et al., 2003a; Hains et al., 2005). When anti-sense oligonucleotides against this sodium channel were administered in the lumbar intrathecal space at four weeks after thoracic spinal cord injury in the rat, hyperactivity of spinal and thalamic neurons to noxious and innocuous input was reduced and pain hypersensitivity was attenuated (Hains et al., 2003a, 2005); these effects were lost when treatment was ceased.

In an animal model of thoracic spinal cord injury, cervical and lumbar DRG neurons were also found to display ectopia; these cells included neurons of the C fiber population as evidenced by IB4 binding-affinity and capsaicin-sensitivity (Bedi et al., 2010). The ectopia was found to originate immediately proximal to the DRG and persist for at least 8 months after induction of spinal cord injury (Bedi et al., 2010). The finding of spontaneously active DRG neurons is suggestive of a novel and exciting concept that central nerve injury has retrograde effects on peripheral neurons, which may then act to provide a positive feedback of re-sensitization of the CNS. As such, the use of local anesthetics might have beneficial effects in management of SCI-induced pain hypersensitivity. Indeed, a case-report showed that relief of at-level neuropathic pain could be achieved using topical lidocaine in a patient with thoracic spinal cord injury (Wasner et al., 2007).

7.2. *Peripheral sensitization as a therapeutic target*

Besides displaying ectopia, cervical DRG neurons have also been reported to show hyperexcitability to mechanical and thermal stimuli after experimental thoracic spinal cord injury (Carlton et al., 2009). This hyperexcitability was also observed in the peripheral nerve branches after these were disconnected from DRG and spinal cord, thus suggesting an intrinsic molecular change in the fibers themselves as being the consequence of the hyperexcitability (Carlton et al., 2009). However, since the molecular and/or cellular mechanisms for

this phenomenon remain unknown at this stage (Hulsebosch et al., 2009), related therapeutic options are not being currently considered.

7.3. *Structural plasticity as a therapeutic target*

Spinal cord injury-induced structural plasticity within the nociceptive system is a very likely mechanism of pain hypersensitivity. As mentioned in the earlier section 'Therapeutic approaches in painful peripheral neuropathies', GAP-43 is considered a marker of neuronal plasticity such as fiber sprouting. Experimental spinal cord injury is known to trigger an increased GAP-43 protein expression between days and weeks after injury (Christensen and Hulsebosch, 1997a). This expression is observed in many locations, but is most prominent in the superficial dorsal horn and can extend over several segments of the spinal cord (see also Christensen and Hulsebosch, 1997b). GAP-43 expression occurs in peptidergic fibers, most likely of the C fiber population, as GAP-43 co-localizes with CGRP (Christensen and Hulsebosch, 1997a). Inhibition of CGRP via intrathecal delivery of the highly selective CGRP receptor antagonist $CGRP_{8-37}$ resulted in reversal of established pain hypersensitivity when administered four weeks after injury (Bennett et al., 2000). As peptidergic C fibers express the receptor trkC (Hunt and Mantyh, 2001), their sprouting responses may be mediated by enhanced levels of NGF, a factor being produced by inflammatory cells (such as microglia) upon spinal injury (Krenz and Weaver, 2000). Indeed, NGF can induce sprouting of nociceptive fibers (Romero et al., 2001; Tang et al., 2004) and delivery of anti-NGF antibodies for two weeks after spinal cord injury resulted in a significant attenuation of CGRP fiber sprouting in the spinal dorsal horn (Christensen and Hulsebosch, 1997a). Besides neurotrophin expression, also other molecules have been linked to CGRP fiber sprouting following spinal cord injury including the cell adhesion molecule L1 (Deumens et al., 2007). Structural changes following spinal cord injury are possibly long lasting as GAP-43 expression in sprouting fibers is likely followed by the formation of new synaptic contacts, just as observed during neurodevelopment (Fitzgerald et al., 1991).

The issue of undesired sprouting of nociceptive fibers following spinal cord injury is particularly relevant in light of current experimental therapies for spinal cord injury which aim to enhance plasticity in severed motor fibers (for improved motor outcome). A therapy which induces significant motor outcome but elicits or enhances pain hypersensitivity due to the provocation of non-specific fiber sprouting is doomed to fail (Deumens et al., 2008). An elegant study by Christoph Hofstetter et al. (2005) showed that transplantation of adult neural stem cells into a contused rat spinal cord only showed beneficial effects on motor outcome and pain hypersensitivity when these stem cells were pre-destined to enter a non-astroglial lineage upon transplantation. This effect was achieved by genetically modifying the stem cells (transduction with neurogenin-2) before transplantation. Transplantation of non-modified stem cells was found to induce pain hypersensitivity, an effect which was considered to depend on CGRP fiber sprouting in the dorsal horn, possibly mediated by a predominant astroglial differentiation of transplanted stem cells (Hofstetter et al., 2005). A later study showed that induction of pain hypersensitivity is specifically dependent on the astroglial subtype (Davies et al., 2008). Induction of pain hypersensitivity by cell grafting into injured spinal cords is not specific to stem cell grafts or astrocytes, however. Also other cell types including olfactory ensheathing cells, which are enthusiastically used in regeneration studies on experimentally injured spinal cords (Deumens et al., 2006a, 2006b; Li et al., 1997; Raisman, 2001; Ramon-Cueto et al., 2000), have been suggested to elicit pain hypersensitivity (Richter et al., 2005). This observation is even more relevant since transplantation of autologous olfactory ensheathing cells has already made it to clinical trials (Mackay-Sim et al., 2008). Another open-label phase I clinical trial is currently recruiting participants for transplantation of autologous olfactory ensheathing cells in treatment of complete human spinal cord injuries. Also, several phase I and phase II clinical trials have just been completed in which spinally injured patients were treated with autologous bone marrow stromal cells or mesenchymal stem cells, and one such study is currently recruiting participants (see http://clinicaltrials.gov/). With respect to regenerative approaches to treat the injured spinal cord, it should be noted that the various fiber tracts each have their specific requirements for regeneration, strongly depending on the repertoire of receptor proteins expressed (Deumens et al., 2005). Hence, specific induction of plasticity in motor fibers may be feasible for regenerative approaches to treat the injured spinal cord.

Besides structural plasticity in primary afferent fibers after experimental spinal cord injury, dendritic re-modeling of WDR neurons in the lumbar spinal cord has been reported several weeks after thoracic spinal cord injury in the rat (Tan et al., 2008). Such structural changes may heavily impact on the processing of noxious and innocuous signals (Tan et al., 2009). An intracellular small GTP-binding protein, Rac1, was found to be a vital player in this re-modeling. The documented injury-induced increase in the density of dendritic spines, enhanced maturity of these spines, as well as the associated neuronal hyperactivity to incoming signals and pain hypersensitivity were strongly attenuated when the Rac1-specific inhibitor NSC23777 was administered intrathecally at four weeks after injury (Tan et al., 2008). This latter study by the group of Bryan Hains, thus suggests that unwanted structural changes are to some extent reversible and hence offers the option of therapeutic approaches to antagonize such effects besides preventive approaches.

7.4. *Treatment of spinal disinhibition*

As mentioned in the earlier section 'Therapeutic approaches in painful peripheral neuropathies', spinal nociceptive processing is modulated via non-segmental and segmental systems. The serotonergic (5-HT) system is a main non-segmental system modulating nociceptive signaling (Suzuki et al., 2004). 5-HT innervations are decreased below spinal cord injury sites and this denervation is thought to partly explain the hyperactive state of dorsal horn neurons and, thus, spinal cord injury-induced pain hypersensitivity (Hains et al., 2002, 2003b). Administering a rat neuronal cell line synthesizing and releasing 5-HT into the lumbar intrathecal space at four weeks after spinal cord hemisection injury reversed

mechanical and thermal hypersensitivity (Hains et al., 2001a). Chronic pharmacological interference with the serotonergic system (using a 5-HT_{1A} receptor agonist) was also found to attenuate pain hypersensitivity after photochemically-induced spinal cord injury, when this intervention was commenced pre-emptively (Wu et al., 2003).

The GABAergic system is a main segmental system, which is compromised following spinal cord injury (Gwak and Hulsebosch, 2011). The malfunctioning of the GABAergic system has been explained by (1) apoptosis of GABAergic interneurons in the superficial spinal dorsal horn below the spinal level of injury (Meisner et al., 2010), (2) a down-regulation in the expression of the rate-limiting enzymes in GABA synthesis, GAD-65 and GAD-67, which has been reported to occur for at least six weeks following spinal cord contusion in the rat (Meisner et al., 2010), and (3) excitatory rather than inhibitory GABA-induced currents (Price et al., 2005).

A gene therapy has been employed in a rat model of spinal cord hemisection to increase GABA protein levels. Transduction of lumbar DRG neurons with a viral vector encoding human GAD-67 partly reversed below-level pain hypersensitivity, an effect which could be blocked using the $GABA_A$ receptor-selective and $GABA_B$ receptor selective antagonists bicuculline and phaclofen, respectively (Liu et al., 2004). Interestingly, the spinal cord injury-induced down-regulation of spinal GAD-65 protein expression has also been successfully prevented using intrathecal treatment with the glial modulator propentofylline (Gwak et al., 2008).

As already discussed in the section on 'Therapeutic approaches in painful peripheral neuropathies', changes in the expression of the two cation-chloride co-transporters NKCC1 and KCC2 can dramatically affect the effects of GABA-binding to the $GABA_A$ receptor. As in peripheral neuropathies, also spinal cord injury has been reported to trigger a down-regulation in the expression of the potassium-chloride co-transporter KCC2 at and below the spinal level of injury (Boulenguez et al., 2010; Cramer et al., 2008); a phenomenon also observed in the spinal dorsal horn (Boulenguez et al., 2010; Lu et al., 2008). KCC2 is responsible for transporting chloride out of the cell, while the sodium–potassium-chloride co-transporter NKCC1 takes part in transportation of chloride into the cell. Since spinal cord injury also induces an up-regulation of NKCC1 at the spinal lesion site (Cramer et al., 2008), intracellular chloride levels will be increased (Hasbargen et al., 2010). These molecular changes are thought to underlie the positive shift of GABA-induced currents as observed in the superficial dorsal horn after spinal cord injury (Lu et al., 2008). The injury-induced regulation of NKCC1 and KCC2 was observed at two weeks after spinal cord injury, and may hence participate in the pain hypersensitivity observed at that time point (Cramer et al., 2008). A single intraperitoneal injection with bumetanide, a potent NKCC1 inhibitor, significantly reversed thermal hypersensitivity already at 1 h after the drug injection (Cramer et al., 2008). Although these data are promising and may open new therapeutic opportunities for management of neuropathic pain, the data do not necessarily imply that GABA only has a pain-enhancing mode of action after spinal cord injury. Indeed, it has been shown that intrathecal bolus injections of GABA reduced spinal cord injury-induced hyperactivity of WDR neurons to incoming signals and reversed established pain hypersensitivity below the spinal level of injury (Gwak et al., 2008) (Fig. 4). Also, intrathecal transplantation with a human neuronal cell line (synthesizing and secreting GABA) at two weeks after an excitotoxic injury of the rat spinal cord completely reversed established mechanically-induced and thermally-induced pain hypersensitivity (Eaton et al., 2007; Eaton and Wolfe, 2009). Despite of the fact that these promising findings could be reproduced with a human cell line (Eaton et al., 2007; Eaton and Wolfe, 2009), the clinical feasibility of cell transplantation remains unclear as it is highly invasive and potential adverse side-effects are only poorly described. Clinical trials have not yet been initiated to the best of our knowledge.

7.5. *Modulation of the neuroinflammatory response*

Spinal cord injury induces a very rapid inflammatory response, which is characterized by responses of immuno-competent cells resident in the spinal tissue, such as microglia and astrocytes, but also by the infiltration of blood-borne immune cells due to a disrupted BSCB (Donnelly and Popovich, 2008). Glial responses extend over time several segments distant from the spinal injury site, both rostrally and caudally (Detloff et al., 2008). The introduction of the term 'gliopathy' in the field of spinal cord injury highlights the persistent nature of these neuropathy-induced glial responses (Hulsebosch, 2008). The molecular character of the glial responses mediating spinal cord injury pain is being increasingly explored.

7.5.1. *Chemokine receptors*

Within the neuron-to-glia communication triggering neuroinflammatory responses after experimental spinal cord injury, CCL21 has been shown to play a key role. CCL21 release, previously associated with neuronal injury (de Jong et al., 2005), was found to be elevated in the lumbar dorsal horn and in the ventro-postero-lateral nucleus of the thalamus at 4 weeks after spinal cord contusion (Zhao et al., 2007b). CCL21 protein expression was predominantly found to be neuronal, while its receptor is expressed by microglial cells (Rappert et al., 2002). Importantly, it was found that thalamic CCL21 expression could be induced by unilateral stimulation of the spinothalamic tract in normal rats, suggesting that a pathologically-enhanced neuronal activity in this tract may cause thalamic CCL21 expression after spinal cord injury (Zhao et al., 2007b). Blocking of thalamic CCL21 by injecting antibodies against CCL21 directly into the ventro-postero-lateral nucleus of the thalamus reduced the hyperactivity of thalamic neurons to incoming signals and reversed below-level pain hypersensitivity; these effects were lost when treatment was stopped (Zhao et al., 2007b). The hyperactivity of thalamic neurons and lumbar spinal cord neurons to incoming signals observed after thoracic spinal cord injury is thought to be mediated by an indirect effect of CCL21 on microglial cells. Thalamic and lumbar dorsal horn microglial responses to thoracic spinal cord injury involve the production of prostaglandin E2 (PGE2) by microglia, via an ERK-dependent mechanism (Zhao et al., 2007a). It has been proposed that microglial PGE2 binding to the prostaglandin receptor E-prostanoid 2 (EP2) on spinal neurons is at least partly responsible for the hyperactivity of WDR neurons to incoming signals (Zhao et al., 2007a). Pharmacological interference with

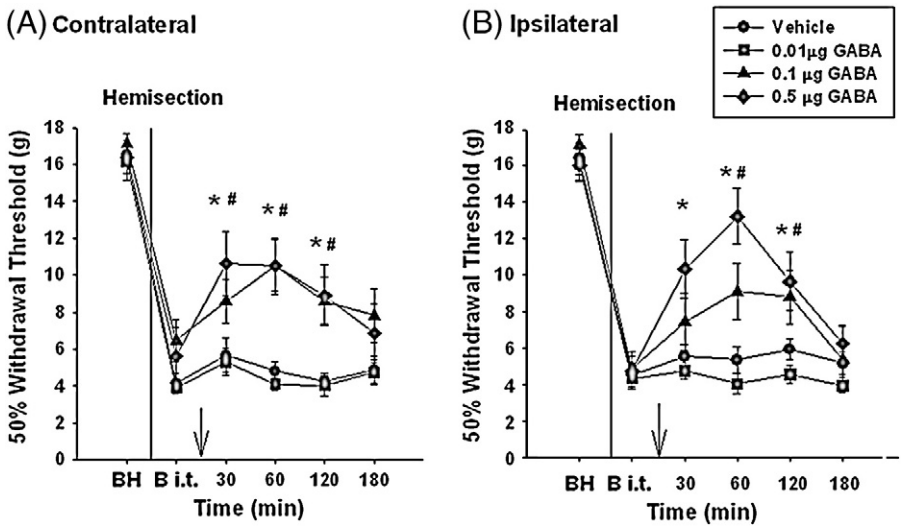

Fig. 4 – Intrathecal spinal administration of GABA immediately after SCI attenuates mechanical allodynia measured 28 days later. Unilateral SCI ($n=5$) results in bilateral mechanical allodynia on the contralateral (uninjured side) and the ipsilateral (injured side) hindlimbs compared to pre-injury values before spinal hemisection (BH). On post-operation day 28 (B i.t, before intrathecal administration), intrathecal 0.1 (#) and 0.5 (*) µg GABA administration significantly affects the mechanical allodynia on both hindlimbs compared to before intrathecal administration ($p<0.05$). Arrow reflects the time point of intrathecal administration.
Adapted from Gwak et al. (2008) with permission.

ERK phosphorylation or the EP2 receptor indeed showed that established below-level pain hypersensitivity at four weeks after spinal cord contusion could be reversed (Zhao et al., 2007a). Moreover, inhibition of microglial responses to spinal cord injury using intrathecal minocycline or Mac-1-SAP, a microglial immunotoxin, also significantly reduced spinal PGE2 protein expression, dorsal horn neuron hyperactivity to incoming signals, and below-level pain hypersensitivity (Hains and Waxman, 2006; Zhao et al., 2007a). Lowering spinal PGE2 expression has also been achieved using a cycloxygenase inhibitor, a treatment which also attenuated pain hypersensitivity (Hains et al., 2001b).

7.5.2. *Broad-spectrum immunomodulatory drugs*

General anti-inflammatory substances which have proven their therapeutic efficacy in treatment of pain hypersensitivity using animal models of spinal cord injury include propentofylline and minocycline (Gwak et al., 2008; Hains and Waxman, 2006). However, novel insights into the cellular and molecular mechanisms underlying spinal cord injury-induced pain hypersensitivity allow for more specific therapeutic approaches (Hulsebosch et al., 2009). A preventive immunomodulatory approach is not quite feasible in the setting of spinal cord injury. Rather, interference with the rapidly changing nature of the dynamic inflammatory reaction after spinal cord injury (Donnelly and Popovich, 2008) may show its merits in management of pain hypersensitivity.

7.5.3. *Novel approaches to achieve anti-inflammatory effects*

Anti-inflammatory effects may also be achieved using drugs interfering with cellular and/or molecular drives of neuroinflammation, and in particular glial responses following spinal cord injury. A first drive of neuroinflammation following injury to the CNS involves intercellular signaling between astroglial cells. Gap junction proteins play an important role in the communication between astroglia. Interference with intercellular astroglial signaling via gap junctions can be achieved using the gap junction decoupler carbenoxolone. Carbonexolone treatment has been described to prevent microglial responses to laser-induced brain damage (Davalos et al., 2005). Importantly, astroglia are reported to also play a critical role in pain hypersensitivity (Nesic et al., 2005). The functionality of gap junction proteins may be very important in relation to pain hypersensitivity. Indeed, gap junctions are linked to neuropathic pain following peripheral nerve injury (Spataro et al., 2004). A recent investigation showed that intrathecal treatment with carbenoxolone partly prevented the onset of below-level pain hypersensitivity following spinal cord hemisection, but did not reverse this hypersensitivity once established (after 10 days) (Roh et al., 2010). The exact triggers of astroglial responses to spinal cord injury remain largely unknown, although the signaling between cytokines and their receptors on astrocytes may be involved (Pineau et al., 2010) as well as the presence of aquaporins on astrocytes (Kigerl et al., 2007; Nesic et al., 2008). Although some of these molecular changes have been found to prevail up to weeks or even months after spinal cord injury, more investigations are needed to provide a clear profile of these mechanisms in relation to time and place. Moreover, the insights obtained so far support the need for future research to address the role of these molecular pathways in spinal cord injury-induced pain hypersensitivity.

A second drive of neuroinflammation following injury to the spinal cord is the presence of blood-borne leukocytes

penetrating the spinal cord parenchyma via a leaking BSCB (Donnelly and Popovich, 2008). The expression of integrin receptors on the membrane of these leukocytes is important for the extravasation of these cells. Intravenous delivery of a monoclonal antibody against the CD11d/CD18-integrin within two days after spinal cord injury was indeed effective in reducing the amount of neutrophils and macrophages at the injury site at 3 days after injury (Saville et al., 2004). More importantly, mechanical and thermal pain hypersensitivity was reduced for at least 10 weeks after injury (Gris et al., 2004).

Many inflammatory mechanisms following spinal cord injury converge on the intracellular signaling cascade of the MAPK (Ji et al., 2009). Work from the group of Claire Hulsebosch showed that the activation of several MAPK members closely correlated with the presence of at-level pain hypersensitivity following spinal cord contusion injury (Crown et al., 2006). The about 50% of animals developing at-level pain hypersensitivity showed a strong increase in phosphorylated ERK and p38, which was not observed in the remaining animals who did not develop at-level pain hypersensitivity (Crown et al., 2006). Both neurons and glial cells were found to activate MAPK following spinal cord contusion (Crown et al., 2008). It was further shown that treatment with the p38 MAPK inhibitor SB203580 could completely reverse established at-level pain hypersensitivity (Crown et al., 2008).

The severity of spinal cord injury is considered as one of the predictors for the presence of pain hypersensitivity following contusion injury of the rat spinal cord (Kloos et al., 2005).

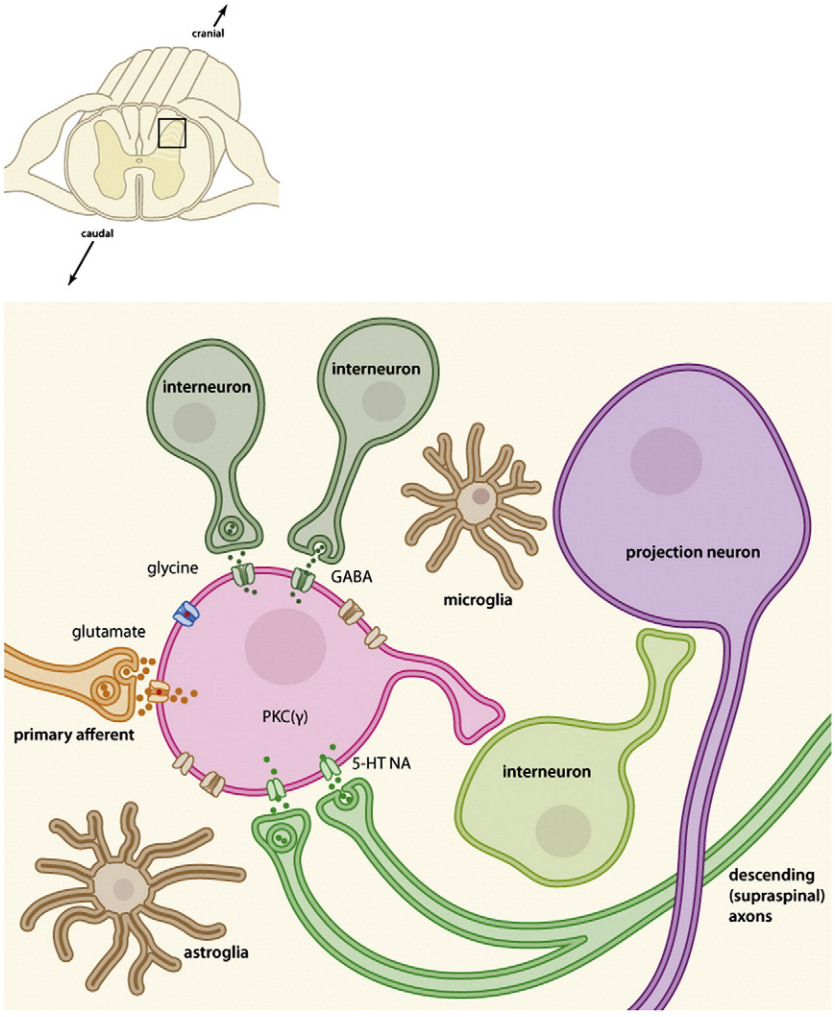

Fig. 5 – Simplified drawing of the superficial dorsal horn in physiological situations. Within the superficial dorsal horn, a silent circuit exists between low-threshold primary afferent fibers which are polysynaptically connected to NS projection neurons. Within this network, interneurons expressing PKC-γ play a key role. In physiological conditions, this network is thought to be kept inactive by inhibitory systems including (segmental) GABAergic and/or glycinergic interneurons. Modulation of this circuit may derive not only from segmental systems but also from non-segmental systems such as descending (supraspinal) axons including serotonergic and noradrenergic ones. Moreover, surrounding glial cells are thought to play regulatory roles in neuronal signal transmission with a predominant role for astroglia, which are typically associated with neuronal synapses (not shown). Note that this drawing is an oversimplification and other primary afferent input (such as nociceptive afferent innervations of NS projection neurons) is not indicated. PKC(γ), protein kinase C-γ; GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine/serotonin; NA, noradrenaline/norepinephrine; NS, nociception-specific.
Art-design: Rogier Trompert, medical illustrator.

Hence, neuroprotective and immune suppressive therapies enhancing tissue preservation may be particularly relevant to the attenuation of pain hypersensitivity (Koopmans et al., 2009; Nesic et al., 2010; Pacini et al., 2010; Sweitzer and De Leo, 2011; van Neerven et al., 2010).

Finally, drugs specifically interfering with pro-inflammatory cytokines may be of potential benefit to treatment of spinal cord injury pain hypersensitivity. However, the therapeutic window-of-opportunity for such treatments may be limited as cytokines are mainly increased in the early phase after spinal cord injury (Donnelly and Popovich, 2008). The pro-inflammatory cytokine TNF-α has been found necessary in the development of below-level pain hypersensitivity (Peng et al., 2006). Intrathecal treatment with the TNF decoy receptor etanercept has recently been reported to prevent microglial responses to spinal cord injury and, more importantly, below-level pain hypersensitivity (Marchand et al., 2009). When etanercept treatment was commenced after two weeks, these effects were absent (Marchand et al., 2009).

8. Conclusion

A better management of neuropathic pain is most likely within reach with the upcoming view on mechanism-based treatment approaches. Lots of progress are being made in

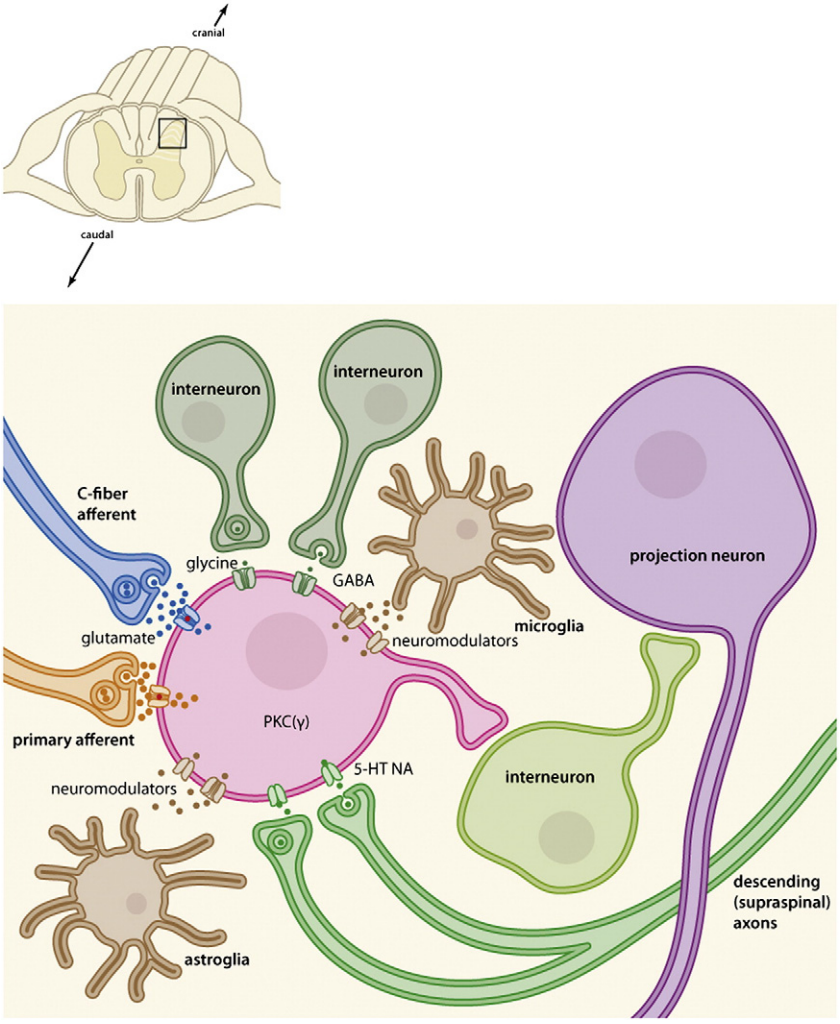

Fig. 6 – Simplified drawing of the superficial dorsal horn in neuropathic situations. Neuropathy induced a wide range of pathological changes in the spinal dorsal horn including changes in the activity of primary afferent fibers (e.g. as a result of ectopia), structural changes (e.g. sprouting of primary afferents), disinhibition (malfunctioning segmental as well as non-segmental systems), and the action of neuromodulatory substances (mainly related to inflammatory responses). As a result of these pathological changes, the input to the spinal dorsal horn is substantially changed in favor of excitation, which has substantial effects on the activity as well as excitability of dorsal horn neurons. One of the putative consequences of the enhanced excitation of the spinal dorsal horn is the activation of the normally silent circuit existing between low-threshold primary afferent fibers and NS projection neurons. Activity of this circuit has been proposed to 'turn touch into pain'. Note that this drawing is an oversimplification and other primary afferent input (such as nociceptive afferent innervations of NS projection neurons) is not indicated. PKC(γ), protein kinase C-γ; GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine/serotonin; NA, noradrenaline/norepinephrine; NS, nociception-specific.
Art-design: Rogier Trompert, medical illustrator.

filling the many caveats in our knowledge about the persistence of novel and already described cellular and/or molecular mechanisms involved in neuropathy-induced pain hypersensitivity and many potential therapeutic targets have consequently been identified. One of the strengths of a mechanism-based treatment approach is its narrowed-down specificity to pain hypersensitivity, and thus, a decreased risk of side effects. Indeed, less specific drugs may prove to be less efficient in treatment of neuropathic pain than hoped or perhaps even anticipated. As an example, immune suppressant therapies receive more and more attention, but inflammatory processes can have beneficial as well as detrimental effects on the outcome in neuropathies (including effects on pain) (Deumens et al., 2009; Faulkner et al., 2004; Milligan and Watkins, 2009; Myer et al., 2006; Sofroniew, 2009). More detailed knowledge about the specific cells and molecules responsible for pain hypersensitivity will decrease risks on failed clinical trials. If not yet developed, drugs can now be designed that specifically interfere with processes that are causally related to neuropathic pain. Such processes include specific changes in peripheral nociceptors (such as ectopia, peripheral sensitization, phenotypic switching and structural changes), altered inhibitory systems (disinhibition), and neuromodulatory events (with a main role for inflammatory reactions) (Figs. 5 and 6). However, a few side remarks need to be made. First, the success of mechanism-based treatment approaches heavily depends on the identification of windows-of-opportunity in relation to the specific neuropathic indication. Although many pathological processes are similar between various neuropathic conditions such as peripheral and central neuropathies, essential differences in the timing as well as in the molecular character of these processes exist. As an example, pain-related neuroinflammatory events occur both after peripheral nerve injury and spinal cord injury, but the specific molecules (e.g. chemokine type) identified as contributing to pain hypersensitivity are very different between these two pathologies. Hence, immunomodulation can be anticipated to be a promising approach in treatment of both peripheral and central neuropathic pain, but the exact immune-related molecule(s) targeted will most likely differ between these two neuropathic indications. Second, drugs that may be of particular use to effective treatment of neuropathic pain are those acting simultaneously on several of the mechanisms implicated in neuropathy-induced pain hypersensitivity. Local anesthetics are a typical example of such drugs as they do not only block voltage-gated ion channels, but (possibly as a consequence of channel blocking) also interfere with neuroinflammatory responses following nerve injury, and may even act preventive to leakage of the BSCB after nerve injury. The efficacy of local anesthetics in treatment of neuropathic pain is being tested in multiple clinical trials. Time will tell whether other, currently still mainly experimental, therapeutic targets will make it to clinical trials as well.

Acknowledgments

The authors are very grateful to Prof. Marshall Devor for critically reading this manuscript. This work is financially supported by a Young Investigator Grant by the Dutch Society for Anesthesiology (YIG/NVA grant to R.D.) and a grant from the Dutch Government (SENTERNOVEM grant # IS 041064 to E.A.J.J. and M.A.E.M.).

REFERENCES

Abbadie, C., Lindia, J.A., Cumiskey, A.M., Peterson, L.B., Mudgett, J.S., Bayne, E.K., DeMartino, J.A., MacIntyre, D.E., Forrest, M.J., 2003. Impaired neuropathic pain responses in mice lacking the chemokine receptor CCR2. Proc. Natl Acad. Sci. U. S. A. 100, 7947–7952.

Abrahamsen, B., Zhao, J., Asante, C.O., Cendan, C.M., Marsh, S., Martinez-Barbera, J.P., Nassar, M.A., Dickenson, A.H., Wood, J.N., 2008. The cell and molecular basis of mechanical, cold, and inflammatory pain. Science 321, 702–705.

Ahlgren, S.C., Levine, J.D., 1993. Mechanical hyperalgesia in streptozotocin-diabetic rats. Neuroscience 52, 1049–1055.

Amir, R., Michaelis, M., Devor, M., 1999. Membrane potential oscillations in dorsal root ganglion neurons: role in normal electrogenesis and neuropathic pain. J. Neurosci. 19, 8589–8596.

Amir, R., Liu, C.N., Kocsis, J.D., Devor, M., 2002a. Oscillatory mechanism in primary sensory neurones. Brain 125, 421–435.

Amir, R., Michaelis, M., Devor, M., 2002b. Burst discharge in primary sensory neurons: triggered by subthreshold oscillations, maintained by depolarizing afterpotentials. J. Neurosci. 22, 1187–1198.

Arner, S., Lindblom, U., Meyerson, B.A., Molander, C., 1990. Prolonged relief of neuralgia after regional anesthetic blocks. A call for further experimental and systematic clinical studies. Pain 43, 287–297.

Attal, N., Mazaltarine, G., Perrouin-Verbe, B., Albert, T., 2009. Chronic neuropathic pain management in spinal cord injury patients. What is the efficacy of pharmacological treatments with a general mode of administration? (Oral, transdermal, intravenous). Ann. Phys. Rehabil. Med. 52, 124–141.

Austin, P.J., Moalem-Taylor, G., 2010. The neuro-immune balance in neuropathic pain: involvement of inflammatory immune cells, immune-like glial cells and cytokines. J. Neuroimmunol. 229, 26–50.

Bao, L., Wang, H.F., Cai, H.J., Tong, Y.G., Jin, S.X., Lu, Y.J., Grant, G., Hokfelt, T., Zhang, X., 2002. Peripheral axotomy induces only very limited sprouting of coarse myelinated afferents into inner lamina II of rat spinal cord. Eur. J. Neurosci. 16, 175–185.

Barclay, J., Patel, S., Dorn, G., Wotherspoon, G., Moffatt, S., Eunson, L., Abdel'al, S., Natt, F., Hall, J., Winter, J., Bevan, S., Wishart, W., Fox, A., Ganju, P., 2002. Functional downregulation of P2X3 receptor subunit in rat sensory neurons reveals a significant role in chronic neuropathic and inflammatory pain. J. Neurosci. 22, 8139–8147.

Baron, R., Tolle, T.R., Gockel, U., Brosz, M., Freynhagen, R., 2009. A cross-sectional cohort survey in 2100 patients with painful diabetic neuropathy and postherpetic neuralgia: differences in demographic data and sensory symptoms. Pain 146, 34–40.

Baron, R., Binder, A., Wasner, G., 2010. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. Lancet Neurol. 9, 807–819.

Bedi, S.S., Yang, Q., Crook, R.J., Du, J., Wu, Z., Fishman, H.M., Grill, R.J., Carlton, S.M., Walters, E.T., 2010. Chronic spontaneous activity generated in the somata of primary nociceptors is associated with pain-related behavior after spinal cord injury. J. Neurosci. 30, 14870–14882.

Beggs, S., Liu, X.J., Kwan, C., Salter, M.W., 2010. Peripheral nerve injury and TRPV1-expressing primary afferent C-fibers cause opening of the blood-brain barrier. Mol. Pain 6, 74.

Benn, S.C., Perrelet, D., Kato, A.C., Scholz, J., Decosterd, I., Mannion, R.J., Bakowska, J.C., Woolf, C.J., 2002. Hsp27 upregulation and

phosphorylation is required for injured sensory and motor neuron survival. Neuron 36, 45–56.
Bennett, G.J., Xie, Y.K., 1988. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 33, 87–107.
Bennett, A.D., Chastain, K.M., Hulsebosch, C.E., 2000. Alleviation of mechanical and thermal allodynia by CGRP(8–37) in a rodent model of chronic central pain. Pain 86, 163–175.
Bettoni, I., Comelli, F., Rossini, C., Granucci, F., Giagnoni, G., Peri, F., Costa, B., 2008. Glial TLR4 receptor as new target to treat neuropathic pain: efficacy of a new receptor antagonist in a model of peripheral nerve injury in mice. Glia 56, 1312–1319.
Boulenguez, P., Liabeuf, S., Bos, R., Bras, H., Jean-Xavier, C., Brocard, C., Stil, A., Darbon, P., Cattaert, D., Delpire, E., Marsala, M., Vinay, L., 2010. Down-regulation of the potassium-chloride cotransporter KCC2 contributes to spasticity after spinal cord injury. Nat. Med. 16, 302–307.
Bruce, J.C., Oatway, M.A., Weaver, L.C., 2002. Chronic pain after clip-compression injury of the rat spinal cord. Exp. Neurol. 178, 33–48.
Calcutt, N.A., Jorge, M.C., Yaksh, T.L., Chaplan, S.R., 1996. Tactile allodynia and formalin hyperalgesia in streptozotocin-diabetic rats: effects of insulin, aldose reductase inhibition and lidocaine. Pain 68, 293–299.
Cao, L., DeLeo, J.A., 2008. CNS-infiltrating CD4+ T lymphocytes contribute to murine spinal nerve transection-induced neuropathic pain. Eur. J. Immunol. 38, 448–458.
Cao, L., Tanga, F.Y., Deleo, J.A., 2009. The contributing role of CD14 in toll-like receptor 4 dependent neuropathic pain. Neuroscience 158, 896–903.
Carlton, S.M., Du, J., Tan, H.Y., Nesic, O., Hargett, G.L., Bopp, A.C., Yamani, A., Lin, Q., Willis, W.D., Hulsebosch, C.E., 2009. Peripheral and central sensitization in remote spinal cord regions contribute to central neuropathic pain after spinal cord injury. Pain 147, 265–276.
Caterina, M.J., Leffler, A., Malmberg, A.B., Martin, W.J., Trafton, J., Petersen-Zeitz, K.R., Koltzenburg, M., Basbaum, A.I., Julius, D., 2000. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288, 306–313.
Cavaliere, C., Cirillo, G., Rosaria Bianco, M., Rossi, F., De Novellis, V., Maione, S., Papa, M., 2007. Gliosis alters expression and uptake of spinal glial amino acid transporters in a mouse neuropathic pain model. Neuron Glia Biol. 3, 141–153.
Chaplan, S.R., Bach, F.W., Shafer, S.L., Yaksh, T.L., 1995. Prolonged alleviation of tactile allodynia by intravenous lidocaine in neuropathic rats. Anesthesiology 83, 775–785.
Cheng, H.T., Dauch, J.R., Hayes, J.M., Hong, Y., Feldman, E.L., 2009. Nerve growth factor mediates mechanical allodynia in a mouse model of type 2 diabetes. J. Neuropathol. Exp. Neurol. 68, 1229–1243.
Chessell, I.P., Hatcher, J.P., Bountra, C., Michel, A.D., Hughes, J.P., Green, P., Egerton, J., Murfin, M., Richardson, J., Peck, W.L., Grahames, C.B., Casula, M.A., Yiangou, Y., Birch, R., Anand, P., Buell, G.N., 2005. Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. Pain 114, 386–396.
Christensen, M.D., Hulsebosch, C.E., 1997a. Spinal cord injury and anti-NGF treatment results in changes in CGRP density and distribution in the dorsal horn in the rat. Exp. Neurol. 147, 463–475.
Christensen, M.D., Hulsebosch, C.E., 1997b. Chronic central pain after spinal cord injury. J. Neurotrauma 14, 517–537.
Christensen, M.D., Everhart, A.W., Pickelman, J.T., Hulsebosch, C.E., 1996. Mechanical and thermal allodynia in chronic central pain following spinal cord injury. Pain 68, 97–107.
Chung, J.M., Kim, H.K., Chung, K., 2004. Segmental spinal nerve ligation model of neuropathic pain. Methods Mol. Med. 99, 35–45.
Clark, A.K., Yip, P.K., Grist, J., Gentry, C., Staniland, A.A., Marchand, F., Dehvari, M., Wotherspoon, G., Winter, J., Ullah, J., Bevan, S., Malcangio, M., 2007. Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain. Proc. Natl Acad. Sci. U. S. A. 104, 10655–10660.
Clark, A.K., Yip, P.K., Malcangio, M., 2009. The liberation of fractalkine in the dorsal horn requires microglial cathepsin S. J. Neurosci. 29, 6945–6954.
Clark, A.K., Staniland, A.A., Marchand, F., Kaan, T.K., McMahon, S.B., Malcangio, M., 2010. P2X7-dependent release of interleukin-1beta and nociception in the spinal cord following lipopolysaccharide. J. Neurosci. 30, 573–582.
Clark, A.K., Wodarski, R., Guida, F., Sasso, O., Malcangio, M., 2011. Cathepsin S release from primary cultured microglia is regulated by the P2X7 receptor. Glia 58, 1710–1726.
Colburn, R.W., Rickman, A.J., DeLeo, J.A., 1999. The effect of site and type of nerve injury on spinal glial activation and neuropathic pain behavior. Exp. Neurol. 157, 289–304.
Costigan, M., Moss, A., Latremoliere, A., Johnston, C., Verma-Gandhu, M., Herbert, T.A., Barrett, L., Brenner, G.J., Vardeh, D., Woolf, C.J., Fitzgerald, M., 2009. T-cell infiltration and signaling in the adult dorsal spinal cord is a major contributor to neuropathic pain-like hypersensitivity. J. Neurosci. 29, 14415–14422.
Coull, J.A., Boudreau, D., Bachand, K., Prescott, S.A., Nault, F., Sik, A., De Koninck, P., De Koninck, Y., 2003. Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. Nature 424, 938–942.
Coull, J.A., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., Gravel, C., Salter, M.W., De Koninck, Y., 2005. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 438, 1017–1021.
Coyle, D.E., 1998. Partial peripheral nerve injury leads to activation of astroglia and microglia which parallels the development of allodynic behavior. Glia 23, 75–83.
Cramer, S.W., Baggott, C., Cain, J., Tilghman, J., Allcock, B., Miranpuri, G., Rajpal, S., Sun, D., Resnick, D., 2008. The role of cation-dependent chloride transporters in neuropathic pain following spinal cord injury. Mol. Pain 4, 36.
Cregg, R., Momin, A., Rugiero, F., Wood, J.N., Zhao, J., 2010. Pain channelopathies. J. Physiol. 588, 1897–1904.
Crown, E.D., Ye, Z., Johnson, K.M., Xu, G.Y., McAdoo, D.J., Hulsebosch, C.E., 2006. Increases in the activated forms of ERK 1/2, p38 MAPK, and CREB are correlated with the expression of at-level mechanical allodynia following spinal cord injury. Exp. Neurol. 199, 397–407.
Crown, E.D., Gwak, Y.S., Ye, Z., Johnson, K.M., Hulsebosch, C.E., 2008. Activation of p38 MAP kinase is involved in central neuropathic pain following spinal cord injury. Exp. Neurol. 213, 257–267.
Cui, J.G., O'Connor, W.T., Ungerstedt, U., Linderoth, B., Meyerson, B.A., 1997. Spinal cord stimulation attenuates augmented dorsal horn release of excitatory amino acids in mononeuropathy via a GABAergic mechanism. Pain 73, 87–95.
Cummins, T.R., Waxman, S.G., 1997. Downregulation of tetrodotoxin-resistant sodium currents and upregulation of a rapidly repriming tetrodotoxin-sensitive sodium current in small spinal sensory neurons after nerve injury. J. Neurosci. 17, 3503–3514.
Dansereau, M.A., Gosselin, R.D., Pohl, M., Pommier, B., Mechighel, P., Mauborgne, A., Rostene, W., Kitabgi, P., Beaudet, N., Sarret, P., Melik-Parsadaniantz, S., 2008. Spinal CCL2 pronociceptive action is no longer effective in CCR2 receptor antagonist-treated rats. J. Neurochem. 106, 757–769.
Davalos, D., Grutzendler, J., Yang, G., Kim, J.V., Zuo, Y., Jung, S., Littman, D.R., Dustin, M.L., Gan, W.B., 2005. ATP mediates rapid microglial response to local brain injury in vivo. Nat. Neurosci. 8, 752–758.
Davies, J.E., Proschel, C., Zhang, N., Noble, M., Mayer-Proschel, M., Davies, S.J., 2008. Transplanted astrocytes derived from BMP- or CNTF-treated glial-restricted precursors have opposite effects on recovery and allodynia after spinal cord injury. J. Biol. 7, 24.
de Jong, E.K., Dijkstra, I.M., Hensens, M., Brouwer, N., van Amerongen, M., Liem, R.S., Boddeke, H.W., Biber, K., 2005.

Vesicle-mediated transport and release of CCL21 in endangered neurons: a possible explanation for microglia activation remote from a primary lesion. J. Neurosci. 25, 7548–7557.
De Leo, J.A., Tawfik, V.L., LaCroix-Fralish, M.L., 2006. The tetrapartite synapse: path to CNS sensitization and chronic pain. Pain 122, 17–21.
De Vry, J., Kuhl, E., Franken-Kunkel, P., Eckel, G., 2004. Pharmacological characterization of the chronic constriction injury model of neuropathic pain. Eur. J. Pharmacol. 491, 137–148.
Decosterd, I., Woolf, C.J., 2000. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain 87, 149–158.
Deng, Y.S., Zhong, J.H., Zhou, X.F., 2000. Effects of endogenous neurotrophins on sympathetic sprouting in the dorsal root ganglia and allodynia following spinal nerve injury. Exp. Neurol. 164, 344–350.
Detloff, M.R., Fisher, L.C., McGaughy, V., Longbrake, E.E., Popovich, P.G., Basso, D.M., 2008. Remote activation of microglia and pro-inflammatory cytokines predict the onset and severity of below-level neuropathic pain after spinal cord injury in rats. Exp. Neurol. 212, 337–347.
Deumens, R., Koopmans, G.C., Joosten, E.A., 2005. Regeneration of descending axon tracts after spinal cord injury. Prog. Neurobiol. 77, 57–89.
Deumens, R., Koopmans, G.C., Honig, W.M., Hamers, F.P., Maquet, V., Jerome, R., Steinbusch, H.W., Joosten, E.A., 2006a. Olfactory ensheathing cells, olfactory nerve fibroblasts and biomatrices to promote long-distance axon regrowth and functional recovery in the dorsally hemisected adult rat spinal cord. Exp. Neurol. 200, 89–103.
Deumens, R., Koopmans, G.C., Honig, W.M., Maquet, V., Jerome, R., Steinbusch, H.W., Joosten, E.A., 2006b. Chronically injured corticospinal axons do not cross large spinal lesion gaps after a multifactorial transplantation strategy using olfactory ensheathing cell/olfactory nerve fibroblast-biomatrix bridges. J. Neurosci. Res. 83, 811–820.
Deumens, R., Lubbers, M., Jaken, R.J., Meijs, M.F., Thurlings, R.M., Honig, W.M., Schachner, M., Brook, G.A., Joosten, E.A., 2007. Mice lacking L1 have reduced CGRP fibre in-growth into spinal transection lesions. Neurosci. Lett. 420, 277–281.
Deumens, R., Joosten, E.A., Waxman, S.G., Hains, B.C., 2008. Locomotor dysfunction and pain: the scylla and charybdis of fiber sprouting after spinal cord injury. Mol. Neurobiol. 37, 52–63.
Deumens, R., Jaken, R.J., Knaepen, L., van der Meulen, I., Joosten, E.A., 2009. Inverse relation between intensity of GFAP expression in the substantia gelatinosa and degree of chronic mechanical allodynia. Neurosci. Lett. 452, 101–105.
Deumens, R., Bozkurt, A., Meek, M.F., Marcus, M.A., Joosten, E.A., Weis, J., Brook, G.A., 2010. Repairing injured peripheral nerves: bridging the gap. Prog. Neurobiol. 92, 245–276.
Devor, M., 2006a. Centralization, central sensitization and neuropathic pain. Focus on "sciatic chronic constriction injury produces cell-type-specific changes in the electrophysiological properties of rat substantia gelatinosa neurons". J. Neurophysiol. 96, 522–523.
Devor, M., 2006b. Sodium channels and mechanisms of neuropathic pain. J. Pain 7, S3–S12.
Devor, M., 2009. Ectopic discharge in Abeta afferents as a source of neuropathic pain. Exp. Brain Res. 196, 115–128.
Diamond, J., Holmes, M., Coughlin, M., 1992. Endogenous NGF and nerve impulses regulate the collateral sprouting of sensory axons in the skin of the adult rat. J. Neurosci. 12, 1454–1466.
Doble, A., 1996. The pharmacology and mechanism of action of riluzole. Neurology 47, S233–S241.
Donnelly, D.J., Popovich, P.G., 2008. Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. Exp. Neurol. 209, 378–388.
Doubell, T.P., Woolf, C.J., 1997. Growth-associated protein 43 immunoreactivity in the superficial dorsal horn of the rat spinal cord is localized in atrophic C-fiber, and not in sprouted A-fiber, central terminals after peripheral nerve injury. J. Comp. Neurol. 386, 111–118.
Doubell, T.P., Mannion, R.J., Woolf, C.J., 1997. Intact sciatic myelinated primary afferent terminals collaterally sprout in the adult rat dorsal horn following section of a neighbouring peripheral nerve. J. Comp. Neurol. 380, 95–104.
Dowdall, T., Robinson, I., Meert, T.F., 2005. Comparison of five different rat models of peripheral nerve injury. Pharmacol. Biochem. Behav. 80, 93–108.
Dworkin, R.H., O'Connor, A.B., Backonja, M., Farrar, J.T., Finnerup, N.B., Jensen, T.S., Kalso, E.A., Loeser, J.D., Miaskowski, C., Nurmikko, T.J., Portenoy, R.K., Rice, A.S., Stacey, B.R., Treede, R.D., Turk, D.C., Wallace, M.S., 2007. Pharmacologic management of neuropathic pain: evidence-based recommendations. Pain 132, 237–251.
Eaton, M.J., Wolfe, S.Q., 2009. Clinical feasibility for cell therapy using human neuronal cell line to treat neuropathic behavioral hypersensitivity following spinal cord injury in rats. J. Rehabil. Res. Dev. 46, 145–165.
Eaton, M.J., Martinez, M.A., Karmally, S., 1999a. A single intrathecal injection of GABA permanently reverses neuropathic pain after nerve injury. Brain Res. 835, 334–339.
Eaton, M.J., Plunkett, J.A., Martinez, M.A., Lopez, T., Karmally, S., Cejas, P., Whittemore, S.R., 1999b. Transplants of neuronal cells bioengineered to synthesize GABA alleviate chronic neuropathic pain. Cell Transplant. 8, 87–101.
Eaton, M.J., Wolfe, S.Q., Martinez, M., Hernandez, M., Furst, C., Huang, J., Frydel, B.R., Gomez-Marin, O., 2007. Subarachnoid transplant of a human neuronal cell line attenuates chronic allodynia and hyperalgesia after excitotoxic spinal cord injury in the rat. J. Pain 8, 33–50.
Eijkelkamp, N., Heijnen, C.J., Willemen, H.L., Deumens, R., Joosten, E.A., Kleibeuker, W., den Hartog, I.J., van Velthoven, C.T., Nijboer, C., Nassar, M.A., Dorn 2nd, G.W., Wood, J.N., Kavelaars, A., 2010. GRK2: a novel cell-specific regulator of severity and duration of inflammatory pain. J. Neurosci. 30, 2138–2149.
Eng, L.F., Ghirnikar, R.S., Lee, Y.L., 2000. Glial fibrillary acidic protein: GFAP-thirty-one years (1969–2000). Neurochem. Res. 25, 1439–1451.
Engle, M.P., Gassman, M., Sykes, K.T., Bettler, B., Hammond, D.L., 2006. Spinal nerve ligation does not alter the expression or function of GABA(B) receptors in spinal cord and dorsal root ganglia of the rat. Neuroscience 138, 1277–1287.
Faulkner, J.R., Herrmann, J.E., Woo, M.J., Tansey, K.E., Doan, N.B., Sofroniew, M.V., 2004. Reactive astrocytes protect tissue and preserve function after spinal cord injury. J. Neurosci. 24, 2143–2155.
Fernyhough, P., Diemel, L.T., Brewster, W.J., Tomlinson, D.R., 1995. Altered neurotrophin mRNA levels in peripheral nerve and skeletal muscle of experimentally diabetic rats. J. Neurochem. 64, 1231–1237.
Ferrari, D., Pizzirani, C., Adinolfi, E., Lemoli, R.M., Curti, A., Idzko, M., Panther, E., Di Virgilio, F., 2006. The P2X7 receptor: a key player in IL-1 processing and release. J. Immunol. 176, 3877–3883.
Finnerup, N.B., Jensen, T.S., 2004. Spinal cord injury pain—mechanisms and treatment. Eur. J. Neurol. 11, 73–82.
Finnerup, N.B., Sindrup, S.H., Jensen, T.S., 2010. The evidence for pharmacological treatment of neuropathic pain. Pain 150, 573–581.
Fitzgerald, M., Reynolds, M.L., Benowitz, L.I., 1991. GAP-43 expression in the developing rat lumbar spinal cord Neuroscience 41, 187–199.
Gao, X., Kim, H.K., Chung, J.M., Chung, K., 2005. Enhancement of NMDA receptor phosphorylation of the spinal dorsal horn and nucleus gracilis neurons in neuropathic rats. Pain 116, 62–72.
Gao, Y.J., Zhang, L., Samad, O.A., Suter, M.R., Yasuhiko, K., Xu, Z.Z., Park, J.Y., Lind, A.L., Ma, Q., Ji, R.R., 2009. JNK-induced MCP-1 production in spinal cord astrocytes contributes to central sensitization and neuropathic pain. J. Neurosci. 29, 4096–4108.

Garrison, C.J., Dougherty, P.M., Carlton, S.M., 1994. GFAP expression in lumbar spinal cord of naive and neuropathic rats treated with MK-801. Exp. Neurol. 129, 237–243.
Gordh, T., Chu, H., Sharma, H.S., 2006. Spinal nerve lesion alters blood–spinal cord barrier function and activates astrocytes in the rat. Pain 124, 211–221.
Govrin-Lippmann, R., Devor, M., 1978. Ongoing activity in severed nerves: source and variation with time. Brain Res. 159, 406–410.
Gris, D., Marsh, D.R., Oatway, M.A., Chen, Y., Hamilton, E.F., Dekaban, G.A., Weaver, L.C., 2004. Transient blockade of the CD11d/CD18 integrin reduces secondary damage after spinal cord injury, improving sensory, autonomic, and motor function. J. Neurosci. 24, 4043–4051.
Gruner, J.A., 1992. A monitored contusion model of spinal cord injury in the rat. J. Neurotrauma 9, 123–126 discussion 126–8.
Gu, X., Ding, F., Yang, Y., Liu, J., 2010. Construction of tissue engineered nerve grafts and their application in peripheral nerve regeneration. Prog Neurobiol.
Gwak, Y.S., Hulsebosch, C.E., 2011. GABA and central neuropathic pain following spinal cord injury. Neuropharmacology. 60, 799–808.
Gwak, Y.S., Crown, E.D., Unabia, G.C., Hulsebosch, C.E., 2008. Propentofylline attenuates allodynia, glial activation and modulates GABAergic tone after spinal cord injury in the rat. Pain 138, 410–422.
Hains, B.C., Waxman, S.G., 2006. Activated microglia contribute to the maintenance of chronic pain after spinal cord injury. J. Neurosci. 26, 4308–4317.
Hains, B.C., Johnson, K.M., McAdoo, D.J., Eaton, M.J., Hulsebosch, C.E., 2001a. Engraftment of serotonergic precursors enhances locomotor function and attenuates chronic central pain behavior following spinal hemisection injury in the rat. Exp. Neurol. 171, 361–378.
Hains, B.C., Yucra, J.A., Hulsebosch, C.E., 2001b. Reduction of pathological and behavioral deficits following spinal cord contusion injury with the selective cyclooxygenase-2 inhibitor NS-398. J. Neurotrauma 18, 409–423.
Hains, B.C., Everhart, A.W., Fullwood, S.D., Hulsebosch, C.E., 2002. Changes in serotonin, serotonin transporter expression and serotonin denervation supersensitivity: involvement in chronic central pain after spinal hemisection in the rat. Exp. Neurol. 175, 347–362.
Hains, B.C., Klein, J.P., Saab, C.Y., Craner, M.J., Black, J.A., Waxman, S.G., 2003a. Upregulation of sodium channel Nav1.3 and functional involvement in neuronal hyperexcitability associated with central neuropathic pain after spinal cord injury. J. Neurosci. 23, 8881–8892.
Hains, B.C., Willis, W.D., Hulsebosch, C.E., 2003b. Serotonin receptors 5-HT1A and 5-HT3 reduce hyperexcitability of dorsal horn neurons after chronic spinal cord hemisection injury in rat. Exp. Brain Res. 149, 174–186.
Hains, B.C., Saab, C.Y., Klein, J.P., Craner, M.J., Waxman, S.G., 2004. Altered sodium channel expression in second-order spinal sensory neurons contributes to pain after peripheral nerve injury. J. Neurosci. 24, 4832–4839.
Hains, B.C., Saab, C.Y., Waxman, S.G., 2005. Changes in electrophysiological properties and sodium channel Nav1.3 expression in thalamic neurons after spinal cord injury. Brain 128, 2359–2371.
Hanani, M., 2005. Satellite glial cells in sensory ganglia: from form to function. Brain Res. Brain Res. Rev. 48, 457–476.
Hao, J.X., Xu, X.J., Aldskogius, H., Seiger, A., Wiesenfeld-Hallin, Z., 1991. Allodynia-like effects in rat after ischaemic spinal cord injury photochemically induced by laser irradiation. Pain 45, 175–185.
Hasbargen, T., Ahmed, M.M., Miranpuri, G., Li, L., Kahle, K.T., Resnick, D., Sun, D., 2010. Role of NKCC1 and KCC2 in the development of chronic neuropathic pain following spinal cord injury. Ann. N Y Acad. Sci. 1198, 168–172.
Haydon, P.G., Carmignoto, G., 2006. Astrocyte control of synaptic transmission and neurovascular coupling. Physiol. Rev. 86, 1009–1031.
Haynes, S.E., Hollopeter, G., Yang, G., Kurpius, D., Dailey, M.E., Gan, W.B., Julius, D., 2006. The P2Y12 receptor regulates microglial activation by extracellular nucleotides. Nat. Neurosci. 9, 1512–1519.
Hofstetter, C.P., Holmstrom, N.A., Lilja, J.A., Schweinhardt, P., Hao, J., Spenger, C., Wiesenfeld-Hallin, Z., Kurpad, S.N., Frisen, J., Olson, L., 2005. Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome. Nat. Neurosci. 8, 346–353.
Honda, S., Sasaki, Y., Ohsawa, K., Imai, Y., Nakamura, Y., Inoue, K., Kohsaka, S., 2001. Extracellular ATP or ADP induce chemotaxis of cultured microglia through Gi/o-coupled P2Y receptors. J. Neurosci. 21, 1975–1982.
Honore, P., Kage, K., Mikusa, J., Watt, A.T., Johnston, J.F., Wyatt, J.R., Faltynek, C.R., Jarvis, M.F., Lynch, K., 2002. Analgesic profile of intrathecal P2X(3) antisense oligonucleotide treatment in chronic inflammatory and neuropathic pain states in rats. Pain 99, 11–19.
Hu, S.J., Xing, J.L., 1998. An experimental model for chronic compression of dorsal root ganglion produced by intervertebral foramen stenosis in the rat. Pain 77, 15–23.
Hu, P., Bembrick, A.L., Keay, K.A., McLachlan, E.M., 2007. Immune cell involvement in dorsal root ganglia and spinal cord after chronic constriction or transection of the rat sciatic nerve. Brain Behav. Immun. 21, 599–616.
Hu, Y., Li, W., Lu, L., Cai, J., Xian, X., Zhang, M., Li, Q., Li, L., 2010. An anti-nociceptive role for ceftriaxone in chronic neuropathic pain in rats. Pain 148, 284–301.
Hughes, D.I., Scott, D.T., Todd, A.J., Riddell, J.S., 2003. Lack of evidence for sprouting of Abeta afferents into the superficial laminas of the spinal cord dorsal horn after nerve section. J. Neurosci. 23, 9491–9499.
Hughes, D.I., Scott, D.T., Riddell, J.S., Todd, A.J., 2007. Upregulation of substance P in low-threshold myelinated afferents is not required for tactile allodynia in the chronic constriction injury and spinal nerve ligation models. J. Neurosci. 27, 2035–2044.
Hulsebosch, C.E., 2005. From discovery to clinical trials: treatment strategies for central neuropathic pain after spinal cord injury. Curr. Pharm. Des. 11, 1411–1420.
Hulsebosch, C.E., 2008. Gliopathy ensures persistent inflammation and chronic pain after spinal cord injury. Exp. Neurol. 214, 6–9.
Hulsebosch, C.E., Xu, G.Y., Perez-Polo, J.R., Westlund, K.N., Taylor, C.P., McAdoo, D.J., 2000. Rodent model of chronic central pain after spinal cord contusion injury and effects of gabapentin. J. Neurotrauma 17, 1205–1217.
Hulsebosch, C.E., Hains, B.C., Crown, E.D., Carlton, S.M., 2009. Mechanisms of chronic central neuropathic pain after spinal cord injury. Brain Res. Rev. 60, 202–213.
Hunt, S.P., Mantyh, P.W., 2001. The molecular dynamics of pain control. Nat. Rev. Neurosci. 2, 83–91.
Hutchinson, M.R., Ramos, K.M., Loram, L.C., Wieseler, J., Sholar, P.W., Kearney, J.J., Lewis, M.T., Crysdale, N.Y., Zhang, Y., Harrison, J.A., Maier, S.F., Rice, K.C., Watkins, L.R., 2009. Evidence for a role of heat shock protein-90 in toll like receptor 4 mediated pain enhancement in rats. Neuroscience 164, 1821–1832.
Ito, D., Imai, Y., Ohsawa, K., Nakajima, K., Fukuuchi, Y., Kohsaka, S., 1998. Microglia-specific localisation of a novel calcium binding protein, Iba1. Brain Res. Mol. Brain Res. 57, 1–9.
Jang, J.H., Kim, K.H., Nam, T.S., Lee, W.T., Park, K.A., Kim, D.W., Leem, J.W., 2007. The role of uninjured C-afferents and injured afferents in the generation of mechanical hypersensitivity after partial peripheral nerve injury in the rat. Exp. Neurol. 204, 288–298.
Jarvis, M.F., 2010. The neural–glial purinergic receptor ensemble in chronic pain states. Trends Neurosci. 33, 48–57.
Jeon, S.M., Lee, K.M., Cho, H.J., 2009. Expression of monocyte chemoattractant protein-1 in rat dorsal root ganglia and spinal cord in experimental models of neuropathic pain. Brain Res. 1251, 103–111.
Ji, R.R., Kawasaki, Y., Zhuang, Z.Y., Wen, Y.R., Decosterd, I., 2006. Possible role of spinal astrocytes in maintaining chronic pain

sensitization: review of current evidence with focus on bFGF/JNK pathway. Neuron Glia Biol. 2, 259–269.
Ji, R.R., Gereau, R.W.t., Malcangio, M., Strichartz, G.R., 2009. MAP kinase and pain. Brain Res. Rev. 60, 135–148.
Jin, S.X., Zhuang, Z.Y., Woolf, C.J., Ji, R.R., 2003. 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. 23, 4017–4022.
Jolivalt, C.G., Lee, C.A., Ramos, K.M., Calcutt, N.A., 2008. Allodynia and hyperalgesia in diabetic rats are mediated by GABA and depletion of spinal potassium-chloride co-transporters. Pain 140, 48–57.
Jones, M.G., Munson, J.B., Thompson, S.W., 1999. A role for nerve growth factor in sympathetic sprouting in rat dorsal root ganglia. Pain 79, 21–29.
Jung, H., Toth, P.T., White, F.A., Miller, R.J., 2008. Monocyte chemoattractant protein-1 functions as a neuromodulator in dorsal root ganglia neurons. J. Neurochem. 104, 254–263.
Kato, K., Kikuchi, S., Shubayev, V.I., Myers, R.R., 2009. Distribution and tumor necrosis factor-alpha isoform binding specificity of locally administered etanercept into injured and uninjured rat sciatic nerve. Neuroscience 160, 492–500.
Kawasaki, Y., Xu, Z.Z., Wang, X., Park, J.Y., Zhuang, Z.Y., Tan, P.H., Gao, Y.J., Roy, K., Corfas, G., Lo, E.H., Ji, R.R., 2008a. Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. Nat. Med. 14, 331–336.
Kawasaki, Y., Zhang, L., Cheng, J.K., Ji, R.R., 2008b. Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. J. Neurosci. 28, 5189–5194.
Kawate, T., Michel, J.C., Birdsong, W.T., Gouaux, E., 2009. Crystal structure of the ATP-gated P2X(4) ion channel in the closed state. Nature 460, 592–598.
Keller, A.F., Beggs, S., Salter, M.W., De Koninck, Y., 2007. Transformation of the output of spinal lamina I neurons after nerve injury and microglia stimulation underlying neuropathic pain. Mol. Pain 3, 27.
Kemler, M.A., Barendse, G.A., van Kleef, M., de Vet, H.C., Rijks, C.P., Furnee, C.A., van den Wildenberg, F.A., 2000. Spinal cord stimulation in patients with chronic reflex sympathetic dystrophy. N Engl J. Med. 343, 618–624.
Kemler, M.A., de Vet, H.C., Barendse, G.A., van den Wildenberg, F.A., van Kleef, M., 2008. Effect of spinal cord stimulation for chronic complex regional pain syndrome Type I: five-year final follow-up of patients in a randomized controlled trial. J. Neurosurg. 108, 292–298.
Keurentjes, J.T., Kemmere, M.F., Bruinewoud, H., Vertommen, M.A., Rovers, S.A., Hoogenboom, R., Stemkens, L.F., Peters, F.L., Tielen, N.J., van Asseldonk, D.T., Gabriel, A.F., Joosten, E.A., Marcus, M.A., 2009. Externally triggered glass transition switch for localized on-demand drug delivery. Angew. Chem. Int. Ed. Engl. 48, 9867–9870.
Kigerl, K.A., Lai, W., Rivest, S., Hart, R.P., Satoskar, A.R., Popovich, P.G., 2007. Toll-like receptor (TLR)-2 and TLR-4 regulate inflammation, gliosis, and myelin sparing after spinal cord injury. J. Neurochem. 102, 37–50.
Kim, S.H., Chung, J.M., 1992. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 50, 355–363.
Kim, D., Kim, M.A., Cho, I.H., Kim, M.S., Lee, S., Jo, E.K., Choi, S.Y., Park, K., Kim, J.S., Akira, S., Na, H.S., Oh, S.B., Lee, S.J., 2007. A critical role of toll-like receptor 2 in nerve injury-induced spinal cord glial cell activation and pain hypersensitivity. J. Biol. Chem. 282, 14975–14983.
Kim, D.S., Figueroa, K.W., Li, K.W., Boroujerdi, A., Yolo, T., Luo, Z.D., 2009. Profiling of dynamically changed gene expression in dorsal root ganglia post peripheral nerve injury and a critical role of injury-induced glial fibrillary acidic protein in maintenance of pain behaviors. Pain 143, 114–122 [corrected].
Kingery, W.S., Vallin, J.A., 1989. The development of chronic mechanical hyperalgesia, autotomy and collateral sprouting following sciatic nerve section in rat. Pain 38, 321–332.
Kloos, A.D., Fisher, L.C., Detloff, M.R., Hassenzahl, D.L., Basso, D.M., 2005. Stepwise motor and all-or-none sensory recovery is associated with nonlinear sparing after incremental spinal cord injury in rats. Exp. Neurol. 191, 251–265.
Klusakova, I., Dubovy, P., 2009. Experimental models of peripheral neuropathic pain based on traumatic nerve injuries — an anatomical perspective. Ann. Anat. 191, 248–259.
Kobayashi, K., Yamanaka, H., Fukuoka, T., Dai, Y., Obata, K., Noguchi, K., 2008. P2Y12 receptor upregulation in activated microglia is a gateway of p38 signaling and neuropathic pain. J. Neurosci. 28, 2892–2902.
Koopmans, G.C., Deumens, R., Buss, A., Geoghegan, L., Myint, A.M., Honig, W.H., Kern, N., Joosten, E.A., Noth, J., Brook, G.A., 2009. Acute rolipram/thalidomide treatment improves tissue sparing and locomotion after experimental spinal cord injury. Exp. Neurol. 216, 490–498.
Kovalsky, Y., Amir, R., Devor, M., 2008. Subthreshold oscillations facilitate neuropathic spike discharge by overcoming membrane accommodation. Exp. Neurol. 210, 194–206.
Kovalsky, Y., Amir, R., Devor, M., 2009. Simulation in sensory neurons reveals a key role for delayed Na+current in subthreshold oscillations and ectopic discharge: implications for neuropathic pain. J. Neurophysiol. 102, 1430–1442.
Kozai, T., Yamanaka, H., Dai, Y., Obata, K., Kobayashi, K., Mashimo, T., Noguchi, K., 2007. Tissue type plasminogen activator induced in rat dorsal horn astrocytes contributes to mechanical hypersensitivity following dorsal root injury. Glia 55, 595–603.
Krenz, N.R., Weaver, L.C., 2000. Nerve growth factor in glia and inflammatory cells of the injured rat spinal cord. J. Neurochem. 74, 730–739.
Kreutzberg, G.W., 1996. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 19, 312–318.
Krueger-Naug, A.M., Hopkins, D.A., Armstrong, J.N., Plumier, J.C., Currie, R.W., 2000. Hyperthermic induction of the 27-kDa heat shock protein (Hsp27) in neuroglia and neurons of the rat central nervous system. J. Comp. Neurol. 428, 495–510.
Latremoliere, A., Woolf, C.J., 2009. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J. Pain 10, 895–926.
Ledeboer, A., Liu, T., Shumilla, J.A., Mahoney, J.H., Vijay, S., Gross, M.I., Vargas, J.A., Sultzbaugh, L., Claypool, M.D., Sanftner, L.M., Watkins, L.R., Johnson, K.W., 2006. The glial modulatory drug AV411 attenuates mechanical allodynia in rat models of neuropathic pain. Neuron Glia Biol. 2, 279–291.
Lekan, H.A., Carlton, S.M., Coggeshall, R.E., 1996. Sprouting of A beta fibers into lamina II of the rat dorsal horn in peripheral neuropathy. Neurosci. Lett. 208, 147–150.
Lever, I., Cunningham, J., Grist, J., Yip, P.K., Malcangio, M., 2003. Release of BDNF and GABA in the dorsal horn of neuropathic rats. Eur. J. Neurosci. 18, 1169–1174.
Li, Y., Field, P.M., Raisman, G., 1997. Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. Science 277, 2000–2002.
Li, L., Xian, C.J., Zhong, J.H., Zhou, X.F., 2002. Effect of lumbar 5 ventral root transection on pain behaviors: a novel rat model for neuropathic pain without axotomy of primary sensory neurons. Exp. Neurol. 175, 23–34.
Lindia, J.A., McGowan, E., Jochnowitz, N., Abbadie, C., 2005. Induction of CX3CL1 expression in astrocytes and CX3CR1 in microglia in the spinal cord of a rat model of neuropathic pain. J. Pain 6, 434–438.
Ling, E.A., Kaur, L.C., Yick, T.Y., Wong, W.C., 1990. Immunocytochemical localization of CR3 complement receptors with OX-42 in amoeboid microglia in postnatal rats. Anat. Embryol. (Berl). 182, 481–486.
Liu, C.N., Michaelis, M., Amir, R., Devor, M., 2000. Spinal nerve injury enhances subthreshold membrane potential

oscillations in DRG neurons: relation to neuropathic pain. J. Neurophysiol. 84, 205–215.
Liu, C.N., Devor, M., Waxman, S.G., Kocsis, J.D., 2002. Subthreshold oscillations induced by spinal nerve injury in dissociated muscle and cutaneous afferents of mouse DRG. J. Neurophysiol. 87, 2009–2017.
Liu, J., Wolfe, D., Hao, S., Huang, S., Glorioso, J.C., Mata, M., Fink, D.J., 2004. Peripherally delivered glutamic acid decarboxylase gene therapy for spinal cord injury pain. Mol. Ther. 10, 57–66.
Loeser, J.D., Ward Jr., A.A., White Jr., L.E., 1968. Chronic deafferentation of human spinal cord neurons. J. Neurosurg. 29, 48–50.
Lu, Y., Zheng, J., Xiong, L., Zimmermann, M., Yang, J., 2008. Spinal cord injury-induced attenuation of GABAergic inhibition in spinal dorsal horn circuits is associated with down-regulation of the chloride transporter KCC2 in rat. J. Physiol. 586, 5701–5715.
Ma, W., Ramer, M.S., Bisby, M.A., 1999. Increased calcitonin gene-related peptide immunoreactivity in gracile nucleus after partial sciatic nerve injury: age-dependent and originating from spared sensory neurons. Exp. Neurol. 159, 459–473.
Mackay-Sim, A., Feron, F., Cochrane, J., Bassingthwaighte, L., Bayliss, C., Davies, W., Fronek, P., Gray, C., Kerr, G., Licina, P., Nowitzke, A., Perry, C., Silburn, P.A., Urquhart, S., Geraghty, T., 2008. Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical trial. Brain 131, 2376–2386.
Malcangio, M., Ramer, M.S., Jones, M.G., McMahon, S.B., 2000. Abnormal substance P release from the spinal cord following injury to primary sensory neurons. Eur. J. Neurosci. 12, 397–399.
Malmberg, A.B., Chen, C., Tonegawa, S., Basbaum, A.I., 1997. Preserved acute pain and reduced neuropathic pain in mice lacking PKCgamma. Science 278, 279–283.
Mao, Q.X., Yang, T.D., 2010. Amitriptyline upregulates EAAT1 and EAAT2 in neuropathic pain rats. Brain Res. Bull. 81, 424–427.
Marchand, J.E., Wurm, W.H., Kato, T., Kream, R.M., 1994. Altered tachykinin expression by dorsal root ganglion neurons in a rat model of neuropathic pain. Pain 58, 219–231.
Marchand, F., Tsantoulas, C., Singh, D., Grist, J., Clark, A.K., Bradbury, E.J., McMahon, S.B., 2009. Effects of Etanercept and Minocycline in a rat model of spinal cord injury. Eur. J. Pain 13, 673–681.
McGaraughty, S., Chu, K.L., Namovic, M.T., Donnelly-Roberts, D.L., Harris, R.R., Zhang, X.F., Shieh, C.C., Wismer, C.T., Zhu, C.Z., Gauvin, D.M., Fabiyi, A.C., Honore, P., Gregg, R.J., Kort, M.E., Nelson, D.W., Carroll, W.A., Marsh, K., Faltynek, C.R., Jarvis, M.F., 2007. P2X7-related modulation of pathological nociception in rats. Neuroscience 146, 1817–1828.
McMahon, S.B., Malcangio, M., 2009. Current challenges in glia–pain biology. Neuron 64, 46–54.
Meisner, J.G., Marsh, A.D., Marsh, D.R., 2010. Loss of GABAergic interneurons in laminae I–III of the spinal cord dorsal horn contributes to reduced GABAergic tone and neuropathic pain after spinal cord injury. J. Neurotrauma 27, 729–737.
Michael, G.J., Averill, S., Shortland, P.J., Yan, Q., Priestley, J.V., 1999. Axotomy results in major changes in BDNF expression by dorsal root ganglion cells: BDNF expression in large trkB and trkC cells, in pericellular baskets, and in projections to deep dorsal horn and dorsal column nuclei. Eur. J. Neurosci. 11, 3539–3551.
Mika, J., Korostynski, M., Kaminska, D., Wawrzczak-Bargiela, A., Osikowicz, M., Makuch, W., Przewlocki, R., Przewlocka, B., 2008. Interleukin-1 alpha has antiallodynic and antihyperalgesic activities in a rat neuropathic pain model. Pain 138, 587–597.
Millan, M.J., 2002. Descending control of pain. Prog. Neurobiol. 66, 355–474.
Milligan, E.D., Watkins, L.R., 2009. Pathological and protective roles of glia in chronic pain. Nat. Rev. Neurosci. 10, 23–36.
Milligan, E.D., Zapata, V., Chacur, M., Schoeniger, D., Biedenkapp, J., O'Connor, K.A., Verge, G.M., Chapman, G., Green, P., Foster, A.C., Naeve, G.S., Maier, S.F., Watkins, L.R., 2004. Evidence that exogenous and endogenous fractalkine can induce spinal nociceptive facilitation in rats. Eur. J. Neurosci. 20, 2294–2302.
Miraucourt, L.S., Dallel, R., Voisin, D.L., 2007. Glycine inhibitory dysfunction turns touch into pain through PKCgamma interneurons. PLoS One 2, e1116.
Miraucourt, L.S., Moisset, X., Dallel, R., Voisin, D.L., 2009. Glycine inhibitory dysfunction induces a selectively dynamic, morphine-resistant, and neurokinin 1 receptor-independent mechanical allodynia. J. Neurosci. 29, 2519–2527.
Mirzaei, V., Manaheji, H., Maghsoudi, N., Zaringhalam, J., 2010. Comparison of changes in mRNA expression of spinal glutamate transporters following induction of two neuropathic pain models. Spinal Cord 48, 791–797.
Miyoshi, K., Obata, K., Kondo, T., Okamura, H., Noguchi, K., 2008. Interleukin-18-mediated microglia/astrocyte interaction in the spinal cord enhances neuropathic pain processing after nerve injury. J. Neurosci. 28, 12775–12787.
Moore, K.A., Kohno, T., Karchewski, L.A., Scholz, J., Baba, H., Woolf, C.J., 2002. Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord. J. Neurosci. 22, 6724–6731.
Mukhida, K., Mendez, I., McLeod, M., Kobayashi, N., Haughn, C., Milne, B., Baghbaderani, B., Sen, A., Behie, L.A., Hong, M., 2007. Spinal GABAergic transplants attenuate mechanical allodynia in a rat model of neuropathic pain. Stem Cells 25, 2874–2885.
Myer, D.J., Gurkoff, G.G., Lee, S.M., Hovda, D.A., Sofroniew, M.V., 2006. Essential protective roles of reactive astrocytes in traumatic brain injury. Brain 129, 2761–2772.
Nagata, K., Imai, T., Yamashita, T., Tsuda, M., Tozaki-Saitoh, H., Inoue, K., 2009. Antidepressants inhibit P2X4 receptor function: a possible involvement in neuropathic pain relief. Mol. Pain 5, 20.
Nagy, G.G., Watanabe, M., Fukaya, M., Todd, A.J., 2004. Synaptic distribution of the NR1, NR2A and NR2B subunits of the N-methyl-D-aspartate receptor in the rat lumbar spinal cord revealed with an antigen-unmasking technique. Eur. J. Neurosci. 20, 3301–3312.
Nakamura, S., Myers, R.R., 1999. Myelinated afferents sprout into lamina II of L3–5 dorsal horn following chronic constriction nerve injury in rats. Brain Res. 818, 285–290.
Nassar, M.A., Stirling, L.C., Forlani, G., Baker, M.D., Matthews, E.A., Dickenson, A.H., Wood, J.N., 2004. Nociceptor-specific gene deletion reveals a major role for Nav1.7 (PN1) in acute and inflammatory pain. Proc. Natl Acad. Sci. U. S. A. 101, 12706–12711.
Nassar, M.A., Levato, A., Stirling, L.C., Wood, J.N., 2005. Neuropathic pain develops normally in mice lacking both Na(v)1.7 and Na(v)1.8. Mol. Pain 1, 24.
Nassar, M.A., Baker, M.D., Levato, A., Ingram, R., Mallucci, G., McMahon, S.B., Wood, J.N., 2006. Nerve injury induces robust allodynia and ectopic discharges in Nav1.3 null mutant mice. Mol. Pain 2, 33.
Nasu-Tada, K., Koizumi, S., Tsuda, M., Kunifusa, E., Inoue, K., 2006. Possible involvement of increase in spinal fibronectin following peripheral nerve injury in upregulation of microglial P2X4, a key molecule for mechanical allodynia. Glia 53, 769–775.
Nesic, O., Lee, J., Johnson, K.M., Ye, Z., Xu, G.Y., Unabia, G.C., Wood, T.G., McAdoo, D.J., Westlund, K.N., Hulsebosch, C.E., Regino Perez-Polo, J., 2005. Transcriptional profiling of spinal cord injury-induced central neuropathic pain. J. Neurochem. 95, 998–1014.
Nesic, O., Lee, J., Unabia, G.C., Johnson, K., Ye, Z., Vergara, L., Hulsebosch, C.E., Perez-Polo, J.R., 2008. Aquaporin 1 — a novel player in spinal cord injury. J. Neurochem. 105, 628–640.
Nesic, O., Sundberg, L.M., Herrera, J.J., Mokkapati, V.U., Lee, J., Narayana, P.A., 2010. Vascular endothelial growth factor and spinal cord injury pain. J. Neurotrauma 27, 1793–1803.
Neumann, S., Braz, J.M., Skinner, K., Llewellyn-Smith, I.J., Basbaum, A.I., 2008. Innocuous, not noxious, input activates PKCgamma interneurons of the spinal dorsal horn via myelinated afferent fibers. J. Neurosci. 28, 7936–7944.
Nie, H., Zhang, H., Weng, H.R., 2010. Minocycline prevents impaired glial glutamate uptake in the spinal sensory synapses of neuropathic rats. Neuroscience 170, 901–912.

Nimmerjahn, A., Kirchhoff, F., Helmchen, F., 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308, 1314–1318.
Novakovic, S.D., Kassotakis, L.C., Oglesby, I.B., Smith, J.A., Eglen, R. M., Ford, A.P., Hunter, J.C., 1999. Immunocytochemical localization of P2X3 purinoceptors in sensory neurons in naive rats and following neuropathic injury. Pain 80, 273–282.
Obata, K., Katsura, H., Miyoshi, K., Kondo, T., Yamanaka, H., Kobayashi, K., Dai, Y., Fukuoka, T., Akira, S., Noguchi, K., 2008. Toll-like receptor 3 contributes to spinal glial activation and tactile allodynia after nerve injury. J. Neurochem. 105, 2249–2259.
Oestreicher, A.B., De Graan, P.N., Gispen, W.H., Verhaagen, J., Schrama, L.H., 1997. B-50, the growth associated protein-43: modulation of cell morphology and communication in the nervous system. Prog. Neurobiol. 53, 627–686.
Okamoto, K., Martin, D.P., Schmelzer, J.D., Mitsui, Y., Low, P.A., 2001. Pro- and anti-inflammatory cytokine gene expression in rat sciatic nerve chronic constriction injury model of neuropathic pain. Exp. Neurol. 169, 386–391.
Oku, R., Satoh, M., Fujii, N., Otaka, A., Yajima, H., Takagi, H., 1987. Calcitonin gene-related peptide promotes mechanical nociception by potentiating release of substance P from the spinal dorsal horn in rats. Brain Res. 403, 350–354.
Owolabi, S.A., Saab, C.Y., 2006. Fractalkine and minocycline alter neuronal activity in the spinal cord dorsal horn. FEBS Lett. 580, 4306–4310.
Pacini, A., Di Cesare Mannelli, L., Bonaccini, L., Ronzoni, S., Bartolini, A., Ghelardini, C., 2010. Protective effect of alpha7 nAChR: behavioural and morphological features on neuropathy. Pain 150, 542–549.
Peltier, A.C., Russell, J.W., 2002. Recent advances in drug-induced neuropathies. Curr. Opin. Neurol. 15, 633–638.
Peng, X.M., Zhou, Z.G., Glorioso, J.C., Fink, D.J., Mata, M., 2006. Tumor necrosis factor-alpha contributes to below-level neuropathic pain after spinal cord injury. Ann. Neurol. 59, 843–851.
Perea, G., Navarrete, M., Araque, A., 2009. Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci. 32, 421–431.
Persson, M., Sandberg, M., Hansson, E., Ronnback, L., 2006. Microglial glutamate uptake is coupled to glutathione synthesis and glutamate release. Eur. J. Neurosci. 24, 1063–1070.
Pineau, I., Sun, L., Bastien, D., Lacroix, S., 2010. Astrocytes initiate inflammation in the injured mouse spinal cord by promoting the entry of neutrophils and inflammatory monocytes in an IL-1 receptor/MyD88-dependent fashion. Brain Behav. Immun. 24, 540–553.
Polgar, E., Todd, A.J., 2008. Tactile allodynia can occur in the spared nerve injury model in the rat without selective loss of GABA or GABA(A) receptors from synapses in laminae I–II of the ipsilateral spinal dorsal horn. Neuroscience 156, 193–202.
Polgar, E., Hughes, D.I., Riddell, J.S., Maxwell, D.J., Puskar, Z., Todd, A.J., 2003. Selective loss of spinal GABAergic or glycinergic neurons is not necessary for development of thermal hyperalgesia in the chronic constriction injury model of neuropathic pain. Pain 104, 229–239.
Polgar, E., Hughes, D.I., Arham, A.Z., Todd, A.J., 2005. Loss of neurons from laminas I–III of the spinal dorsal horn is not required for development of tactile allodynia in the spared nerve injury model of neuropathic pain. J. Neurosci. 25, 6658–6666.
Price, T.J., Cervero, F., de Koninck, Y., 2005. Role of cation-chloride-cotransporters (CCC) in pain and hyperalgesia. Curr. Top. Med. Chem. 5, 547–555.
Priest, B.T., Murphy, B.A., Lindia, J.A., Diaz, C., Abbadie, C., Ritter, A.M., Liberator, P., Iyer, L.M., Kash, S.F., Kohler, M.G., Kaczorowski, G.J., MacIntyre, D.E., Martin, W.J., 2005. Contribution of the tetrodotoxin-resistant voltage-gated sodium channel NaV1.9 to sensory transmission and nociceptive behavior. Proc. Natl Acad. Sci. U. S. A. 102, 9382–9387.
Qin, X., Wan, Y., Wang, X., 2005. CCL2 and CXCL1 trigger calcitonin gene-related peptide release by exciting primary nociceptive neurons. J. Neurosci. Res. 82, 51–62.
Qu, X.X., Cai, J., Li, M.J., Chi, Y.N., Liao, F.F., Liu, F.Y., Wan, Y., Han, J.S., Xing, G.G., 2009. Role of the spinal cord NR2B-containing NMDA receptors in the development of neuropathic pain. Exp. Neurol. 215, 298–307.
Radhakrishnan, K., Litchy, W.J., O'Fallon, W.M., Kurland, L.T., 1994. Epidemiology of cervical radiculopathy. A population-based study from Rochester, Minnesota, 1976 through 1990. Brain 117 (Pt 2), 325–335.
Raghavendra, V., Tanga, F., DeLeo, J.A., 2003a. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. J. Pharmacol. Exp. Ther. 306, 624–630.
Raghavendra, V., Tanga, F., Rutkowski, M.D., DeLeo, J.A., 2003b. Anti-hyperalgesic and morphine-sparing actions of propentofylline following peripheral nerve injury in rats: mechanistic implications of spinal glia and proinflammatory cytokines. Pain 104, 655–664.
Raisman, G., 2001. Olfactory ensheathing cells — another miracle cure for spinal cord injury? Nat. Rev. Neurosci. 2, 369–375.
Ramer, M.S., Thompson, S.W., McMahon, S.B., 1999. Causes and consequences of sympathetic basket formation in dorsal root ganglia. Pain Suppl. 6, S111–S120.
Ramon-Cueto, A., Cordero, M.I., Santos-Benito, F.F., Avila, J., 2000. Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia. Neuron 25, 425–435.
Ransohoff, R.M., Perry, V.H., 2009. Microglial physiology: unique stimuli, specialized responses. Annu. Rev. Immunol. 27, 119–145.
Rappert, A., Biber, K., Nolte, C., Lipp, M., Schubel, A., Lu, B., Gerard, N.P., Gerard, C., Boddeke, H.W., Kettenmann, H., 2002. Secondary lymphoid tissue chemokine (CCL21) activates CXCR3 to trigger a Cl– current and chemotaxis in murine microglia. J. Immunol. 168, 3221–3226.
Reeve, A.J., Patel, S., Fox, A., Walker, K., Urban, L., 2000. Intrathecally administered endotoxin or cytokines produce allodynia, hyperalgesia and changes in spinal cord neuronal responses to nociceptive stimuli in the rat. Eur. J. Pain 4, 247–257.
Richter, M.W., Fletcher, P.A., Liu, J., Tetzlaff, W., Roskams, A.J., 2005. Lamina propria and olfactory bulb ensheathing cells exhibit differential integration and migration and promote differential axon sprouting in the lesioned spinal cord. J. Neurosci. 25, 10700–10711.
Ro, L.S., Chen, S.T., Tang, L.M., Jacobs, J.M., 1999. Effect of NGF and anti-NGF on neuropathic pain in rats following chronic constriction injury of the sciatic nerve. Pain 79, 265–274.
Roh, D.H., Yoon, S.Y., Seo, H.S., Kang, S.Y., Han, H.J., Beitz, A.J., Lee, J.H., 2010. Intrathecal injection of carbenoxolone, a gap junction decoupler, attenuates the induction of below-level neuropathic pain after spinal cord injury in rats. Exp. Neurol. 224, 123–132.
Romero, M.I., Rangappa, N., Garry, M.G., Smith, G.M., 2001. Functional regeneration of chronically injured sensory afferents into adult spinal cord after neurotrophin gene therapy. J. Neurosci. 21, 8408–8416.
Romero-Sandoval, A., Chai, N., Nutile-McMenemy, N., Deleo, J.A., 2008. A comparison of spinal Iba1 and GFAP expression in rodent models of acute and chronic pain. Brain Res. 1219, 116–126.
Rothman, S.M., Huang, Z., Lee, K.E., Weisshaar, C.L., Winkelstein, B.A., 2009. Cytokine mRNA expression in painful radiculopathy. J. Pain 10, 90–99.
Rothstein, J.D., Dykes-Hoberg, M., Pardo, C.A., Bristol, L.A., Jin, L., Kuncl, R.W., Kanai, Y., Hediger, M.A., Wang, Y., Schielke, J.P., Welty, D.F., 1996. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16, 675–686.

Russell, J.W., Gill, J.S., Sorenson, E.J., Schultz, D.A., Windebank, A.J., 2001. Suramin-induced neuropathy in an animal model. J. Neurol. Sci. 192, 71–80.
Saade, N.E., Jabbur, S.J., 2008. Nociceptive behavior in animal models for peripheral neuropathy: spinal and supraspinal mechanisms. Prog. Neurobiol. 86, 22–47.
Sandkuhler, J., 2009. Models and mechanisms of hyperalgesia and allodynia. Physiol. Rev. 89, 707–758.
Saville, L.R., Pospisil, C.H., Mawhinney, L.A., Bao, F., Simedrea, F.C., Peters, A.A., O'Connell, P.J., Weaver, L.C., Dekaban, G.A., 2004. A monoclonal antibody to CD11d reduces the inflammatory infiltrate into the injured spinal cord: a potential neuroprotective treatment. J. Neuroimmunol. 156, 42–57.
Schafers, M., Geis, C., Brors, D., Yaksh, T.L., Sommer, C., 2002. Anterograde transport of tumor necrosis factor-alpha in the intact and injured rat sciatic nerve. J. Neurosci. 22, 536–545.
Schafers, M., Lee, D.H., Brors, D., Yaksh, T.L., Sorkin, L.S., 2003a. Increased sensitivity of injured and adjacent uninjured rat primary sensory neurons to exogenous tumor necrosis factor-alpha after spinal nerve ligation. J. Neurosci. 23, 3028–3038.
Schafers, M., Svensson, C.I., Sommer, C., Sorkin, L.S., 2003b. Tumor necrosis factor-alpha induces mechanical allodynia after spinal nerve ligation by activation of p38 MAPK in primary sensory neurons. J. Neurosci. 23, 2517–2521.
Scherrer, G., Low, S.A., Wang, X., Zhang, J., Yamanaka, H., Urban, R., Solorzano, C., Harper, B., Hnasko, T.S., Edwards, R.H., Basbaum, A.I., 2010. VGLUT2 expression in primary afferent neurons is essential for normal acute pain and injury-induced heat hypersensitivity. Proc. Natl Acad. Sci. U. S. A. 107, 22296–22301.
Schmidli, R.S., Faulkner-Jones, B.E., Harrison, L.C., James, R.F., DeAizpurua, H.J., 1996. Cytokine regulation of glutamate decarboxylase biosynthesis in isolated rat islets of Langerhans. Biochem. J. 317 (Pt 3), 713–719.
Schmidt, Y., Unger, J.W., Bartke, I., Reiter, R., 1995. Effect of nerve growth factor on peptide neurons in dorsal root ganglia after taxol or cisplatin treatment and in diabetic (*db*/*db*) mice. Exp. Neurol. 132, 16–23.
Schoffnegger, D., Heinke, B., Sommer, C., Sandkuhler, J., 2006. Physiological properties of spinal lamina II GABAergic neurons in mice following peripheral nerve injury. J. Physiol. 577, 869–878.
Scholz, J., Woolf, C.J., 2007. The neuropathic pain triad: neurons, immune cells and glia. Nat. Neurosci. 10, 1361–1368.
Scholz, J., Broom, D.C., Youn, D.H., Mills, C.D., Kohno, T., Suter, M.R., Moore, K.A., Decosterd, I., Coggeshall, R.E., Woolf, C.J., 2005. Blocking caspase activity prevents transsynaptic neuronal apoptosis and the loss of inhibition in lamina II of the dorsal horn after peripheral nerve injury. J. Neurosci. 25, 7317–7323.
Scholz, J., Abele, A., Marian, C., Haussler, A., Herbert, T.A., Woolf, C.J., Tegeder, I., 2008. Low-dose methotrexate reduces peripheral nerve injury-evoked spinal microglial activation and neuropathic pain behavior in rats. Pain 138, 130–142.
Seal, R.P., Wang, X., Guan, Y., Raja, S.N., Woodbury, C.J., Basbaum, A.I., Edwards, R.H., 2009. Injury-induced mechanical hypersensitivity requires C-low threshold mechanoreceptors. Nature 462, 651–655.
Seltzer, Z., Dubner, R., Shir, Y., 1990. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. Pain 43, 205–218.
Serrano, A., Pare, M., McIntosh, F., Elmes, S.J., Martino, G., Jomphe, C., Lessard, E., Lembo, P.M., Vaillancourt, F., Perkins, M.N., Cao, C.Q., 2010. Blocking spinal CCR2 with AZ889 reversed hyperalgesia in a model of neuropathic pain. Mol. Pain 6, 90.
Seybold, V.S., 2009. The role of peptides in central sensitization. Handb. Exp. Pharmacol. 451–491.
Seybold, V.S., McCarson, K.E., Mermelstein, P.G., Groth, R.D., Abrahams, L.G., 2003. Calcitonin gene-related peptide regulates expression of neurokinin1 receptors by rat spinal neurons. J. Neurosci. 23, 1816–1824.
Shiokawa, H., Kaftan, E.J., MacDermott, A.B., Tong, C.K., 2010. NR2 subunits and NMDA receptors on lamina II inhibitory and excitatory interneurons of the mouse dorsal horn. Mol. Pain 6, 26.
Shortland, P., Kinman, E., Molander, C., 1997. Sprouting of A-fibre primary afferents into lamina II in two rat models of neuropathic pain. Eur. J. Pain 1, 215–227.
Siddall, P.J., McClelland, J.M., Rutkowski, S.B., Cousins, M.J., 2003. A longitudinal study of the prevalence and characteristics of pain in the first 5 years following spinal cord injury. Pain 103, 249–257.
Skoff, A.M., Zhao, C., Adler, J.E., 2009. Interleukin-1alpha regulates substance P expression and release in adult sensory neurons. Exp. Neurol. 217, 395–400.
Sofroniew, M.V., 2009. Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci. 32, 638–647.
Spataro, L.E., Sloane, E.M., Milligan, E.D., Wieseler-Frank, J., Schoeniger, D., Jekich, B.M., Barrientos, R.M., Maier, S.F., Watkins, L.R., 2004. Spinal gap junctions: potential involvement in pain facilitation. J. Pain 5, 392–405.
Staniland, A.A., Clark, A.K., Wodarski, R., Sasso, O., Maione, F., D'Acquisto, F., Malcangio, M., 2010. Reduced inflammatory and neuropathic pain and decreased spinal microglial response in fractalkine receptor (CX3CR1) knockout mice. J. Neurochem. 114, 1143–1157.
Stiller, C.O., Cui, J.G., O'Connor, W.T., Brodin, E., Meyerson, B.A., Linderoth, B., 1996. Release of gamma-aminobutyric acid in the dorsal horn and suppression of tactile allodynia by spinal cord stimulation in mononeuropathic rats. Neurosurgery 39, 367–374 discussion 374–5.
Streit, W.J., Semple-Rowland, S.L., Hurley, S.D., Miller, R.C., Popovich, P.G., Stokes, B.T., 1998. Cytokine mRNA profiles in contused spinal cord and axotomized facial nucleus suggest a beneficial role for inflammation and gliosis. Exp. Neurol. 152, 74–87.
Stubley, L.A., Martinez, M.A., Karmally, S., Lopez, T., Cejas, P., Eaton, M.J., 2001. Only early intervention with gamma-aminobutyric acid cell therapy is able to reverse neuropathic pain after partial nerve injury. J. Neurotrauma 18, 471–477.
Sukhotinsky, I., Ben-Dor, E., Raber, P., Devor, M., 2004. Key role of the dorsal root ganglion in neuropathic tactile hypersensibility. Eur. J. Pain 8, 135–143.
Sun, J.H., Yang, B., Donnelly, D.F., Ma, C., LaMotte, R.H., 2006. MCP-1 enhances excitability of nociceptive neurons in chronically compressed dorsal root ganglia. J. Neurophysiol. 96, 2189–2199.
Sung, B., Lim, G., Mao, J., 2003. Altered expression and uptake activity of spinal glutamate transporters after nerve injury contribute to the pathogenesis of neuropathic pain in rats. J. Neurosci. 23, 2899–2910.
Suter, M.R., Berta, T., Gao, Y.J., Decosterd, I., Ji, R.R., 2009. Large A-fiber activity is required for microglial proliferation and p38 MAPK activation in the spinal cord: different effects of resiniferatoxin and bupivacaine on spinal microglial changes after spared nerve injury. Mol. Pain 5, 53.
Suzuki, R., Rygh, L.J., Dickenson, A.H., 2004. Bad news from the brain: descending 5-HT pathways that control spinal pain processing. Trends Pharmacol. Sci. 25, 613–617.
Sweitzer, S., De Leo, J., 2011. Propentofylline: glial modulation, neuroprotection, and alleviation of chronic pain. Handb. Exp. Pharmacol. 235–250.
Sweitzer, S., Martin, D., DeLeo, J.A., 2001a. 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 103, 529–539.
Sweitzer, S.M., Schubert, P., DeLeo, J.A., 2001b. Propentofylline, a glial modulating agent, exhibits antiallodynic properties in a rat model of neuropathic pain. J. Pharmacol. Exp. Ther. 297, 1210–1217.

Tan, A.M., Stamboulian, S., Chang, Y.W., Zhao, P., Hains, A.B., Waxman, S.G., Hains, B.C., 2008. Neuropathic pain memory is maintained by Rac1-regulated dendritic spine remodeling after spinal cord injury. J. Neurosci. 28, 13173–13183.
Tan, A.M., Choi, J.S., Waxman, S.G., Hains, B.C., 2009. Dendritic spine remodeling after spinal cord injury alters neuronal signal processing. J. Neurophysiol. 102, 2396–2409.
Tang, X.Q., Tanelian, D.L., Smith, G.M., 2004. Semaphorin3A inhibits nerve growth factor-induced sprouting of nociceptive afferents in adult rat spinal cord. J. Neurosci. 24, 819–827.
Tanga, F.Y., Nutile-McMenemy, N., DeLeo, J.A., 2005. The CNS role of Toll-like receptor 4 in innate neuroimmunity and painful neuropathy. Proc. Natl Acad. Sci. U. S. A. 102, 5856–5861.
Tarulli, A.W., Raynor, E.M., 2007. Lumbosacral radiculopathy. Neurol. Clin. 25, 387–405.
Teasell, R.W., Mehta, S., Aubut, J.A., Foulon, B., Wolfe, D.L., Hsieh, J.T., Townson, A.F., Short, C., 2010. A systematic review of pharmacologic treatments of pain after spinal cord injury. Arch. Phys. Med. Rehabil. 91, 816–831.
Thacker, M.A., Clark, A.K., Bishop, T., Grist, J., Yip, P.K., Moon, L.D., Thompson, S.W., Marchand, F., McMahon, S.B., 2009. CCL2 is a key mediator of microglia activation in neuropathic pain states. Eur. J. Pain 13, 263–272.
Tian, J., Gu, Y., Su, D., Wu, Y., Wang, X., 2009. Effects of intrathecal lidocaine on hyperalgesia and allodynia following chronic constriction injury in rats. Eur. J. Pain 13, 130–137.
Tilleux, S., Hermans, E., 2008. Down-regulation of astrocytic GLAST by microglia-related inflammation is abrogated in dibutyryl cAMP-differentiated cultures. J. Neurochem. 105, 2224–2236.
Tilleux, S., Berger, J., Hermans, E., 2007. Induction of astrogliosis by activated microglia is associated with a down-regulation of metabotropic glutamate receptor 5. J. Neuroimmunol. 189, 23–30.
Tilleux, S., Goursaud, S., Hermans, E., 2009. Selective up-regulation of GLT-1 in cultured astrocytes exposed to soluble mediators released by activated microglia. Neurochem. Int. 55, 35–40.
Toda, S., Sakai, A., Ikeda, Y., Sakamoto, A., Suzuki, H., 2011. A local anesthetic, ropivacaine, suppresses activated microglia via a nerve growth factor-dependent mechanism and astrocytes via a nerve growth factor-independent mechanism in neuropathic pain. Mol. Pain 7, 2.
Tozaki-Saitoh, H., Tsuda, M., Miyata, H., Ueda, K., Kohsaka, S., Inoue, K., 2008. P2Y12 receptors in spinal microglia are required for neuropathic pain after peripheral nerve injury. J. Neurosci. 28, 4949–4956.
Trang, T., Beggs, S., Wan, X., Salter, M.W., 2009. P2X4-receptor-mediated synthesis and release of brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated protein kinase activation. J. Neurosci. 29, 3518–3528.
Treede, R.D., Jensen, T.S., Campbell, J.N., Cruccu, G., Dostrovsky, J.O., Griffin, J.W., Hansson, P., Hughes, R., Nurmikko, T., Serra, J., 2008. Neuropathic pain: redefinition and a grading system for clinical and research purposes. Neurology 70, 1630–1635.
Tsuda, M., Shigemoto-Mogami, Y., Koizumi, S., Mizokoshi, A., Kohsaka, S., Salter, M.W., Inoue, K., 2003. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature 424, 778–783.
Tsuda, M., Mizokoshi, A., Shigemoto-Mogami, Y., Koizumi, S., Inoue, K., 2004. Activation of p38 mitogen-activated protein kinase in spinal hyperactive microglia contributes to pain hypersensitivity following peripheral nerve injury. Glia 45, 89–95.
Tsuda, M., Toyomitsu, E., Komatsu, T., Masuda, T., Kunifusa, E., Nasu-Tada, K., Koizumi, S., Yamamoto, K., Ando, J., Inoue, K., 2008a. Fibronectin/integrin system is involved in P2X(4) receptor upregulation in the spinal cord and neuropathic pain after nerve injury. Glia 56, 579–585.
Tsuda, M., Tozaki-Saitoh, H., Masuda, T., Toyomitsu, E., Tezuka, T., Yamamoto, T., Inoue, K., 2008b. Lyn tyrosine kinase is required for P2X(4) receptor upregulation and neuropathic pain after peripheral nerve injury. Glia 56, 50–58.
Tsuda, M., Kuboyama, K., Inoue, T., Nagata, K., Tozaki-Saitoh, H., Inoue, K., 2009. Behavioral phenotypes of mice lacking purinergic P2X4 receptors in acute and chronic pain assays. Mol. Pain 5, 28.
Ullrich, P.M., 2007. Pain following spinal cord injury. Phys. Med. Rehabil. Clin. N Am. 18, 217–233.
Ultenius, C., Linderoth, B., Meyerson, B.A., Wallin, J., 2006. Spinal NMDA receptor phosphorylation correlates with the presence of neuropathic signs following peripheral nerve injury in the rat. Neurosci. Lett. 399, 85–90.
van Neerven, S., Joosten, E.A., Brook, G.A., Lambert, C.A., Mey, J., Weis, J., Marcus, M.A., Steinbusch, H.W., van Kleef, M., Patijn, J., Deumens, R., 2010. Repetitive intrathecal VEGF(165) treatment has limited therapeutic effects after spinal cord injury in the rat. J. Neurotrauma 27, 1781–1791.
Veves, A., Backonja, M., Malik, R.A., 2008. Painful diabetic neuropathy: epidemiology, natural history, early diagnosis, and treatment options. Pain Med. 9, 660–674.
Wall, P.D., Devor, M., Inbal, R., Scadding, J.W., Schonfeld, D., Seltzer, Z., Tomkiewicz, M.M., 1979. Autotomy following peripheral nerve lesions: experimental anaesthesia dolorosa. Pain 7, 103–111.
Wallace, V.C., Blackbeard, J., Segerdahl, A.R., Hasnie, F., Pheby, T., McMahon, S.B., Rice, A.S., 2007. Characterization of rodent models of HIV-gp120 and anti-retroviral-associated neuropathic pain. Brain 130, 2688–2702.
Wang, R., Guo, W., Ossipov, M.H., Vanderah, T.W., Porreca, F., Lai, J., 2003. Glial cell line-derived neurotrophic factor normalizes neurochemical changes in injured dorsal root ganglion neurons and prevents the expression of experimental neuropathic pain. Neuroscience 121, 815–824.
Wang, W., Mei, X., Huang, J., Wei, Y., Wang, Y., Wu, S., Li, Y., 2009. Crosstalk between spinal astrocytes and neurons in nerve injury-induced neuropathic pain. PLoS One 4, e6973.
Wang, W., Petralia, R.S., Takamiya, K., Xia, J., Li, Y.Q., Huganir, R.L., Tao, Y.X., Yaster, M., 2011. Preserved acute pain and impaired neuropathic pain in mice lacking protein interacting with C Kinase 1. Mol. Pain 7, 11.
Wasner, G., Naleschinski, D., Baron, R., 2007. A role for peripheral afferents in the pathophysiology and treatment of at-level neuropathic pain in spinal cord injury? A case report. Pain 131, 219–225.
Watkins, L.R., Maier, S.F., 2002. Beyond neurons: evidence that immune and glial cells contribute to pathological pain states. Physiol. Rev. 82, 981–1011.
Wei, F., Dubner, R., Zou, S., Ren, K., Bai, G., Wei, D., Guo, W., 2010. Molecular depletion of descending serotonin unmasks its novel facilitatory role in the development of persistent pain. J. Neurosci. 30, 8624–8636.
Weissner, W., Winterson, B.J., Stuart-Tilley, A., Devor, M., Bove, G.M., 2006. Time course of substance P expression in dorsal root ganglia following complete spinal nerve transection. J. Comp. Neurol. 497, 78–87.
Wen, Y.R., Suter, M.R., Kawasaki, Y., Huang, J., Pertin, M., Kohno, T., Berde, C.B., Decosterd, I., Ji, R.R., 2007. 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 107, 312–321.
White, F.A., Sun, J., Waters, S.M., Ma, C., Ren, D., Ripsch, M., Steflik, J., Cortright, D.N., Lamotte, R.H., Miller, R.J., 2005. 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. 102, 14092–14097.
Willemen, H.L., Eijkelkamp, N., Wang, H., Dantzer, R., Dorn 2nd, G.W., Kelley, K.W., Heijnen, C.J., Kavelaars, A., 2010. Microglial/macrophage GRK2 determines duration of peripheral IL-1beta-induced hyperalgesia: contribution of spinal cord CX3CR1, p38 and IL-1 signaling. Pain 150, 550–560.

Woolf, C.J., 2011. Central sensitization: implications for the diagnosis and treatment of pain. Pain 152, S2–15.
Woolf, C.J., Reynolds, M.L., Molander, C., O'Brien, C., Lindsay, R.M., Benowitz, L.I., 1990. The growth-associated protein GAP-43 appears in dorsal root ganglion cells and in the dorsal horn of the rat spinal cord following peripheral nerve injury. Neuroscience 34, 465–478.
Woolf, C.J., Shortland, P., Coggeshall, R.E., 1992. Peripheral nerve injury triggers central sprouting of myelinated afferents. Nature 355, 75–78.
Woolf, C.J., Shortland, P., Reynolds, M., Ridings, J., Doubell, T., Coggeshall, R.E., 1995. Reorganization of central terminals of myelinated primary afferents in the rat dorsal horn following peripheral axotomy. J. Comp. Neurol. 360, 121–134.
Wu, G., Ringkamp, M., Hartke, T.V., Murinson, B.B., Campbell, J.N., Griffin, J.W., Meyer, R.A., 2001. Early onset of spontaneous activity in uninjured C-fiber nociceptors after injury to neighboring nerve fibers. J. Neurosci. 21, RC140.
Wu, W.P., Hao, J.X., Xu, X.J., Wiesenfeld-Hallin, Z., Koek, W., Colpaert, F.C., 2003. The very-high-efficacy 5-HT1A receptor agonist, F 13640, preempts the development of allodynia-like behaviors in rats with spinal cord injury. Eur. J. Pharmacol. 478, 131–137.
Wu, L.J., Vadakkan, K.I., Zhuo, M., 2007. ATP-induced chemotaxis of microglial processes requires P2Y receptor-activated initiation of outward potassium currents. Glia 55, 810–821.
Wu, S.X., Wang, W., Li, H., Wang, Y.Y., Feng, Y.P., Li, Y.Q., 2010. The synaptic connectivity that underlies the noxious transmission and modulation within the superficial dorsal horn of the spinal cord. Prog. Neurobiol. 91, 38–54.
Xie, W., Strong, J.A., Meij, J.T., Zhang, J.M., Yu, L., 2005. Neuropathic pain: early spontaneous afferent activity is the trigger. Pain 116, 243–256.
Xie, W., Strong, J.A., Zhang, J.M., 2009. Early blockade of injured primary sensory afferents reduces glial cell activation in two rat neuropathic pain models. Neuroscience 160, 847–857.
Xin, W.J., Weng, H.R., Dougherty, P.M., 2009. Plasticity in expression of the glutamate transporters GLT-1 and GLAST in spinal dorsal horn glial cells following partial sciatic nerve ligation. Mol. Pain 5, 15.
Xu, X.J., Hao, J.X., Aldskogius, H., Seiger, A., Wiesenfeld-Hallin, Z., 1992. Chronic pain-related syndrome in rats after ischemic spinal cord lesion: a possible animal model for pain in patients with spinal cord injury. Pain 48, 279–290.
Xu, J.T., Xin, W.J., Wei, X.H., Wu, C.Y., Ge, Y.X., Liu, Y.L., Zang, Y., Zhang, T., Li, Y.Y., Liu, X.G., 2007. 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. 204, 355–365.
Xu, Z.Z., Zhang, L., Liu, T., Park, J.Y., Berta, T., Yang, R., Serhan, C.N., Ji, R.R., 2010. Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nat. Med. 16, 592–597 1p following 597.
Yezierski, R.P., Liu, S., Ruenes, G.L., Kajander, K.J., Brewer, K.L., 1998. Excitotoxic spinal cord injury: behavioral and morphological characteristics of a central pain model. Pain 75, 141–155.
Youn, D.H., Wang, H., Jeong, S.J., 2008. Exogenous tumor necrosis factor-alpha rapidly alters synaptic and sensory transmission in the adult rat spinal cord dorsal horn. J. Neurosci. Res. 86, 2867–2875.
Zhang, J., De Koninck, Y., 2006. Spatial and temporal relationship between monocyte chemoattractant protein-1 expression and spinal glial activation following peripheral nerve injury. J. Neurochem. 97, 772–783.
Zhang, E.T., Ossipov, M.H., Zhang, D.Q., Lai, J., Porreca, F., 2007a. Nerve injury-induced tactile allodynia is present in the absence of FOS labeling in retrogradely labeled post-synaptic dorsal column neurons. Pain 129, 143–154.
Zhang, J., Shi, X.Q., Echeverry, S., Mogil, J.S., De Koninck, Y., Rivest, S., 2007b. Expression of CCR2 in both resident and bone marrow-derived microglia plays a critical role in neuropathic pain. J. Neurosci. 27, 12396–12406.
Zhao, J., Seereeram, A., Nassar, M.A., Levato, A., Pezet, S., Hathaway, G., Morenilla-Palao, C., Stirling, C., Fitzgerald, M., McMahon, S.B., Rios, M., Wood, J.N., 2006. Nociceptor-derived brain-derived neurotrophic factor regulates acute and inflammatory but not neuropathic pain. Mol. Cell Neurosci. 31, 539–548.
Zhao, P., Waxman, S.G., Hains, B.C., 2007a. Extracellular signal-regulated kinase-regulated microglia–neuron signaling by prostaglandin E2 contributes to pain after spinal cord injury. J. Neurosci. 27, 2357–2368.
Zhao, P., Waxman, S.G., Hains, B.C., 2007b. Modulation of thalamic nociceptive processing after spinal cord injury through remote activation of thalamic microglia by cysteine cysteine chemokine ligand 21. J. Neurosci. 27, 8893–8902.
Zhuang, Z.Y., Gerner, P., Woolf, C.J., Ji, R.R., 2005. ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model. Pain 114, 149–159.
Zhuang, Z.Y., Wen, Y.R., Zhang, D.R., Borsello, T., Bonny, C., Strichartz, G.R., Decosterd, I., Ji, R.R., 2006. 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. 26, 3551–3560.
Zhuang, Z.Y., Kawasaki, Y., Tan, P.H., Wen, Y.R., Huang, J., Ji, R.R., 2007. 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. 21, 642–651.
Zimmermann, M., 2001. Pathobiology of neuropathic pain. Eur. J. Pharmacol. 429, 23–37.