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REVIEW

Neuroplasticity of ascending and descending pathways after somatosensory system injury: reviewing knowledge to identify neuropathic pain therapeutic targets

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Study design: This is a narrative review of the literature.
Objectives: This review aims to be useful in identifying therapeutic targets. It focuses on the molecular and biochemical neuroplasticity changes that occur in the somatosensory system, including ascending and descending pathways, during the development of neuropathic pain. Furthermore, it highlights the latest experimental strategies, based on the changes reported in the damaged nociceptive neurons during neuropathic pain states.
Setting: This study was conducted in Girona, Catalonia, Spain.
Methods: A MEDLINE search was performed using the following terms: descending pain pathways; ascending pain pathways; central sensitization; molecular pain; and neuropathic pain pharmacological treatment.
Results and conclusion: Neuropathic pain triggered by traumatic lesions leads to sensitization and hyperexcitability of nociceptors and projection neurons of the dorsal horn, a strengthening in the descendent excitatory pathway and an inhibition of the descending inhibitory pathway of pain. These functional events are associated with molecular plastic changes such as overexpression of voltage-gated ion channels, algogen-sensitive receptors and synthesis of several neurotransmitters. Molecular studies on the plastic changes in the nociceptive somatosensory system enable the development of new pharmacological treatments against neuropathic pain, with higher specificity and effectiveness than classical drug treatments. Although research efforts have already focused on these aspects, additional research may be necessary to further explore the potential therapeutic targets in neuropathic pain involved in the neuroplasticity changes of neuropathological pathways from the injured somatosensory system.
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INTRODUCTION

The International Association for the Study of Pain describes the term of neuropathic pain as 'the pain initiated or caused by a primary lesion or dysfunction in the nervous system'.[1] However, the Special Interest Group 'Neuropathic Pain' from the International Association for the Study of Pain has proposed a new definition, in which neuropathic pain is stated as 'the pain initiated or caused by a lesion or disease of the somatosensory system'.[2] This new definition replaces the word 'dysfunction' for 'disease' in order to underline the difference between neuropathic pain and the pain caused by the plastic changes that occur in response to an intense nociceptive stimulation. Similarly, the replacement of the 'nervous system' concept by the term 'somatosensory system' has emerged as a need to differentiate neuropathic pain from the pain caused by an eventual lesion in other specific areas of the nervous system (for example, pain associated with the muscle spasticity caused by injuries in the motor tract). In summary, the new proposed definition postulates that lesions and/or diseases of the peripheral and central somatosensory system cause neuropathic pain.[2,3] The results of this system can be because of traumatic lesions, inflammation, infections, cancer infiltrations, as well as because of

consequences derived from pharmacological treatments (for example, chemotherapy and anti-retroviral therapy).[4–7]

Traumatic lesions of the somatosensory system lead to the onset of neuropathic pain, characterized by spontaneous pain, allodynia and hyperalgesia.[8] Traffic collisions are the main cause of nerve injury, affecting over 3.2 million people worldwide. Pain is involved in 65% of people with traumatic neuropathies, and 50% of them are diagnosed with neuropathic pain. Most of these traumatic peripheral neuropathies affect young men (20–40 years old), and the damage is mainly located in the upper limb nerves.[9] Traffic accidents are also the leading cause of spinal cord lesion in young people, among which two-thirds of patients develop neuropathic pain.[10–13] In the United States, the annual socioeconomic cost of neuropathic pain derived from traumatic injuries in the somatosensory system is ~100 billion US dollars.[14]

Some experimental studies have shown that the physiopathological mechanisms of neuropathic pain are associated with nociceptor sensitization, spontaneous and ectopic firing of afferent nociceptive fibers and with changes in the molecular expression of ion channels, neurotransmitters and receptors in the nociceptive axons, as well as in

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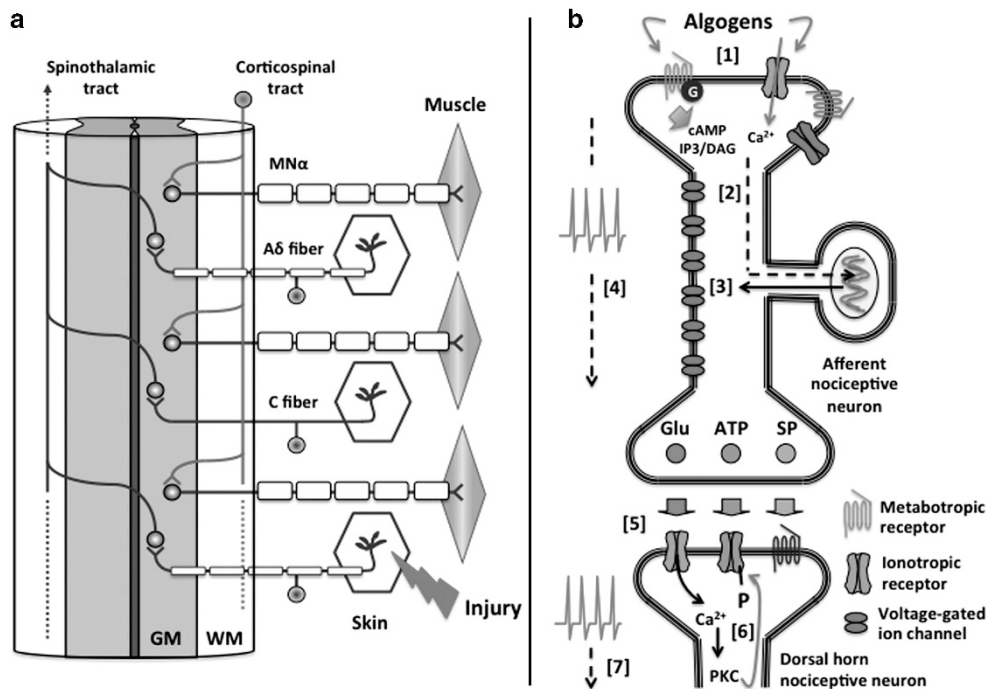E-mail: pere.boadas@udg.edu

Figure 1 Schematic representation of nociceptive ascending somatosensory pathways. (**a**) Afferent Aδ and C nerve fibers from skin project to the nociceptive neurons located in the gray matter (GM) of the dorsal horn of spinal cord. Part of projection neurons of dorsal horn constitutes the spinothalamic tract that transmits pain up to thalamic neurons. Other nociceptive ascending pathways are not represented. Injury of the skin or other tissues causes the activation of afferent nociceptive fibers. (**b**) Tissue injury causes the generation of several algogenic mediators that bind to ion channels or metabotropic receptors of nociceptors, and improve an influx of calcium ions and the generation of second-messengers [1]. Both intracellular mediators are able to facilitate the transcription of target genes [2] and the translation of new proteins such as voltage-gated ion channels [3] that contribute to the hyperexcitability of afferent nociceptive nerve fibers [4]. Excited afferent nociceptive neurons release more neurotransmitters over spinal cord neurons [5], inducing not only the excitation of these nociceptive neurons but also contributing to the sensitization of central neurons [6], and the hyperexcitability of these neurons from the spinothalamic tract.

the dorsal root ganglia neurons. At the spinal cord level, sensitization of second-order nociceptive neurons (central sensitization) and changes in the neurotransmitters, neuropeptides and receptor expression are the main changes established within the framework of neuropathic pain. Moreover, additional changes may involve the dysregulation of the inhibitory interneurons in the dorsal horn and the descending modulatory pathways, glial cell reactivation following the synthesis and release of proinflammatory cytokines and, finally, a morphological and functional reorganization of the afferent projections in the dorsal horn.[15–18]

Despite the fact that neuropathic pain physiopathology has been thoroughly studied, a detailed study focusing on the plastic processes affecting nociceptive neurons in an injured somatosensorial system could involve a double implication: (1) a novel approach in the identification of either molecular or cellular targets involved in neuropathic pain; and (2) it could also shed some light in the design of new therapeutic strategies for neuropathic pain states. Therefore, the aim of the present review is to look over the molecular and biochemical neuroplasticity changes that occur in the somatosensory system during the development of neuropathic pain.

MOLECULAR NEUROPLASTICITY OF ASCENDING PAIN PATHWAY IN INJURED SOMATOSENSORY SYSTEM: SPINAL AND SUPRA-SPINAL CENTERS

The nociceptive somatosensory pathway is initially composed of nociceptors or nociceptive neurons of first order, which are free nerve endings (type Aδ and C sensory fibers) that transduce noxious stimulus from damaged peripheral tissues to the spinal cord and/or

the brainstem. Primary afferent nociceptive neurons (type Aδ and C fibers) synapse with second-order neurons (specific nociceptive neurons and wide-dynamic range neurons) and inhibitory/excitatory interneurons located in the first layers of the dorsal horn. The axons of these spinal neurons are considered to form the spinothalamic tract, ending as synapses to thalamic neurons that in turn eventually send projections to different brain cortical areas. It is important to note that the spinothalamic tract, in its upward pathway, also generates collateral projections to neuronal groups located in the brainstem.[19] Primary nociceptive neurons release glutamate, substance P (SP), calcitonin gene-related peptide and adenosine triphosphate (ATP) that act as neurotransmitters and neuromodulators, distributed either in axon terminal projections from the dorsal horn of the spinal cord or in peripheral endings of primary sensory Aδ/C nerve fibers (Figure 1). Similarly, these neurons have a wide variety of ionic channels and membrane receptors on which several algogen molecules interact[20,21] (Table 1).

Several experimental studies have shown that lesions in peripheral nerves and inflammation of skin, muscle joints and internal organs cause phenotypic changes in sensory afferent fibers, induced by an excessive calcium influx in these afferent fibers or by the interaction of inflammatory mediators with their respective receptors located in the cell membrane of these afferent fibers. All these extracellular signals induce gene expression changes in sensory nociceptive afferent neurons, resulting in voltage-gated ion channels, ligands and metabotropic receptor overexpression[22,23] (Table 2).

Some functional consequences stemming from the aforementioned phenotypic changes include increased sensitization and depolarization

of nociceptors to different algogens by overexpression of acid-sensing ion channels (ASIC), hyperpolarization-activated cyclic nucleotide-gated channels and transient receptor potential ion channels (TRP), whereas increased action potential generation and its extensive

propagation through nociceptive fibers by overexpression of voltage-gated sodium channels might also be another functional consequence. Nociceptor hyperexcitability results in a greater release of neurotransmitters (glutamate, SP, calcitonin gene-related peptide, ATP) to second-order nociceptive neurons in the spinal cord (Figure 1; Table 2), including projection neurons nociceptive specific neurons called NS neurons and wide dynamic range neurons called WDR neurons and interneurons (excitatory and inhibitory). All the physiological processes taking place within injured nociceptive fibers also contribute to the reduction of K_{ATP} channels. At physiological levels of intracellular ATP concentration, K_{ATP} channels are inhibited, whereas activation is stated whenever a decrease of intracellular ATP occurs as a consequence of energy depletion in the injured neuron. Activation subsequently causes an outflow of potassium ions that leads to neuronal hyperpolarization, as well as to a decrease in the neuronal excitability and to a reduction in the release of neurotransmitters. The decrease in K_{ATP} expression, after a peripheral nerve axotomy in myelinated nociceptive fibers (Aδ type), enhances the hyperexcitability of the damaged nociceptive fibers.[24,25] Sensitization and hyperexcitability of injured peripheral nociceptors also induce hyperexcitability of nociceptive spinal neurons, triggering within an increase of electric activity, the expansion of their receptor field and a threshold decrease to afferent inputs as a result of overexpression of ionic channels and receptors. The explained plastic changes in spinal nociceptive neurons are known as 'central sensitization'.[26,27]

The aforementioned neurotransmitters interact with N-methyl-D-aspartate receptor (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA), metabotropic receptors of glutamate (mGluR), neurokinin-1 receptor (NK1R) and purinergic receptors (P2X) of spinal nociceptive projection neurons[28–35] causing their depolarization and the generation of painful signals to be scattered throughout the nociceptive spinothalamic pathway. In turn, this chemical neurotransmission causes an influx of calcium ions in the spinal nociceptive neurons of second order,[36] a process that activates calcium-dependent intracellular cascades, inducing phosphorylation sensitization[37] and ionic channel and membrane receptor overexpression (Table 3). Moreover, the neurotransmitters and

Table 1 Main algogens that interact with nociceptive neurons of somatosensory system

Algogen	Receptor	Action
(A) Algogenic substances that stimulate tissue nociceptors or first-order nociceptive neurons after injury and/or tissue inflammation		
Acetylcholine	Muscarinic and nicotinic	Activation after injury
ATP	P2X	Activation
Bradykinin	B1 and B2	Activation
Capsaicin	TRPV1	Activation
Catecholamines	α-Adrenergic	Activation after injury
CGRP	CGRP-R	Activation/sensitization
H+	ASIC channels	Activation/sensitization
Histamine	H1 receptors	Activation/sensitization
Nitric oxide	Diffusion	Sensitization
Prostaglandins	Prostaglandin receptor	Activation/sensitization
Serotonin	5-HT1A, 5-HT3	Activation/sensitization
Substance P	NK1	Activation/sensitization
(B) Algogenic substances in the spinal cord parenchyma that stimulate nociceptive neurons of the second order (spinothalamic tract) following nerve injury and/or spinal cord		
ATP	P2X	Activation/sensitization
CCL2 o MCP1	CCR2	Activation/sensitization
CGRP	CGRP-R	Activation/sensitization
IL-1 and IL-6	Interleukin receptor	Activation/sensitization
Nitric oxide	Diffusion	Sensitization
Substance P	NK1	Activation/sensitization
TNF-α	TNF-R	Activation/sensitization

Abbreviations: ASIC, acid-sensing ion channels; ATP, adenosine triphosphate; CGRP, calcitonin gene-related peptide; IL, interleukin; TNF, tumor necrosis factor.
Source: Gao and Ji[51] and Xifró et al.[18].

Table 2 Main molecular changes of nociceptors after injury and/or tissue inflammation

Type of channel and/or receptor	Class of receptor or ion channel	Result of the change	Reference (s)
Voltage-gated ion channels (increase of their expression)	Sodium ions: Nav1.3, Nav1.7, Nav1.8 and Nav1.9	Hyperexcitability of fibers Aδ and C. Increase of nerve conduction of these nerve fibers. Generation of ectopic discharges.	Zimmermann,[15] Lai et al.,[119] Cummins et al.,[120] Wood et al.,[121] Lampert et al.[122] and Berger et al.[123]
	Calcium ions: N, P/Q	Increase of neurotransmitter release on nociceptive spinal cord neurons. Sensitization of nociceptors.	Cao[124]
Ligand-gated ion channels (increase of their expression)	Gated by nucleosides: HCN	Easy to depolarize by inflammatory stimuli (prostaglandins, nitric oxide). Increased release of NTs on spinal neurons.	Jiang et al.[125] and Emery et al.[126]
	Sensitive to acidosis: ASIC	Easily depolarized by hydrogenions. Increased release of NTs on spinal neurons.	Bianchi and Driscoll[127] and Lingueglia[128]
	Purinergic receptors: P2X	Neurotransmission spinal favors.	Chizh and Illes,[28] Ding et al.[129] and Tsuda et al.[130]
	TRP receptors: vanilloid (TRPV1, TRPV2), melastatin (TRPM8) and ankyrin (TRPA1)	Easy to depolarize by inflammatory stimuli. Increased release of NTs on spinal neurons.	Jara-Oseguera et al.[131] and Stucky et al.[132]
Ligand-gated ion channels (decrease of their expression)	Sensitive potassium channels to ATP: K_{ATP}	Enhancing the hyperexcitability of nociceptive fibers.	Zoga et al.[24] and Sarantopoulos et al.[25]

Abbreviations: ASIC, acid-sensing ion channels; ATP, adenosine triphosphate; HCN, hyperpolarization-activated cyclic nucleotide-gated channels; NTs: neurotransmitters; TRP, transient receptor potential ion channels.

Table 3 Main molecular changes of spinothalamic neurons and thalamic neurons that process pain responses

Neuron	Class of receptor or ion channel	Result of the change	Reference (s)
Spinal neuron second order (spinothalamic pathway)	Sodium voltage-gated ion channels (increase of their expression): Nav1.3	Hyperexcitability of spinal nociceptive neurons. Increased nerve conduction in these neurons.	Hains et al.[133] and Spicarova et al.[134]
	Calcium voltage-gated ion channels (increase of their expression): N, P/Q	Increased release of thalamic neurons NTs; Sensitization of spinal nociceptive neurons.	Li et al.[135] and Boroujerdi et al.[136]
	Ligand-gated ion channels: large conductance Ca^{2+}-activated K^+ channel BK_{Ca}		Chen et al.[137]
	Ligand-gated ion channels (increase of their expression): TRPV1, ASIC and glutamatergic receptors	Easily depolarized by hydrogenions, inflammatory mediators and glutamate released during spinal cord injury	Luo et al.,[138] Wu et al.[139] and Galan et al.[140]
	Decreased expression of the KCC2 cotransporter	Disinhibition of spinal action of GABA neurons mediated by GABA-A receptor	Coull et al.,[141] Lu et al.[142] and Price et al.[143]
	Decreased expression of GABAergic receptors	No inhibition of spinal neurons by the action of GABA	Castro-Lopes et al.[144]
Pain thalamic neuron	Voltage-gated ion channels (increase of their expression) for sodium (Nav1.3) and calcium (N, P/Q) ions	Hyperexcitability of thalamic neurons and increased NTs on cortical neurons. Sensitization of thalamic neurons	Hains et al.,[145] Zhao et al.,[146] Todorovic and Jevtovic-Todorovic[147]
	Decreased expression of GABAergic receptors	No inhibition of thalamic neurons by the action of GABA	Ralston et al.,[148] Jeanmonod et al.[149] and Weng et al.[150]

Abbreviations: ASIC, acid-sensing ion channels; ATP, adenosine triphosphate; GABA, γ-aminobutyric acid; HCN, hyperpolarization-activated cyclic nucleotide-gated channels; NTs: neurotransmitters; TRP, transient receptor potential ion channels.

neuromodulators released from nociceptive primary afferent fibers in the dorsal horn interact with glutamate receptors (NMDA, AMPA), SP receptor (NK1), purinergic receptors (P2X, P2Y) and calcitonin gene-related peptide receptors of microglia and astrocytes.[38–42] Reactive microglial cells synthesize and release cytokines (IL1, IL6, TNF-alpha), prostaglandins (PGE2), chemokines (MCP-1 or CCL2) and nitric oxide, which act as chemical mediators that amplify the microglial reactivity in a paracrine manner, favoring the elevation of these mediators in the dorsal horn of the spinal cord.[43] Reactive microglial cells are also responsible for the release of cathepsine-S protease that causes the proteolysis of a trans-membrane glycoprotein called fractalkine (CX3CL1). This protein is located in the plasma membrane of terminal axons in afferent nociceptive fibers (Aδ and C) that project to the dorsal horn, as well as in spinal projection nociceptive neurons. This proteolysis releases the active fragment of fractalkine, which interacts with the fractalkine receptor (CX3XR1) located in the reactive microglial cell membrane, maintaining its reactivity[44–46] and therefore contributing to the preservation of neuropathic pain (Figure 2). This process is because of most of the inflammatory mediators released by reactive microglia interacting with membrane receptors of nociceptive second-order projection neurons in the dorsal horn of the spinal cord, causing hyperexcitability and sensitization among them.[47–50] It is important to mention that traumatic spinal cord injury also induces spinal microglial cell reactivation, which leads to an increase of inflammatory mediators and hyperexcitability in spinal nociceptive projection neurons.[18] After microglial cell reactivation is produced by an injury in the nociceptive somatosensory system, a long-standing reactivation of spinal astrocytes occurs (>150 days post-injury), inducing hypertrophy within (increase in vimentin and GFAP glial fibrillary acidic cytoskeleton proteins) and a release of inflammatory mediators that contribute to hyperexcitability and sensitization of nociceptive neurons in the dorsal horn of the spinal cord.[47–51]

On the other hand, neuropathic pain is also promoted by spinal cord injury. It is well known that spinal cord injury causes the astrocyte and microglial cell reactivation and the generation and release of several soluble factors such as cytokines (IL1, IL6 and TNF-alpha), chemokines (CCL2) and prostaglandins (PGE2) that interact with nociceptive projection neurons and afferent primary nerve fibers at the dorsal horn of spinal cord segments located rostral and caudal to the lesion site, causing hyperexcitability of these nociceptive neurons and pain neurotransmission in the dorsal horn. Spinal cord injury also causes the necrotic death of astrocytes and neurons, with the release of neurotransmitters (glutamate) and potassium ion that also stimulates nociceptive projection neurons.[52] Hyperalgesia and pain following spinal cord injury can result from maladaptive plastic changes throughout the neural axis, including supra-spinal structures. In line with these findings, it has been repeatedly shown that spinal cord injury is associated with increased activity, increased spike bursts and changes in glial activation in the thalamus.[53–55]

The thalamus is an important structure that mediates different components of pain: sensory discriminative (lateral pain pathway) and affective-motivational (medial pain pathway) components. Despite the fact that the thalamus has a key role in modulating nociceptive information,[56] central sensitization and hyperexcitability of neurons from the spinothalamic pathway results not only in a greater stimulation of thalamic neurons via glutamate neurotransmission,[57,58] which interacts with glutamatergic receptors (AMPA and NMDA) from thalamic neurons inducing depolarization, but also sensitization via calcium influx, as described above (Table 3). Some evidence on molecular neuroplasticity changes associated with peripheral and/or spinal cord injuries in the thalamus and other supra-spinal structures related with neuropathic pain has already been provided.[59] In line with this, chronic constriction injury (CCI) of the sciatic nerve induces an overexpression of constitutive isoforms Homer1b/c and Homer2a/b in the spinal dorsal horn and supra-spinal structures involved in nociception (prefrontal cortex and thalamus) that co-occurre with increases in their associated mGluRs and NR2 subunits of the NMDA receptor. These findings suggest that glutamatergic transmission in the ascending pain pathway related to neuropathic pain is mediated by Homer proteins.[59]

Several receptors have analgesic effects in models of neuropathic pain. Upregulation of thalamic nicotinic and cannabinoid receptors
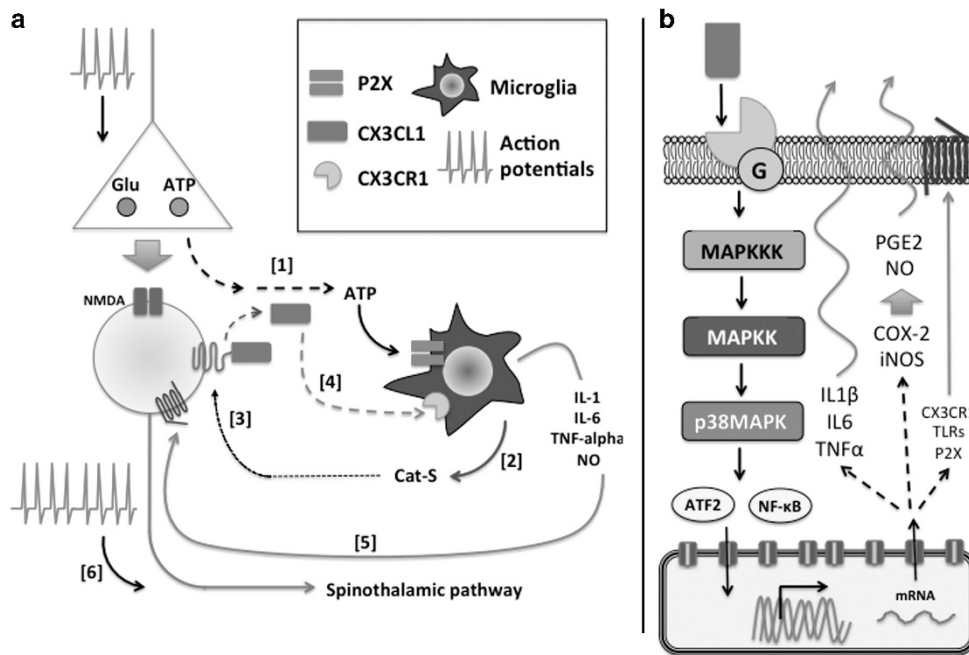
Figure 2 Schematic representation of microglia reactivation by neurotransmitters and neuromodulators released by afferent nociceptive neurons. (**a**) Hyperexcited afferent nociceptive neurons release not only glutamate but also ATP over nociceptive spinal cord neurons, causing their excitability. ATP also promotes the activation of spinal cord microglia cells by means of their binding with P2X receptors (e.g., P2X4 or P2X7) [1]. Activated microglia secrets cathepsin-S [2] that promotes the cleavage of fractalkine (CX3CL1), a glycoprotein expressed in the cell membrane of spinal cord nociceptive neurons [3]. Subsequently, biologically active fractalkine binds with fractalkine receptors (CX3CR1) located in the cell membrane of microglia cells [4], inducing the overexpression of proinflammatory agents such as cytokines and nitric oxide (NO), which also are able to stimulate spinal cord nociceptive neurons [5]. Overall, this feedback between neurons and microglia cells improves the hyperexcitability of spinal cord nociceptive neurons [6]. (**b**) When fractalkine (CX3CL1) binds with fractalkine receptors of microglia cells, it activates p38MAPK, signaling that activates the transcription factors ATF2 and NF-κB that translocate to the nucleus and initiate the transcription of target genes, including proinflammatory cytokines (e.g., IL-1β, Il6 and TNF-α), proinflammatory enzymes (e.g., COX-2 and iNOS) that produce prostaglandins (PGE2) and NO and metabotropic receptors (e.g., CX3CR1 and TLRs, P2X). For further details, please see text.

has been reported after peripheral nerve injury models of neuropathic pain,[60–62] suggesting that supra-spinal nicotinic and cannabinoid receptors in the thalamus may contribute to the modulation of neuropathic pain responses. On the other hand, μ-opioid receptor-mediated G-protein activity was reduced in the thalamus of CCI mice, indicating an apparent desensitization in receptors from this region.[63] A decrease of Na-K-Cl cotransporter-1 (NKCC1) and potassium chloride cotransporter-2 (KCC2) expression, implicated in the GABAergic/glycinergic transmission, has also been reported in the thalamus (ventral posterolateralnucleus) and primary somatosensory cortex (S1) after sciatic nerve section and suture in adult rats that showed mechanical and thermal hyperalgesia, suggesting that peripheral nerve injury induces neuropathic pain by reducing inhibitory inputs at thalamus and cortex.[64]

Neuropathic pain also causes neuroplasticity affecting glial cells located in supra-spinal centers. In line with this, thalamic microglial density increased in streptozotocin (STZ)-diabetic CD1 mice that showed tactile allodynia and thermal hypersensitivity,[65] and in CCI-mice that also showed thermal hyperalgesia.[66] Finally, an increase of IL-1β expression was observed in the thalamus after spared nerve injury in rats.[67] Altogether, these findings suggest that the reactivation of microglia cells and secretion of inflammatory cytokines in the thalamus by peripheral nerve injuries facilitate excitation of pain thalamic neurons and neuropathic pain. These findings are in contrast with another study that showed that microglia was not activated in supra-spinal regions of the central nervous system, including the thalamus, the anterior cingulate cortex, prefrontal cortex, primary and secondary somatosensory cortex (S1 and S2), insular cortex, amygdala, hippocampus, periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) in animals subjected to a peroneal nerve ligation.[68]

MOLECULAR NEUROPLASTICITY OF THE DESCENDING INHIBITORY PAIN PATHWAY IN THE INJURED SOMATOSENSORY SYSTEM

It is well known that several groups of brainstem neurons are related with nociceptive modulation, forming the called 'brainstem pain-modulating system'.[69] Descending control arises from a number of supra-spinal sites, including the midline PAG-RVM system, the more lateral and caudal dorsal reticular nucleus (DRt) and caudal ventro-lateral medulla (cVLM).[70] The neurons from PAG do not project directly to the spinal cord. The main descending projection is the RVM that includes the nucleus raphe magnus and the adjacent reticular formation. RVM neurons receive a dense innervation from the PAG and project to the dorsal horn through the dorsolateral funiculus, forming synapses with spinal cord neurons of the dorsal horn, in both superficial and deep layers.[69–71] The DRt is placed in the reticular formation medial to the spinal trigeminal nucleus, pars caudalis, lateral to the nucleus tractus solitarius, ventral to the cuneate nucleus and dorsal to the ventral reticular nucleus. DRt stimulation causes hyperalgesia in acute pain, whereas its lesion induces analgesia in both the acute and persistent pains.[72,73] The descending pro-nociceptive fibers from the DRt nucleus establish putatively excitatory synaptic contacts upon lamina I neurons that project back to the DRt. Excitatory synaptic contacts also occur between DRt-projecting spinal
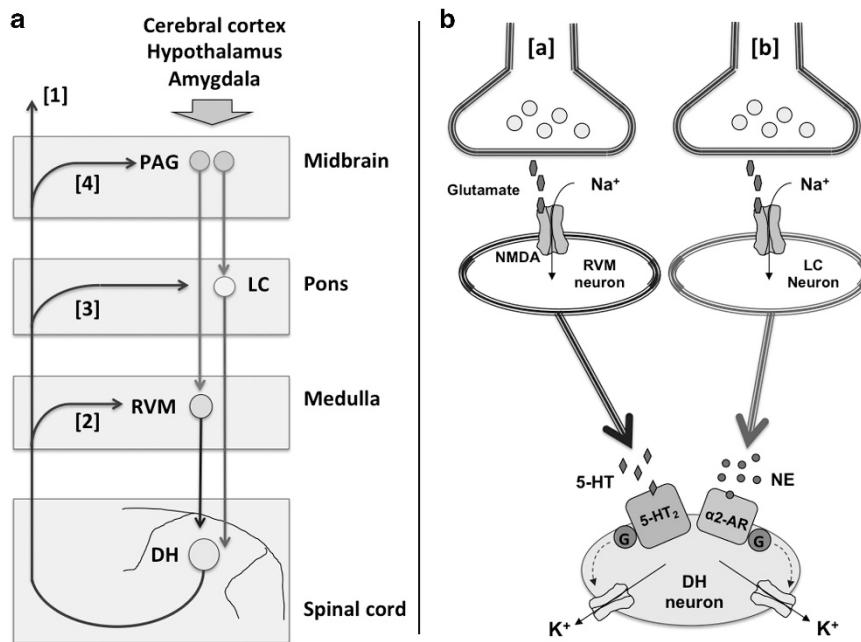
Figure 3 Schematic representation of two major brainstem descending nociceptive somatosensory pathways. (**a**) Nociceptive neurons located in the dorsal horn (DH) project their axons up to thalamic neurons forming not only the spinothalamic pathway [1] but also other nociceptive ascending pathways such as the spinobulbar pathway, which projects to neurons located in the ventrolateral reticular formation, dorsal reticular nucleus, nucleus tractus solitari and rostral ventromedial medulla (RVM) [2], the spinopontine pathway that projects to neurons from the parabranchial nuclei and locus coeruleus (LC) [3], and the spinomesencephalic pathways that project to neurons of periaqueductal gray matter (PAG) [4]. PAG neurons project their descending axons to neurons located in LC and RVM. Neurons located in the RVM and LC directly project their axons to the spinal DH, where the nociceptive input is initially processed. PAG neurons also receive input from forebrain structures including cerebral cortex, hypothalamus and amygdala. (**b**) Spinobulbar pathway (spino-RVM) [a] and spinopontine pathway (spino-LC) [b] release glutamate over RMV and LC neurons. In both groups of neurons, glutamate binds with NMDA receptors, causing an influx of sodium ions and depolarization of these neurons. At the dorsal horn, RVM neurons release serotonin (5-HT), whereas LC neurons release norepinephrine (NE) over the same neuron. Serotonin binds with $5-HT_2$ receptors, whereas NE binds with alpha-2-adrenoreceptors, causing hyperpolarization of the DH neuron by the modulation of potassium ion channels. For more details, please see text.

cells and spinally projecting DRt neurons.[74] cVLM is one of the main inhibitory components of the endogenous pain modulatory system, and its stimulation induces analgesia in acute pain.[74] The reticular formation known as cVLMlat, located in the most lateral part of the cVLM between the spinal trigeminal nucleus (pars caudalis) and the lateral reticular nucleus, appears to be the cVLM region responsible for pain modulation. The analgesia induced upon cVLMlat stimulation is more intense,[75] and the neurons project to the spinal laminae involved in pain transmission (laminae I, IV, V and X).[74,76] The cVLMlat also targets the dorsal horn indirectly through other components of the pain modulatory system, specifically through the pontine A5 noradrenergic cell group.[74,77]

Terminals of descending pathways originating in the rostroventral medulla (RVM) and other brainstem nuclei (for example, nucleus raphe magnus, A5, A6 and A7 nuclei) interact with afferent fibers, interneurons and projection neurons in the dorsal horn.[78] Several neurotransmitters are involved in these descending pathways, but serotonin and noradrenaline are the main neurotransmitters. The predominant source of serotonergic input to the spinal cord arises within the vicinity of the RVM and, most prominently, from the nucleus raphe magnus. Serotonin causes hyperpolarization of afferent nociceptive fiber terminals and dorsal horn projection neurons when interacting with $5-HT_1$ and $5-HT_2$ receptors, and it produces excitation in spinal GABA-ergic interneurons when interacting with $5-HT_3$ receptors.[78] On the other hand, the spinal cord is innervated by both adrenergic cell clusters in medullary C1 and C2 nuclei and by

noradrenergic nuclei localized in A5 and A6 (locus coeruleus) and the pontine A7 (subcoeruleus) regions. Similarly, noradrenaline causes hyperpolarization of projection neurons when interacting with α-2A receptors and over terminals of primary afferent fibers when it interacts with α-2B/C receptors, whereas it induces excitation of dorsal horn inhibitory interneurons via α-1A receptors[78] (Figure 3).

Electrophysiological recordings in the RVM have revealed two types of neurons. One class of RVM neuron, the 'ON' cell, shows a burst of activity beginning just before withdrawal from a noxious stimulus. The other major cell class, the 'OFF' cell, has the opposite firing pattern, pausing during withdrawal from noxious heat. Several studies suggest that 'ON' cells facilitate, while 'OFF' cells inhibit, pain transmission.[79–81]

Several experimental models of neuropathic (for example, nerve ligation) and inflammatory pain have demonstrated that hyperexcitation of specific nociceptive and WDR neurons in the spinal cord causes the hyperexcitability and sensitization of RVM neurons that facilitate the descendent pain signaling toward a dorsal horn level in the spinal cord.[82,83] In particular, in the RVM nucleus, a potentiating of the 'ON' neuron response and a decrease of the 'OFF' neuron response has been observed. 'ON' cells exert a pro-nociceptive effect, whereas 'OFF' cells produce an anti-nociceptive effect. The preferential activation of 'ON' cells located in RVM causes hyperalgesia, whereas hypoalgesia is achieved by the activation of RVM 'OFF' cells.[80,82,84,85]

It is well known that 'ON' cells showed not only mu opioid receptor[81] but also other receptors including cholecystokinin-B
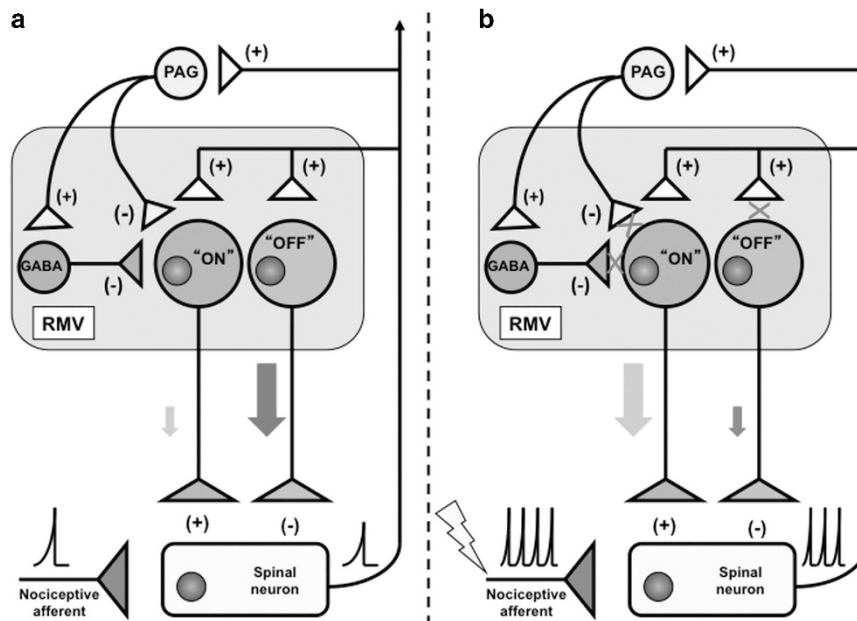
Figure 4 Schematic representation of activation/inactivation of 'ON' and 'OFF' neurons from RVM. (**a**) Under physiological conditions, the firing derived from nociceptors depolarizes spinal cord nociceptive neurons, and pain sensation travels through the spinothalamic pathway. At brainstem level, these action potentials stimulate PAG neurons and 'ON'/'OFF' neurons from RMV. Excited PAG neurons also stimulate GABAergic interneurons located in RMV and inhibit 'ON' neurons. Consequently, 'ON' neurons are inhibited, whereas 'OFF' neurons are excited, and the effect over the spinal nociceptive neuron is a decrease in the firing response. (**b**) After neuropathic pain caused by injuries in the peripheral and/or central nervous system, the hyperexcitability of spinal cord neurons causes changes in the expression of ion channels and receptors, and consequently 'ON' neurons are insensitive to inhibitory inputs from GABAergic interneurons and PAG neurons, whereas they become more sensitive to inputs from the spinothalamic pathway. In addition, these molecular changes also affect 'OFF' neurons that are insensitive to excitatory inputs from the spinothalamic pathway. Therefore, neuropathic pain results in a potentiation of 'ON' neuron inputs over spinal cord neurons that produce descent facilitation from RVM (for details see text).

receptor, NMDA/AMPA receptors, SP-NK1 receptors, brain-derived neurotrophic factor TrK-B receptors and TRPV1 receptors.[86–90] PAG neurons release β-endorphin over 'ON'-RVM neurons causing hyperpolarization via mu opioid receptor, and the descending facilitation from 'ON' neurons is inhibited.[83] In addition, the stimulation of TRPV1 receptor also inhibits 'ON'-RVM neurons (Figure 4).[91]

However, after neuropathic and/or inflammatory pain, the hyperexcitability of nociceptive ascending neurons also causes a sensitization of 'ON'-RVM neurons by overexpression of NMDA/AMPA, Trk-B and NK1 receptors, whereas mu opioid receptor expression decreases. Under these circumstances, 'ON'-RVM neurons do not respond to inhibitory signals from PAG, whereas they are highly stimulated by ascending inputs (Figure 4)[86,92,93] that release glutamate, SP and dynorphin over thalamic and brainstem neurons including 'ON'-RVM cells.[84–96] In summary, all these neurotransmitters released by nociceptive ascending neurons over 'ON'-RVM neurons cause their hyperexcitability (Figure 4).[97]

Neuropathic and inflammatory pain also causes changes in PAG neurons. Under these circumstances, the hyperexcitability of nociceptive ascending neurons not only induces sensitization of PAG neurons causing overexpression of several receptors including NMDA/AMPA and SP/NK1 but also overexpression of glutamate and brain-derived neurotrophic factor. These PAG-brain-derived neurotrophic factor-positive neurons project their axons over the 'ON'-RVM neurons, enhancing their depolarization via Trk-B and NMDA/AMPA receptors.[86]

In summary, several independent lines of evidence suggest that both nociceptive ascending inputs and PAG-brain-derived neurotrophic factor neurons are related with the hyperexcitability of 'ON'-RVM

neurons after neuropathic and inflammatory pain (Figure 4). It is well know that nociceptive ascending pathways include the spinothalamic, spinocervical, spinobulbar, spinopontine, spinomesencephalic, spinodiencephalic and spinothelencephalic pathways.[98] Spinobulbar and spinomesencephalic pathways are ascending nociceptive pathways that project to RVM and PAG, respectively.[98] Spinal cells projecting in the spino-RVM pathway predominate in laminae V, VII, VIII and X, whereas neurons of origin of the spino-PAG pathway are located in laminae I, IV-V, VI-VIII and the lateral spinal nucleus.[98]

Furthermore, 'OFF'-RVM neurons mainly express NMDA/AMPA[69] and TRPV1[99] receptors. Under physiological conditions, PAG neurons release glutamate over 'OFF'-RVM neurons, causing their depolarization via NMDA/AMPA receptors. Consequently, 'OFF'-RVM neurons produce an anti-nociceptive effect in the spinal neurons of dorsal horn.[80] After neuropathic and/or inflammatory pain, the hyperexcitation of ascending nociceptive pathways, including spino-RMV and spino-PAG pathways, causes molecular changes of 'OFF'-RVM neurons such as an overexpression of GABA-A and kappa opioid receptors.[69,100] Under these circumstances, 'OFF'-RVM neurons are more sensitive to the action of GABA released by RVM interneurons, which in turn are stimulated by ascending neurons from the spino-RVM pathway, and by β-endorphins released by PAG neurons, which in turn are excited by ascending neurons from the spino-PAG pathway.[101] Altogether, these results suggest that after neuropathic and/or inflammatory pain 'OFF'-RVM neurons are hyperpolarized and their anti-nociceptive effect on spinal cord neurons is reduced (Figure 4).

On the other hand, several studies focusing on neuropathic pain (for example, chronic constriction injury) and inflammatory pain (for example, peripheral inflammation with carrageenan) showed an

increase of microglial and astroglial reactivity in RVM, releasing several mediators that facilitate the excitation of 'ON'-RVM neurons and their excitatory effect on dorsal horn neurons.[102–104]

In summary, experimental evidence demonstrates that neuropathic pain triggers plastic changes in the descending pain modulatory pathway, which results eventually in 'ON' cell activation and 'OFF cell inactivation from the PAG-RVM system, and RVM glial reactivation, which causes pain facilitation in the spinal cord.

THERAPEUTIC APPROACHES IN NEUROPATHIC PAIN BASED ON THE NEUROPLASTICITY OF THE INJURED SOMATOSENSORY SYSTEM

The overexpression of sodium and calcium voltage-gated ion channels, TRP, ASIC, hyperpolarization-activated cyclic nucleotide-gated channels, TrK-B, ROM, KOM, NK1, purinergic and glutamatergic receptors along with a decrease in endogenous inhibitory inputs and a spinal glia reactivation bring together the main molecular and cellular changes related to neuroplasticity of the injured nociceptive somatosensory system, responsible for triggering neuropathic pain. The use of pharmacological treatment specific to cellular and molecular targets of neuropathic pain constitutes a new therapeutic approach.

Several experimental studies have shown that the use of agonists and antagonists that selectively bind most of the molecular targets that are overexpressed in neuropathic pain would provide a promising new therapy for the treatment of neuropathic pain. In this sense, a first therapeutic approach involves the development and use of new 'antagonists and/or membrane receptor blockers' associated with neuropathic pain. Thus, the administration of H-Arg-15-15C, a TRPV1 selective antagonist, in peripheral inflammation models (for example, formalin, carrageenan Complete Freund's adjuvant) results in a significant reduction of allodynic responses.[105] Similar results are obtained with the administration of AMG-517, another TRPV1 receptor antagonist.[106] Moreover, the administration of isopentenyl pyrophosphate, TRPA1 and TRPV3 inhibitors also reduces mechanical and thermal hypersensitivity related to inflammatory processes.[107] The use of TRPA1 channel inhibitors (for example, AP-18, A-967079, HC-030031 and Chembridge-5861528) reduces hyperalgesic and allodynic symptoms after a peripheral lesion produced by either nerve chronic constriction or inflammation.[108] Blocking purinergic P2X receptors with A-740003 antagonists also reduces neuropathic pain induced by a peripheral inflammation.[109] In CCI models, systemic administration of ZD7288 and Eugenol, two inhibitors of hyperpolarization-activated cyclic nucleotide-gated channels channels, produces a significant decrease in allodynic responses after nerve injury.[110,111] Furthermore, a toxin named APETx2, obtained from a sea anemone *Anthopleura elegantissina*, is a potent selective antagonist of ASIC-3 receptors. Its experimental utilization has shown a significant decrease in painful symptoms related to muscle injuries, inflammatory lesions, visceral pain, migraine and postoperative pain.[112]Alternative drugs or ASIC blockers are amiloride, A-317567, metallic ions (Gd^{3+}, Pb^{2+}, Ni^{2+}, Cd^{2+} and Cu^{2+}) and divalent cations (Ca^{2+}, Mg^{2+} and Zn^{2+}).[113] In peripheral inflammation models, the administration of amiloride and benzamil also inhibits ASIC channels and produces, thus, a decrease in pain responses.[114]

A second therapeutic strategy against neuropathic pain lies in reducing hyperexcitability of the injured nociceptive somatosensory system by 'modulation of voltage-dependent and/or ligand-gated ion channels'. In this sense, in an arthritis experimental model, it has been shown that bisphosphonate compounds (for example, etidronate and alendronate) reduce the associated neuropathic pain. This anti-nociceptive effect could be reversed by glibenclamide and tolbutamide,

which are ATP-gated potassium channel (K^+_{ATP} channels) inhibitors. These results suggest that bisphosphonate compounds enhance the activation of K^+_{ATP} channels located in peripheral nociceptors, causing outward currents of potassium ions and hyperpolarization that result in an analgesic effect.[115] Posterior to a CCI procedure, intrathecalcromakalim administration, a potassium channel-opening drug, also reduced thermal hyperalgesia and mechanical allodynia.[116] In both CCI and inflammatory models, the administration of second-generation voltage-gated sodium channel inhibitors (for example, CDA54, QX-314, A-803467 and V102862) also alleviates hyperalgesia and allodynic symptoms.[117]

An alternative therapeutic approach used to reduce neuropathic pain consists of preventing the expression of ion channels in the plasma membrane of nociceptive neurons. In this sense, the development and administration of TAT-CBD3, a polypeptide that interferes with intracellular transportation of voltage-dependent calcium channel (Cav2.2), reduces hyperalgesia and allodynia responses, after a chronic nerve constriction injury.[118]

CONCLUSIONS

Pain triggered by traumatic lesions, tissular inflammation and/or peripheral nerve injury leads to sodium and calcium ions influx in the nociceptors, resulting in depolarization and sensitization, respectively. Peripheral sensitization activates intracellular cascades that induce ionic channel and membrane receptor phosphorylation, subsequently enhancing nociceptor sensitivity to algogenic mediators, and also molecular changes including overexpression of voltage-gated ion channels, receptors to algogenic mediators and neurotransmitters–neuromodulators. All these changes cause hyperexcitability of peripheral nociceptors that in turn release glutamate and other neurotransmitters over nociceptive neurons located in the dorsal horn of the spinal cord. Hyperexcitation of projection neurons of the dorsal horn results in hyperexcitation of ascending nociceptive pathways, including the spinothalamic pathway that projects pain to both the thalamus and the cortex, but also spino-RVM and spino-PAG pathways that facilitate the excitation of 'ON'-RVM neurons and the hyperpolarization of 'OFF'-RVM neurons. The molecular changes that occurred in PAG and RVM neurons after neuropathic and/or inflammatory pain result in a strenghthening of the descendent excitatory pathway mediated by 'ON'-RVM neurons, and an inhibition of the descending inhibitory pathway of pain, mediated by 'OFF'-RVM neurons. Molecular studies in plastic changes in the nociceptive somatosensory system enable the development of new pharmacological treatments against pain, which will be more specific and effective than classical drug treatments. Although research efforts currently focus on this point, additional research may be necessary to further explore potential therapeutic targets in pain involved in the neuroplasticity changes of neuropathological pathways of the injured somatosensory system.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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