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Review

Chronic pain following spinal cord injury: Current approaches to cellular and molecular mechanisms

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ABSTRACT

Traumatic spinal cord injury (SCI) has devastating implications for patients, including a high prevalence of chronic pain. Despite advancements in our understanding of the mechanisms involved post-SCI, there are no effective treatments for chronic pain following injury. The development of new treatment interventions for pain is needed, but this requires improved models to assess injury-related cellular, neurophysiological and molecular changes in the spinal cord. Here, we will discuss recent animal models for SCI, molecular screening for altered patterns of gene expression, and the importance of injury severity and timing after SCI.

KEYWORDS: dorsal root ganglion, nociceptor, inflammation, neuropathic pain, central sensitization.

1. Introduction

Traumatic spinal cord injury (SCI) has overwhelming implications for patients and caretakers. There are currently over 1 million people affected by SCI in North America, with lifetime costs per patient reaching up to $4.6 million [1-3]. Most treatment options emphasize rehabilitation and neuroprotection; however, an average of 65% of patients report chronic pain, with an estimated 33% describing their pain as severe or excruciating [2]. Animal models have been utilized to study a myriad of therapeutic interventions, with a primary focus on improving neurological outcomes after injury. However, a significant concern in promoting axonal repair within the central nervous system (CNS) is the prospect of the development of neuropathic pain. Neuropathic pain is pain caused by a lesion or disease of the nervous system. It has several distinguishing features, including lesions of nervous tissue, pain in an area of sensory loss, pain in response to a normally non-noxious stimulus (allodynia), increased pain in response to noxious stimuli (hyperalgesia), and unpleasant or abnormal sense of touch (dysesthesia) [3-5]. Despite an improved understanding of the mechanisms involved in the pathophysiology observed following SCI, there are still no effective treatments for chronic pain [6]. This review will discuss the unique features of pain that can accompany SCI as well as current animal models and approaches that are being employed to better develop treatment methods for SCI patients.

2. Gross anatomy of the pain pathway

Sensory fibers that innervate specific regions of the body arise from cell bodies within the trigeminal ganglion and dorsal root ganglia (DRG). Neurons within the DRG (collections of sensory neurons just outside the spinal cord) do not have dendrites, but have a single axon that bifurcates, with one branch projecting to the periphery and the other projecting to the CNS. The peripheral branch is functionally a dendrite, carrying information toward the cell body, while also having "axonal" properties in conducting action potentials. These neurons are therefore considered "pseudo-unipolar" [7].

Conventionally, neurons within the DRG are distinguished by cell body size, degree of myelination, and terminal location within the dorsal horn of the

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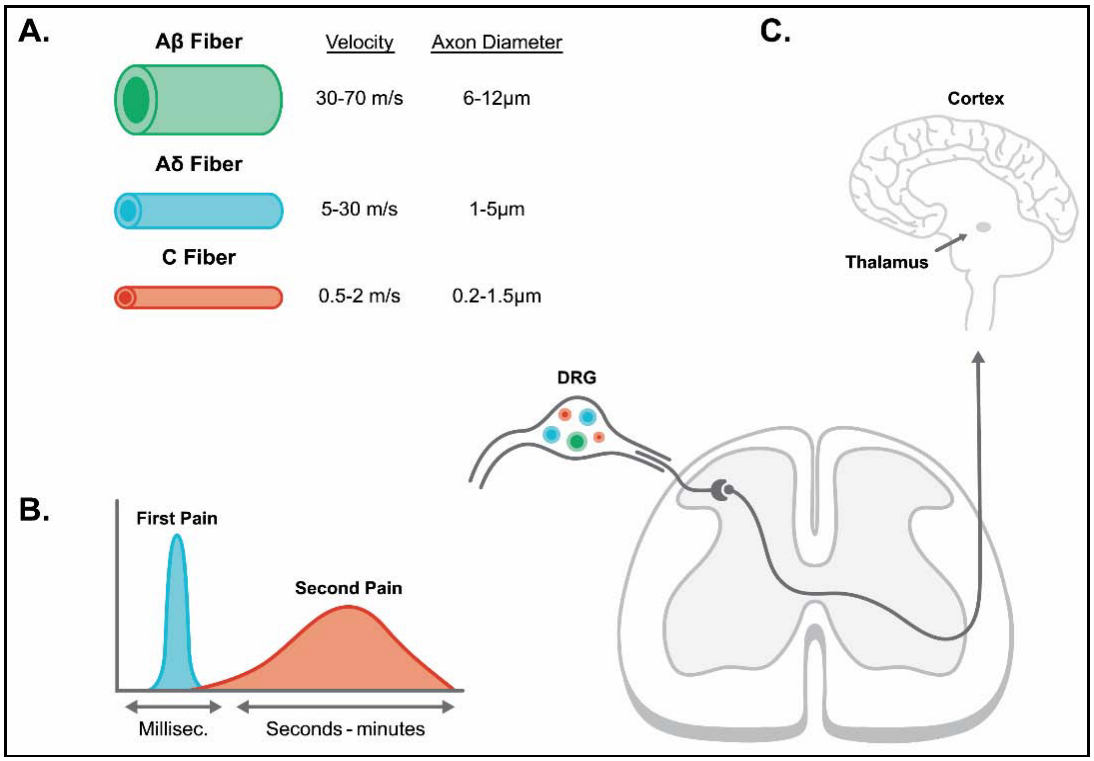

Figure 1

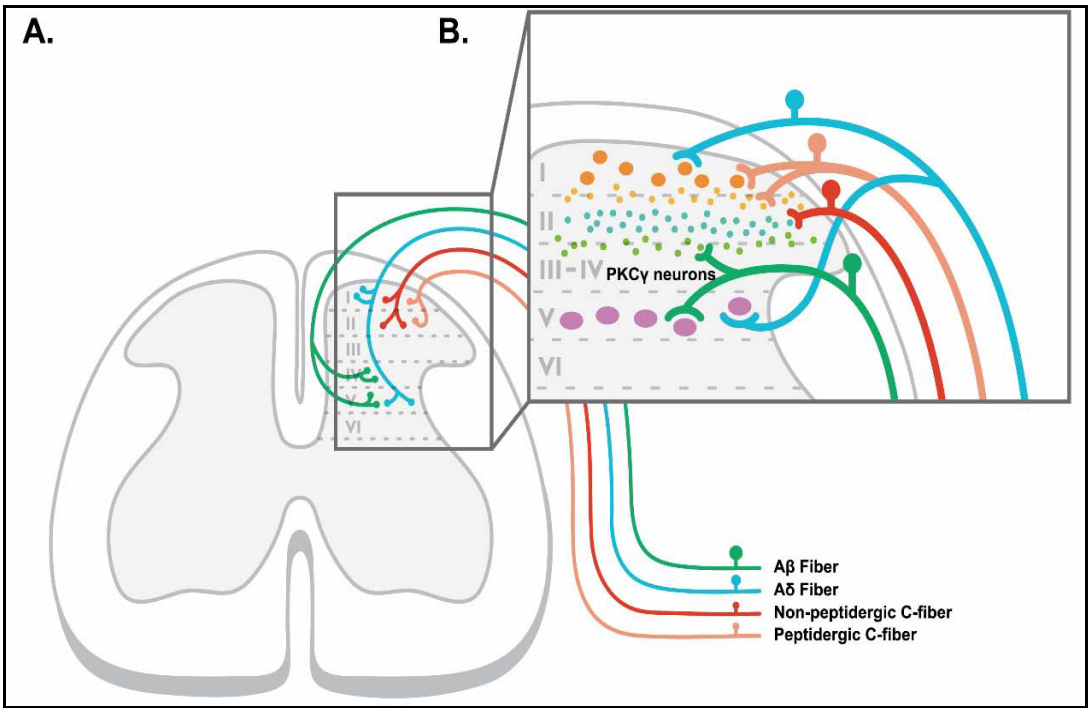

Figure 2

spinal cord [8]. Using these principles, somatosensory neurons have been classified into four fiber types, Aβ, Aδ, C-fibers, and proprioceptors (Figure 1A). Each class has specialized roles in sensation. DRG cell bodies with the largest diameter (>50 μm) represent the myelinated, rapidly conducting (30-70 m/s) Aβ fibers that respond to innocuous stimuli, such as light touch. These fibers do not respond to noxious stimuli. Aδ fibers have medium-diameter cell bodies, are lightly myelinated and are thought to conduct "first" pain, specifically the rapid (5-30 m/s), sharp pain that occurs following noxious stimuli. C-fibers have the smallest cell bodies (10-30 μm), are unmyelinated, slow conducting (0.5-2 m/s) and convey "second" or delayed pain after noxious stimuli (Figure 1B). Most C-fibers are polymodal, and respond to thermal, mechanical, or chemical stimuli [7, 9, 10]. C-fibers are the most abundant neuronal class within the DRG, and make up more than half of all somatosensory neurons [11]. Previous studies have validated that C-fibers, or nociceptors, respond to specific stimuli such as heat or chemicals, but not to non-noxious stimuli, such as light touch [12]. C-fibers project to interneurons within the dorsal horn of the spinal cord that project to the somatosensory cortex *via* the thalamus, transmitting information about painful stimuli [13] (Figure 1C).

3. Cellular anatomy of the pain pathway

DRG neurons have been characterized both by gene expression and protein expression as well as cellular function. Studies have utilized immunofluorescence to broadly distinguish A-fibers using antibodies specific to neurofilament 200 proteins and the antibody peripherin to identify unmyelinated C-fiber subpopulations [14, 15]. C-fibers are further classified into two general categories, peptidergic and non-peptidergic [16]. The peptidergic class is demarcated by the expression of neuropeptides such as calcitonin gene related-peptide (CGRP) or substance P. The non-peptidergic class is distinguished by the binding of isolectin B4 (IB4) to α-D-galactose carbohydrate residues on the cell membrane [17]. Following injury, altered gene expression and protein expression may cause overlap between these groups [18, 19].

C-fibers terminate as free nerve endings on peripheral targets in the skin, organs, and bone. Centrally, they project to the superficial laminae (I, II) of the dorsal horn of the spinal cord and are responsible for the initial stages of pain processing [13]. Previous work has utilized wheat germ agglutinin-horse radish peroxidase conjugate (WGA-HRP) to label this smaller population of cells within the DRG as well as their afferent projections into the superficial laminae of the dorsal horn [20].

Whereas most neurons typically have biochemically distinct dendrites and axons, the unique structure of the DRG allows for uniform protein distribution, since both the central and peripheral terminals send and receive messages. The central terminal projections are dependent on calcium for neurotransmitter release, and the peripheral terminal delivers molecules such as CGRP and substance P to the local tissue [21]. Each afferent fiber type projects to anatomically distinct laminae; Aβ afferents project to lamina III, IV, and V, Aδ fibers project to lamina I and lamina V, and C-fibers project to the superficial laminae I and II (Figure 2A). The

Legend to Figure 1. (A) Somatosensory neurons can be divided by cell body size and degree of myelination. These include large-diameter Aβ myelinated fibers, medium diameter Aδ fibers, and small diameter unmyelinated C-fibers. **(B)** Conduction velocity is related to degree of myelination. Following noxious stimuli, Aδ fibers account for the immediate "fast" pain that occurs within milliseconds, and C-fibers are responsible for secondary "slow" pain in response to noxious stimuli. In neuropathic pain, secondary pain persists, even in the absence of noxious stimuli. **(C)** The dorsal root ganglia (DRG) are comprised of a heterogeneous population of sensory neuron cell bodies that project to both the periphery and the spinal cord. Efferent projections synapse onto second order neurons within the dorsal horn of the spinal cord and project to the thalamus and somatosensory cortex for the perception of pain.

Legend to Figure 2. (A) Different subtypes of DRG neurons terminate within discrete laminae of the dorsal horn of the spinal cord. **(B)** Fast Aβ fibers (green) project onto PKCγ interneurons located within inner laminae II and also project more deeply to lamina V. Slower Aδ fibers (blue) terminate in lamina I and V, while the slowest C fibers (red and orange) project more superficially. Non-peptidergic C-fibers (red) project to interneurons within inner lamina II, and peptidergic C-fibers (orange) terminate onto interneurons in lamina I and the outer lamina II. Figure adapted from [13].

dorsal horn is further organized by C-fiber subtype projections, where the peptidergic class of neurons terminates within lamina I and the outer region of lamina II, and the non-peptidergic afferents terminate in the inner layer of lamina II. These laminae are further organized by electrophysiological characteristics, where the ventral portion of lamina II is composed largely of excitatory interneurons that express protein kinase C (PKC) γ (Figure 2B) [13].

3.1. Nociceptors

Nociceptors are heterogeneous in both their physiology and cellular properties. The sensory endings of their primary afferents project to various peripheral tissues, including skin, muscle, joints, and viscera. During development, immature sensory neurons evolve via dedicated gene programs that orchestrate neuronal subtype specific characteristics. These neurons eventually mature into a diverse population of sensory neurons that respond to a variety of chemical, mechanical, and thermal environmental cues [22]. Each subgroup exhibits stereotypical patterns that terminate within the dorsal horn of the spinal cord; in addition, they terminate in specialized end structures or as free nerve endings in the periphery.

DRG sensory neuron subtypes can be distinguished by their expression of neurotrophic factor receptors: tropomyosin-receptor-kinase A (TrkA), TrkB, TrkC, and Ret (Ret proto-oncogene) bind nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3 [gene name NTF3]) and glial-derived neurotrophic factor (GDNF) family ligands, respectively. These receptors are necessary for appropriate peripheral innervation of tissue targets, cell survival, and the expression of ion channels and receptors. Most neurons with small-diameter, unmyelinated axons develop following the expression of neurogenin 1 (Ngn1) during neurogenesis at embryonic days 9-10 [22]. An increase in TrkA expression prompts the early stages of differentiation, while an increase in runt related transcription factor 1 (Runx1) and either the proto-oncogenes Met, Ret, or protein coding gene transient receptor potential cation channel 8 (Trpm8) further specifies subtypes and central innervation. Additional specification and subclass refinement occurs postnatally [22]. The peptidergic sensory neurons express TrkA and respond to NGF while the non-peptidergic sensory neurons express ATP-gated P2X3 purinergic receptors [9].

A wide range of stimuli can activate sensory neurons. Previous studies have used retrograde tracing, immunohistochemistry, and electrophysiology to identify the various properties of neurons innervating distinct tissues [15]. However, efforts to attribute pain modalities and behavior to individual sensory neurons have proven to be a difficult task. In conjunction with the involvement of multiple systems following injury, the diversity of this population has made it challenging to understand cellular pain mechanisms [23].

3.2. Structural changes following SCI

While a consequence of all types of pain is modification of the pain circuit, it is important to distinguish differences between the outcomes of short-term pain, such as inflammatory pain vs. chronic pain, including neuropathic pain. Following tissue injury, primary sensory neurons exhibit alterations in excitability, largely mediated by the activation of intracellular signaling pathways *via* phosphorylation of receptors or ion channels. These changes cause posttranslational modifications that can alter cell-surface expression of channels within the DRG or dorsal horn of the spinal cord [24]. This form of plasticity is modulatory, and is reversible. Although neuroplasticity is essential in spontaneous recovery following SCI, these compensatory shifts may also produce negative consequences, such as neuropathic pain [25]. Long-term changes within the pain circuit appear during neuropathic pain due to plasticity that causes permanent modifications of the pain pathway. This latter type of plasticity is likely representative of what occurs in SCI patients experiencing chronic pain. These long-lasting shifts are supported by changes in the expression of neurotransmitters, receptors, and ion channels, but also changes due to neuronal survival and subsequently system connectivity [24]. The result of these modifications is a system that no longer has normal stimulus-response characteristics.

Although precise mechanisms responsible for driving chronic pain are not well understood, it is likely that multiple processes are involved. These include functional as well as structural neuroplasticity within the CNS that culminates in increased neuronal

excitability [26]. Variations in neurotrophic factor expression and neuronal damage from an increase of excitatory amino acid levels both play a role in modulating changes after injury [27, 28]. Immunohistochemistry studies have shown that SCI can also physically alter primary sensory neurons; nociceptors immunoreactive for CGRP exhibit sprouting of new branches within the dorsal horn, which also contributes to the development of new abnormal connections [29, 30]. It is important to note that sprouting has not been found in all models of SCI [31, 32].

It is well established that SCI produces increased extracellular concentrations of glutamate, as well as inflammatory cytokines and reactive oxygen species (ROS). SCI not only elicits changes within the neuronal population, it also causes extensive alterations in spinal microglia and astroglia; these changes may promote pain-related behaviors as well [33-36]. Microglia are present after injury, as evidenced by the increased levels of proinflammatory cytokines that are detectable within the first several minutes of injury. These changes promote an increase in extracellular glutamate to excitotoxic levels within a similar timeframe [37]. During the acute phase of injury, astrocytes surround the region of injury and proliferate in an attempt to prevent further damage. However, over time the increased presence of astrocytes becomes detrimental, creating a glial scar and preventing regeneration [6].

4. Clinical presentation of SCI pain

SCI can be categorized into two phases, primary and secondary injury [37-39]. The immediate results following injury are due to direct physical trauma to the spinal cord, and result in spinal shock and complete loss of motor and sensory function below the level of injury. This physiological response is accompanied by loss of tendon reflexes and absence of the sphincter reflex [6]. A cascade of secondary events follows, expanding the region of neural injury and worsening neurological outcomes [40, 41]. This secondary event, or injury, is used to describe the delayed and progressive multitude of physiologic, biochemical, and intracellular changes that occur following the primary spinal cord injury [42].

During the early stages of the secondary response, inflammatory cells (macrophages, microglia, T-cells, neutrophils) enter the site of injury. Studies conducted in rodents have shown that within 6 hours this response triggers the release of cytokines such as interleukin-1α (IL-1α), IL-1β, IL-6, and tumor necrosis factor-α (TNF-α), which can remain elevated for up to 4 days [43, 44]. Phagocytic inflammatory cells (largely macrophages and neutrophils) also release reactive oxygen species, causing oxidative DNA damage and ATP release, ultimately contributing to delayed apoptosis, cord edema and a pro-inflammatory state [45-47]. This disruption of the blood-spinal cord barrier permits the disequilibrium of ionic homeostasis of the cord to go unchecked, and activates calcium-dependent proteases, mitochondrial dysfunction, and finally apoptosis [48].

Oligodendrocytes are among the many cell types susceptible to cell death, and loss of these CNS myelinating cells has been observed both at the site of injury as well as distant to the lesion epicenter [49, 50]. Excitatory amino acids such as glutamate and aspartate are also released as a consequence of cell death, and further propagate excitotoxicity (toxicity to neurons caused by excess calcium entry *via* excitatory neurotransmitters) and glial and neuronal death within the region surrounding the injury [1, 51, 52].

Pathophysiological processes activated by the primary injury contribute to the more protracted secondary injury phase [37]. It is useful to examine secondary injury during different time periods following the primary injury. The immediate 0-2 hours after injury are characterized by swelling of the spinal cord and cell death. From 2-48 hours post injury (acute window), hemorrhaging of the cord, edema, and inflammation occur. From 2 days- 2 weeks post injury (sub-acute window) as well as during a transitional period (2 weeks- 6 months after injury), scarring from astrocytes and axonal sprouting occur. Finally, a chronic secondary injury continuum occurs, with the formation of scars, Wallerian degeneration (nerve process death extending from an injury to the distal process), and injured axons [37]. This prolonged secondary injury cascade produces a harsh post injury environment and results in the unique pathophysiology of SCI that obstructs regeneration and healing and promotes the development of chronic pain [1].

When patients are asked about complications associated with SCI, pain is rated as the third most

important symptom, ranking just behind decreased ability to walk or move and decreased sexual function [53]. The current treatment options for chronic pain consist of systemic steroid therapy, such as methylprednisolone, early surgical decompression, and early mobilization for rehabilitation [54-57]. Because pain is severe, chronic, and resistant to treatment, animal studies are necessary in order to develop strategies for better pain management, or preferably pretreatment of chronic pain. However, there are no predictive measures for chronic pain or effective treatments [58]. It is likely that pain develops within weeks to months after injury, or perhaps even earlier, and that many patients are being treated after the development of pain has already begun [58]. Due to the bi-phasic nature of the injury response following SCI, contiguous delivery interventions during the early post-injury stage would probably have a positive impact on long-term recovery, both functionally but also as a preventative approach to the development of chronic pain.

5. Types of pain following spinal cord injury

Pain elicited by SCI is difficult to manage and is a priority for patient treatment [59, 60]. Patients with chronic pain following SCI most commonly report pain in segments near the site of injury (at-level pain) and below the level of injury (below-level pain) [53, 61]. Pain above the level of injury does occur, but is typically due to upper extremity pain from overuse of muscles, rather than a result of the injury itself [62-64]. The most predominant trait reported for at- and below-level pain is burning pain; however, there are at least five types of pain that may arise after injury, and these are important to consider as they may contribute to long term pain phenotypes [65].

5.1. Acute pain

Acute pain is defined as a cascade of events aimed to fight infection, to prevent further damage, and to initiate repair. This occurs during the primary phase of SCI and involves inflammatory responses, neuronal changes, as well as peripheral and nerve sensitization. These alterations enhance nociceptive responses in an effort to limit further injury to the lesion. Under normal conditions, acute pain functions to prevent further damage and wanes as healing progresses [66].

5.2. Inflammatory pain

Inflammatory pain can be a form of acute pain, which is triggered in response to tissue damage and inflammation, and is characterized by hypersensitivity at the site of injury and nearby tissue as a result of increased excitation of nociceptors [24, 67]. Although inflammatory pain occurs during the primary or acute phase of injury, and may also be a contributing factor to chronic pain, it typically dissipates as the disease process heals. After SCI, cell bodies of nociceptors (sensory neurons responding to painful stimuli) are exposed to the macrophages and T-cells that have infiltrated one or more DRG near the site of the spinal lesion [68]. DRGs have a much higher vascular permeability than the blood-brain or blood-nerve barriers [69, 70], leaving neuronal cell bodies and satellite glial cells within the DRG exposed to both blood and cerebrospinal fluid and the inflammatory factors that are released after SCI [71-73]. Many studies have evaluated the therapeutic efficacy of inhibiting various inflammatory factors to reduce tissue injury and pain associated with spinal cord trauma. One study used TNF-α knockout mice in a vascular clip model of SCI to demonstrate its role in the development of inflammation and the pathogenesis of SCI [74]. Additional studies have shown that specific channels, such as sodium channels $Na_v1.8$ and $Na_v1.9$ (encoded by genes SCN10A and SCN11A) are critical for inflammatory pain, but not neuropathic pain [23, 75]. While certain groups have demonstrated mechanisms that are exclusive to inflammatory pain, others have shown an overlap between inflammatory and chronic pain. Lalisse *et al*. used ATP-gated purinergic receptor P2X4-deficient mice to determine that the receptor is expressed within DRG neurons and to establish a role for P2X4. The study demonstrated that during continued inflammation, P2X4 mediates the release of neuronal BDNF, which contributes to hyperexcitability during chronic inflammatory pain [76].

5.3. Chronic pain

Chronic pain is characterized by persistent activation of nociceptors in the periphery, which produces peripheral and eventually central sensitization [66]. Chronic pain may also result from abnormal firing of myelinated sensory neurons that are not normally responsible for conveying noxious stimuli, that

have defective sodium channels following injury, or from overly active central circuits, possibly *via* wide dynamic range (WDR) neurons in the spinal cord [13]. Although the transition to chronic pain is widely thought to occur after the onset of acute pain, it is possible that both acute and chronic pain mechanisms emerge simultaneously [77]. The shift to chronic pain is not well understood, in part because many different systems contribute to the development of similar phenotypes that present as allodynia and hyperalgesia. Persistent pain can present as both hypersensitivity (allodynia and hyperalgesia) and spontaneous pain. Hypersensitivity results from a decrease in the threshold for nociceptors to fire action potentials in response to normally non-noxious stimuli or to produce an amplified response to noxious stimuli. This is well documented particularly *via* activation of transient receptor potential (TRP) channels after inflammation [67]. These secondary mechanisms not only include changes in the excitability of primary sensory neurons *via* post-translational modifications of receptors and ion channels, but also alterations in expression, connectivity, and neuronal survival, all of which modify normal stimulus-response characteristics of pain [24]. It is unclear whether treatment plans should be aimed at preventatively targeting mechanisms that underlie the transition to chronic pain, or if there is a way to resolve and reverse chronic pain after it has already developed.

5.4. Nociceptive pain

Nociceptive pain is the most prevalent type of pain after SCI and occurs during both the acute and chronic phase [25]. Nociceptive pain is produced by activation of peripheral nociceptors stimulated by continuous tissue damage, and not from persistent painful insults. Analgesics, such as non-steroidal anti-inflammatory drugs (NSAIDs) and physical therapy are effective in treating nociceptive pain, but not neuropathic pain [78].

5.5. Neuropathic pain

Neuropathic pain is elicited by lesions or diseases of the nervous system that alter its function [4, 79]. This syndrome is characterized by spontaneous and evoked pain [80]. Like inflammatory pain, neuropathic pain is described, in part, by hypersensitivity at the site of injury as well as in nearby uninjured tissue. Because this type of pain is triggered by alterations in the function of the somatosensory nervous system, pain is amplified and appears spontaneously [81]. Neuropathic pain frequently results from SCI and develops in over half of SCI patients, typically within the first year after injury, and has a tendency to progress into chronic pain [25]. Neuropathic pain is divided into at-level pain, and below-level pain [4, 82, 83]. At-level pain is demarcated by its existence within a region of one dermatome rostral (up the spinal cord) and three dermatomes caudal (down the spinal cord) to the site of injury. Below-level pain is defined as the presence of pain emanating from three dermatomes below the level of injury. Patients most commonly describe below-level pain as burning, tingling, or pins-and-needles sensation in the absence of sensory stimuli [84].

There are several mechanisms thought to contribute to neuropathic pain including the development of maladaptive plasticity in the nervous system, aberrant sensory neuron activation, increased synaptic transmission, alterations in synaptic connectivity, as well as neuro-immune interactions [78, 80]. Additional factors, such as genetic variations, gender, and age can all impact the development of persistent pain [85, 86]. A defining feature of neuropathic pain is that it remains even after the initial injury has healed, becoming an uncontrolled, inappropriate action of the nervous system rather than an appropriate response to the condition [24]. A better understanding of the mechanisms responsible for abnormal recovery can offer precise therapeutic opportunities to individuals with neuropathic pain.

6. Nociceptor mechanisms in response to SCI

6.1. Central alterations

The somatosensory system is structured in such a way that specialized primary sensory neurons that encode low intensity stimuli only activate pathways in the CNS that convey proprioception, and sensory neurons that encode high intensity stimuli only activate pathways that lead to pain perception [87]. However, following central alterations, sensitization occurs and noxious stimuli are no longer necessary to produce a nociceptive response [88]. Previous studies support this idea of the development of central sensitization of dorsal horn neurons after SCI [89]. If this does occur, this provides an explanation for the development of mechanical and thermal hypersensitivity following injury.

There are several proposed mechanisms for the cause of central sensitization. These include enhanced or prolonged discharge of C-fibers, spontaneous activity from DRG sensory neurons, disinhibition *via* loss of gamma-aminobutyric acid (GABA) and glycine within the spinal cord, increased efficacy of previously weak synapses, and a change in the presence of excitatory amino acids, peptides or other receptors [90-95]. All of these changes might occur over a period of several days or longer [89]. After injury, central sensitization emerges, as the somatosensory system pathways converge and distort or amplify pain signals [85]. This central amplification takes place following reduced inhibition within the central system, and enhances the sensory neuron response in amplitude, duration, and spatial extent, as well as increasing synaptic efficacy, so that low threshold sensory inputs now activate the pain circuit [85, 88].

Other research suggests that central sensitization can begin within seconds after injury by an increase of activity in nociceptors [96]. However, it is likely that prolonged inputs to nociceptors over a period of days to months will produce a more persistent phenotype of hyperexcitability within the central nervous system. These sustained changes may be a greater contributor to the development of central sensitization, because inflammatory factors can activate nociceptors within the DRG, and can also produce signaling molecules, such as NGF, that have downstream effects on sensory neuron behavior [97] (Figure 3A). It is evident that the process of central sensitization is complex and reflects the involvement of numerous mechanisms at multiple sites within the spinal cord, brainstem, and cortex.

The increase of excitatory neurotransmitters, glutamate in particular, is also a contributing factor in the development of central sensitization, and is associated with allodynia and hyperalgesia in patients that report chronic pain [98] (Figure 3B). One study demonstrated the involvement of type 4 metabotropic glutamate receptors (mGluR4 [GRM4]) and thus the ability to regulate glutamatergic signaling in the spinal cord. This was done by inhibiting glutamatergic transmission in both C-fibers and afferent terminals in the inner lamina II of the dorsal horn *via* coupling of Cav2.2 channels [98, 99]. It is possible that the sensory neurons that contribute to the C-fiber population include a variety of chemical and structural differences in their plasma membranes that might affect cell signaling.

6.2. Spontaneous activity

Despite the presence of spontaneous pain as a common problem after injury, it is poorly understood. It has been proposed that prolonged depolarization of resting membrane potential, lowered threshold for action potential propagation, and spontaneous activity of nociceptor sensory terminals could contribute to spontaneous pain [67, 73]. Glutamate is the primary excitatory neurotransmitter in all nociceptors [101]. Regardless of structural changes, the accumulation of excitatory neurotransmitters following SCI, in combination with the loss of normal inhibitory processes such as GABA and glycine, can result in functional changes such as spontaneous activity and increased evoked neuronal activity [26]. Spinal injuries are more likely to elicit multiple central alterations, rather than just one, which can then impact primary afferents and trigger chronic pain. In addition to the increase in excitatory amino acids following tissue damage, SCI also severs descending inhibitory pathways, causing disinhibition and further promoting excitatory transmission within the spinal cord [102, 103]. Loss of inhibitory interneurons reduces the amount of GABA and glycine in the spinal cord, alters the Cl^- potential in neurons receiving inhibitory input, and allows persistent excitability of central neurons within pain pathways [104-106]. An increase in excitability is evident based on the upregulation of Na^+ channels in spinal dorsal horn neurons [107]. It is not surprising that the increase in voltage-gated sodium channels that occurs after injury also contributes to the generation of ectopic activity in the nociceptor population, as evidenced by the robust effects of nonselective sodium channel blockers [108, 109]. Additional studies have highlighted the importance of the voltage gated sodium channel, $Na_v1.7$. Patients with erythromelalgia (burning pain) have a mutation in the *SCN9A* gene, which encodes $Na_v1.7$, and display increased firing in their sensory neurons [110]. Other findings have implicated excess release of glutamate, substance P, and neurotrophic factors such as BDNF or NT-3 in central sensitization after injury [111].

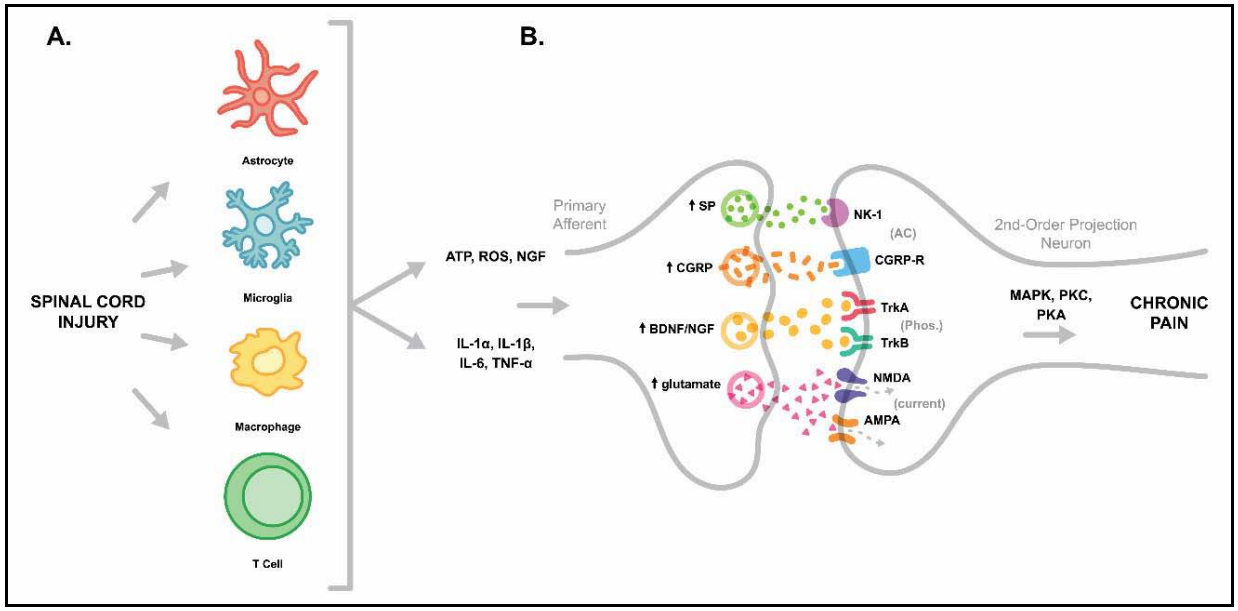

Figure 3. (A) Depiction of the contribution of neuro-immune interactions in the development of pain. Following SCI, mediators such as IL-1α, IL-1β, IL-6, TNF-α, NGF, ATP, and reactive oxygen species (ROS) are released from invading astrocytes, microglia, macrophage, and T-cell populations. Released factors act directly on terminals of primary afferents that express receptors for these mediators. **(B)** After injury, C-fibers and some Aδ-fibers release a variety of neurotransmitters including substance P (SP), calcitonin-gene related peptide (CGRP), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and excess glutamate onto secondary projection neurons located within the superficial dorsal horn. As a result, NMDA and AMPA receptors in the postsynaptic neuron allow an increase in current (Ca^{++} and Na^{+}) to enter the cell. Activation of postsynaptic TrkA and TrkB receptors increases phosphorylation (Phos.) of downstream targets, and activation of CGRP receptors (CGRP-R) and SP receptors (NK-1) increases adenylyl cyclase (AC) activity. This cascade of events activates calcium dependent signaling pathways and second messenger systems including mitogen-activated protein kinase (MAPK), protein kinase C (PKC), and protein Kinase A (PKA), all of which increases neuronal excitability, neuropeptide release, and gene regulation that facilitates the transmission of pain messaging to the somatosensory cortex. Loss of GABAergic and glycinergic inhibition further perpetuates hyperexcitability within the spinal cord (not shown here). Figure adapted from [100].

Work done by Bedi *et al.* used a SCI contusion injury model at T10 (thoracic segment 10) to demonstrate that spontaneous activity occurs in the terminals of nociceptors distal to the site of injury as early as 3 days after injury, continuing for up to 8 months [92]. This suggests that persistent increased excitability above and below the level of injury in nociceptors may support the development or maintenance of chronic pain after SCI [33]. Ectopic discharges of primary afferent fibers projecting to the spinal cord may subsequently result in amplified responses, or sensitization of spinal neurons [112, 113].

Several studies have suggested that an increase of neuronal activity within the dorsal horn is the cause of pain after SCI. However, the underlying mechanisms are still unclear. Many agree that increases in glutamate following injury, as well as the upregulation of NMDA receptors, are important contributors to increased excitability after injury [78]. A role for long-term potentiation (LTP) in the spinal cord dorsal horn after injury has also been proposed as a contributor to pain after SCI, since the increase in excitability of primary sensory neurons activated by tissue injury prompts central sensitization that is analogous to the process of LTP [96]. This model would equate chronic pain after SCI with the LTP that transpires during memory formation. Importantly, both processes involve NMDA receptors.

Studies have shown that inflammation or injury stimulates hypersensitivity, reduces nociceptive thresholds, and induces synaptic potentiation between C-fiber afferents and WDR second-order sensory

neurons within the spinal cord. WDRs receive synaptic input for nociceptor afferent terminals as well as from GABA-releasing neurons and descending inhibitory projections [80]. The loss of GABA after injury, in combination with an increase in AMPA and NMDA receptors after injury, can also trigger an increase in intracellular postsynaptic calcium concentration [111]. Increased intracellular calcium levels are known to activate protein kinases involved in the induction of LTP [111].

Several other mechanisms for spontaneous activity have been proposed, including low-threshold large myelinated sensory neurons, which generate spontaneous pain due to altered connectivity within the spinal cord. In addition, inflammatory signaling between the spinal cord, DRG, and blood is involved in producing hyperexcitability [86, 114]. Satellite glial cells (SGCs) may also play a role in the development or maintenance of chronic pain [73]. SGCs are the primary type of glial cells in sensory ganglia and form a sheath that surrounds neuronal cell bodies [115]. This cell type also expresses several ion channels, receptors, and adhesion molecules [116]. Sensory neurons within the DRG do not form synaptic contacts between each other; however SGCs surrounding these cells may communicate *via* gap junctions [117]. Because there are so many changes that occur after injury, it is challenging to determine which mechanisms are essential contributors to the development of pain. It is also not yet understood why a portion of the SCI patient population do not report pain after similar injuries [26].

6.3. Synaptic changes

It is well established that binding of postsynaptic density protein-95 (PSD-95) to NMDA receptors participates in the downstream intracellular signaling events involved in synaptic plasticity [118, 119]. Using the chronic constriction injury (CCI) model and histochemical staining, Garry *et al*. have shown that NMDA receptors form complexes with PSD-95 in the thoracic and lumbar spinal cord, and that PSD-95 expression is detected specifically in lamina II of the dorsal horn of the spinal cord [120]. In this same study, they demonstrated that mutant mice expressing a truncated form of PSD-95 failed to develop NMDA receptor-dependent hyperalgesia and allodynia after a model of neuropathic pain [120]. Additional work by Lu *et al*. revealed that neuronal activity and pain-related behaviors associated with central sensitization could be altered by blocking the interaction between PSD-95 and the NR2B subunit of the NMDA receptor within the dorsal horn, as well as by lowering the interaction between PSD-95 and the multifunctional scaffold protein Kalirin-7 [121]. This may suggest a role for PSD-95 and Kalirin in central sensitization and chronic pain. Earlier work using antisense oligonucleotides already showed that knockdown of PSD-95 in the spinal cord reduced behavioral hypersensitivity after nerve injury [122-124].

It is apparent that NMDA receptors are critical in the progression of central neuronal hyperexcitability [78, 125, 126]. Inhibition of NMDA receptors further supports the observation of the contribution of NMDA in the development of central neuropathic pain after SCI [127-129]. Additional work indicates the involvement of glutamate in central sensitization as an activator of AMPA receptors in the spinal cord, producing an influx of calcium to cells and irreversible damage following injury. AMPA receptors are also expressed in oligodendrocytes and astrocytes, suggesting a role for glutamate in non-neuronal subtypes and their possible involvement in chronic pain [52, 98].

6.4. Changes in gene expression

The net result of physical tissue damage, inflammation, and central sensitization after injury is an increase in neuronal activity. This hyperexcitability leads to changes in gene expression and consequently even more changes throughout the nervous system. It is entirely possible that neuropathic pain is associated with major changes in gene expression in primary sensory neurons that innervate areas near or at the site of injury in the spinal cord.

Using non-SCI models of injury, studies have shown that specific subtypes of hypersensitivity temporally diverge based on differences in gene expression in sensory neurons; for example, nociceptor transcripts initially change with the onset of cold allodynia, while immune cell transcripts change more slowly as tactile hypersensitivity develops [130]. Although we now know that target-derived growth factors are essential for development

and cell survival, research has established that they also have a role in regulating everyday functional properties of sensory neurons in the adult [131, 132]. Following injury, inflammation triggers an increase in target-derived growth factors, whereas after peripheral axon damage there is a decrease [133]. Both changes produce alterations in the levels of neurotransmitters, ion channels, receptors, and structural proteins [134]. While inflammation may not be sufficient to cause neuropathic pain, chronic inflammation prompts sensory neuron gene expression modification, including changes in genes involved in ion channel expression [135]. Acute sensory neuron sensitization occurs locally, at the site of injury, but long-term sensory changes are dependent on transcriptional changes of ion channels at the cell body in the DRG [135, 136]. These changes occur post-inflammation, outside of the acute phase of injury, due to the inherent delay of changes in gene expression and protein transport [67]. Increased expression of retrograde trafficking of pro-nociceptive molecules such as substance P, CGRP, NGF, and GDNF also results from these changes. This can induce changes in membrane expression of ion channels that increase the excitability of sensory neurons by lowering their sensory thresholds [137-139]. In addition to retrograde signaling of growth factors, increased electrical activity generated by calcium influx through voltage-gated ion channels in the spinal cord can also cause transcriptional changes in sensory neurons [24].

Early studies established the importance of NGF, when mice lacking the growth factor or its receptor TrkA resulted in mice lacking nociceptors [140-142]. In humans, loss-of-function mutations in the *NTRK1* gene (encoding TrkA) manifest as congenital insensitivity to pain syndrome [143, 144]. Injection of NGF produces thermal and mechanical hypersensitivity in both rodents and humans, although over differing time courses [142]. NGF-TrkA interaction on the peptidergic class of nociceptors activates downstream signaling pathways, such as phospholipase C (PLC), mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K) by increasing intracellular free calcium levels within sensory neurons. This activates TRPV1, producing a change in heat sensitivity, and orchestrates further changes in transcription, translation and post-translational modification of sensory neuron ion channels [145]. NGF can also be transported in a retrograde direction to the cell body of nociceptors in the DRG, where it can stimulate expression of substance P, TRPV1, and $Na_v1.8$ voltage-gated sodium channels [146, 147]. Kinase signaling serves two purposes: it allows relatively fast activity-dependent changes by regulating local protein levels and channel activities, while also altering long-term transcriptional changes [67]. The cumulative outcome of changes in gene expression of NGF results in the amplification of neurogenic inflammation [13].

Primary sensory neurons expressing TrkB are also involved in pain signaling. This has been demonstrated following light touch after nerve injury in mice. Mice lacking TrkB display less sensitivity to touch and lack mechanical allodynia in a model of neuropathic pain. Consistent with this observation, activation of TrkB-expressing neurons produces nociceptive behavior in wildtype mice [148]. Additionally, studies have shown that cellular immediate-early genes, such as c-fos, are expressed in spinal cord neurons following inflammation and the activation of nociceptors, suggesting rapid activation of genes and changes in transcription after injury [149]. It is evident that following injury there is an increased expression of sodium channels on damaged C-fibers, as well as the release of growth factors, all of which further trigger changes in channel and receptor expression on both injured and uninjured fibers, and perpetuate the perception of pain [80].

6.5. Supraspinal alterations

While the primary focus of SCI is at or near the site of injury, it is important to discuss changes in the surpraspinal pathway and its involvement in neuropathic pain. Primary afferents communicate noxious information from the periphery to projection neurons within the dorsal horn of the spinal cord. A subset of these projection neurons convey information to the thalamus and finally to the somatosensory cortex [21]. Additional subsets of projection neurons communicate the affective component of pain, or act as a feedback system *via* connections in the rostral ventral medulla, midbrain periaqueductal gray, brainstem, and amygdala [13]. There is evidence to support the idea that the development of persistent pain after SCI is

Table 1. Summary of the diverse models used to study SCI.

Model	Method	Animal	Level of injury	Study
Compression	Clip-compression	Rat, mouse	C6, T3, T4, T9, T12,	[102, 151, 153-158]
Contusion	Impactor	Rat, mouse	T8, T9, T13	[59, 159-163]
Transection	Vanna spring scissors, micro scalpel	Rat, mouse	T9, T10	[164-166]
Excitotoxic	Glutamate, quisqualate, 3-morpholinosydnonimine (SIN-1)	Rat, mouse	T13, L3,	[167-169]
Ischemic	Irradiation, aortic occlusion	Rat, rabbit	T8, T10	[170-174]

dependent on a balance between nociceptive and non-nociceptive sensory inputs at the thalamic level [78]. Neurosurgical studies have found a correlation between neurons in the somatosensory thalamus of patients reporting neuropathic pain and an increase in spontaneous firing rates and evoked responses not normally capable of activating those neurons. This has also been observed in patients with spinal cord transections and subsequent neuronal hyperactivity in thalamic regions denervated of their normal sensory afferent input [125]. Another study utilized transcriptome analysis using publicly available databases to show that certain pathways were enriched in the brain following SCI [150]. These included oxidative phosphorylation, inflammatory response pathways, and endoplasmic reticulum stress-related pathways, among several others. Different pathways were activated at different time periods after injury, suggesting differences in gene expression patterns at acute (3 hours post-injury) and sub-acute (2 weeks post-injury) phases in the brain [150].

7. Animal models of SCI

Models of SCI-induced neuropathic pain focus primarily on injury caused by contusion or weight drop, spinal cord compression, transection, excitotoxic lesions, or ischemic injury [26, 151] (Table 1). Although several models have been developed to better understand the contribution of distinct injury models to the initiation of pain, there is no consensus as to the best model to use for SCI pain research. Given the diversity of human injuries, all of the current animal models have face validity. A practical approach is to utilize several different injury models to find common features that provide more realistic therapeutics that will work for the majority of SCI patients [59, 83, 152].

8. Conclusion

While multiple SCI models exist, assessment of pain remains a challenge. Although insight into changes of the nociceptive system has improved, the mechanisms underlying the transition from acute to chronic pain have yet to be resolved. Such pain is particularly difficult to assess in both humans and rodents because a primary feature is spontaneous pain [82]. Outcome measures in rodent models rely on spinally mediated withdrawal reflexes and assessment of motor activity. These models have a very limited response repertoire compared to human patients, which is further confounded by the differences in the rate and extent of SCI recovery between rodents and humans [85, 175]. A more advantageous approach to circumvent this issue may be to study underlying genetic changes rather than phenotypic responses in rodent models. More recently, an emphasis has been placed on the concept of “time is spine”, which highlights early interventions to improve long-term outcomes [6]. “Time is spine” concentrates on rapid transfer of patients to centers specializing in spinal cord injuries, improving early surgery to accomplish spinal cord decompression, and encouraging additional treatments with proven long-term benefits, such as steroid treatments and blood pressure stabilization. Irrespective of injury model, SCI alters genetic, cellular, and molecular pathways that all contribute to the development of neuropathic pain. Because of this, a multifaceted approach is necessary to better understand mechanisms of SCI pain and for the development of new treatment strategies and models.

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CONFLICT OF INTEREST STATEMENT

The authors have nothing to declare.

GLOSSARY

- *Allodynia:* Painful response to a normally non-noxious stimuli.
- *Cytokines:* Small proteins secreted by cells of the immune system that are involved in the induction of inflammatory responses. Cytokines include chemokines, interferons, and interleukins.
- *Dermatome:* An area of skin supplied by a single spinal nerve.
- *Dysesthesia:* Unpleasant or abnormal sense of touch.
- *Erythromelalgia:* Intense, burning pain, redness (erythema), swelling, and increased skin temperature that primarily affects the extremities.
- *Hyperalgesia:* Increased sensitivity to already painful stimuli.
- *Neuropathic pain:* Pain caused by a lesion or disease of the peripheral or central nervous system.
- *Nociceptor:* Sensory neuron that transduces painful stimuli into transmitted neuronal signals.
- *Pro-inflammatory state:* Period in which cytokines that induce inflammation are released, in addition to the release of reactive oxygen species and ATP, all of which results in a harsh environment that supports inflammation.
- *Wallerian degeneration:* The degeneration of nerve fibers that occurs after injury or disease of the PNS or CNS that begins at the site of injury and continues distal to the site of injury, while the cell body remains intact.

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