

Sensory Neurons, Ion Channels, Inflammation and the Onset of Neuropathic Pain

Patrick L. Stemkowski, Peter A. Smith

ABSTRACT: Neuropathic pain often fails to respond to conventional pain management procedures. Here we review the aetiology of neuropathic pain as would result from peripheral neuropathy or injury. We show that inflammatory mediators released from damaged nerves and tissue are responsible for triggering ectopic activity in primary afferents and that this, in turn, provokes increased spinal cord activity and the development of 'central sensitization'. Although evidence is mounting to support the role of interleukin-1 β , prostaglandins and other cytokines in the onset of neuropathic pain, the clinical efficacy of drugs which antagonize or prevent the actions of these mediators is yet to be determined. Basic science findings do, however, support the use of pre-emptive analgesia during procedures which involve nerve manipulation and the use of anti-inflammatory steroids as soon as possible following traumatic nerve injury.

RÉSUMÉ: Neurones sensitifs, canaux ioniques, inflammation et début de la douleur neuropathique. Il arrive fréquemment que la douleur neuropathique ne réponde pas au traitement conventionnel de la douleur. Nous revoyons l'étiologie de la douleur neuropathique résultant d'une neuropathie périphérique ou d'une blessure. Nous démontrons que les médiateurs de l'inflammation libérés au niveau des nerfs et des tissus lésés sont responsables du déclenchement d'une activité ectopique dans les afférents primaires et que ceci provoque une augmentation de l'activité au niveau de la moelle épinière et entraîne une « sensibilisation centrale ». Bien qu'il existe de plus en plus de données en faveur du rôle de l'interleukine-1 β , des prostaglandines et d'autres cytokines lors du début de la douleur neuropathique, l'efficacité clinique des médicaments qui sont des antagonistes ou qui préviennent l'action de ces médiateurs demeure à déterminer. Les observations faites en sciences fondamentales sont en faveur de l'utilisation de l'analgésie prophylactique pendant les interventions où il y a manipulation de nerfs et de l'utilisation de stéroïdes anti-inflammatoires le plus tôt possible après une lésion nerveuse traumatique.

Can J Neurol Sci. 2012; 39: 416-435

The biology of pain

Pain can be defined as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage'¹. Pain minimizes contact with the injurious stimulus; thus, promoting a protective response². This evokes both a reflex withdrawal from the stimulus and complex behavioural strategies to avoid further contact with such stimuli³. Despite the unpleasant nature of pain, it would be difficult to imagine any organism surviving without it. For instance, what would motivate an organism to limit use of an injured limb and allow healing? How would an organism learn from its surrounding environment? Cox and colleagues studied several families from Northern Pakistan that contained members with a congenital inability to experience pain and illustrated the following: "The index case was a ten-year-old child, well known to the medical service after regularly performing 'street theatre'. He placed knives through his arms and walked on burning coals, but experienced no pain. He died

before being seen on his fourteenth birthday, after jumping off a house roof"⁴. Clearly, without the protection afforded by the pain response, survival is compromised. So long as pain reflects an injury, in space and time, then the benefits of pain outweigh costs.

An important consideration, from the above definition, is that pain has sensory and emotional (affective) dimensions. The sensory dimension corresponds to nociception which is defined as the neural process of encoding and processing noxious or harmful stimuli⁵. Through this process, the nature (chemical, mechanical or thermal), location, intensity and temporal aspects

From the Centre for Neuroscience, University of Alberta, Edmonton Alberta, Canada.
RECEIVED OCTOBER 14, 2011. FINAL REVISIONS SUBMITTED MARCH 1, 2012.
Correspondence to: Peter A Smith, Centre for Neuroscience and Department of Pharmacology, 9-75 Medical Sciences Building, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada. Email: peter.a.smith@ualberta.ca

of the stimulus are communicated to the higher centres⁶. Although this specialized detection system usually initiates pain, it is not synonymous with pain, as this is a conscious experience that can occur in the absence of nociception⁵. For example, post-stroke pain can be independent of peripheral nociceptor activation⁷. The affective dimension is the moment-by-moment unpleasantness of pain, made up of emotional feelings associated with future implications, including distress, fear and suffering⁸. These concerns are the result of pain-related activity in limbic structures, such as the amygdala which elicits complex behaviours leading to escape and avoidance⁸.

The affective dimension suggests psychosocial aspects can influence the perception of pain such that 'pain is whatever the person says it is'⁹. Therefore, what is painful, yet tolerable to one individual or in one circumstance, can be unbearable to or in another. For instance, Rainville and colleagues demonstrated that hypnosis can manipulate the unpleasantness of a given noxious stimulus to individual human subjects¹⁰. Further, while somatosensory regions of the brain always became active in response to the noxious stimulus, activity in affective regions varied with the degree of unpleasantness^{10,11}.

Relative to the physiological contribution of nociception to the pain experience, psychosocial aspects have been poorly addressed in pain research. This disparity is, in part, a consequence of animal models and measures of pain behaviour¹². While reflexive measures, such as paw withdrawal, provide quantitative meaning to nociception, they suggest little about the psychological state of the animal and, thus, under-represent the pain experience⁸. Until recently, measures which include the monitoring of pain affect, such as conditioned place aversion (CPA), have been uncommon¹³. The focus on nociception in pain research has been associated with the clinical failure of several potential pain medicines¹². Thus, an understanding of both sensory and affective dimensions of pain may improve translational research.

Pain in the clinic

Pain can result in hyperalgesia, allodynia and spontaneous pain. These symptoms are the consequence of a heightened state of sensitivity in response to tissue damage. The IASP (International Association for the Study of Pain) defines hyperalgesia as 'an increased response to a stimulus which is normally painful'. It is thought that hyperalgesia is the consequence of sensitized nociceptive nerve endings and, therefore, stimulus modality is harmful and response is painful¹⁴. In contrast, allodynia is defined as 'pain due to a stimulus which does not normally provoke pain' (IASP). Allodynia is generated by a different mechanism than hyperalgesia, where the original stimulus modality is non-harmful but the response has become painful. Therefore, the 'quality' of the sensation has changed. For example, allodynia is observed in patients with lesions of the nervous system where touch, light pressure, or moderate cold or warmth evokes pain when applied to apparently normal skin. The term allodynia is, thus, reserved for situations where it is known that the stimulus is incapable of activating nociceptors¹⁴. Chronic pain resulting from tissue injury is often associated with paroxysmal spontaneous pain¹². Unlike allodynia and hyperalgesia, spontaneous pain is non-evoked and is the most universal clinical symptom in chronic pain states, such as

neuropathic pain¹⁵. In addition, spontaneous pain appears to be a much better predictor of 'average' and 'worst' pain ratings than evoked pain hypersensitivities¹⁵. Paradoxically, research has focussed on behavioural measures of hyperalgesia and allodynia in chronic pain animal models. For instance, in a ten year period, 90% of the papers published in *Pain* reported evoked hypersensitivity data, whereas the remaining 10% reported spontaneous behaviour measurements¹⁶. This reluctance has been attributed to the uncertainty of animal behaviours corresponding to spontaneous pain and, therefore, represents another important challenge in translational pain research¹².

Nociceptive pain is limited in duration, lasting long enough to signal the presence of a noxious stimulus. In response to tissue damage, the protective function of pain is further enhanced by allodynia and hyperalgesia³. Inflammation is critical to this process, whereby, a complex cascade of events (discussed below) leads to the activation and sensitization of sensory nerve fibres¹⁷. This heightened state of sensitivity subsides in the absence of further tissue damage and once the wound has healed³.

Pain symptoms persisting long after an initial insult suggest that the pain response has become maladaptive and, thus, can be considered a disease¹⁴. These chronic pain states can be neuropathic or inflammatory in aetiology^{12,14}. The IASP defines neuropathic pain as 'pain caused by a lesion or disease of the somatosensory nervous system'. This definition encompasses the observation that neuropathic pain can be triggered by a wide variety of insults. These include direct nerve and spinal cord trauma; viral infections including *Herpes zoster* and HIV; or metabolic diseases including diabetes¹⁸. Chronic inflammatory pain is thought to be the consequence of an underlying inflammatory disorder related to tissue pathology such as arthritis, gastritis, colitis or dermatitis¹⁹. Chronic inflammatory pain is also associated with post traumatic and repetitive strain injuries. Although distinct differences between neuropathic and chronic inflammatory pain states have been reported, including neurochemical changes and responses to analgesics²⁰, both pain states are thought to involve nervous system plasticity³. Further understanding of these pain states at a molecular and cellular level, as well as how they relate to one another is, therefore, required. The present review focuses on cutaneous sensory neurons, our understanding of the pathophysiological changes that occur in them following injury and how these changes may lead to the onset of chronic pain.

Primary afferent fibres and Dorsal Root Ganglion neurons

Primary afferent fibres are classified according to size, extent of myelination, conduction velocity and neurochemical phenotype. Conduction velocity is positively correlated with fibre cross-sectional diameter²¹. Large diameter A-fibres are the most rapidly conducting, whereas, small diameter C-fibres are the slowest conducting. The A-fibres are myelinated and have readily definable subgroups. From fastest to slowest, the A-fibre subgroups have been designated the Greek letters: α , β and δ . There is considerable variability in conduction velocity between species and even between nerves of the same species²¹. However, it is known that mammalian A-fibres can conduct up to 100 m/s while C-fibres conduct at <1m/s²². The majority of afferents that transmit painful information are A δ or C, while the

majority of afferents that convey innocuous thermal or mechanical information are A β -fibres. However, thinly myelinated and, possibly, some unmyelinated small afferents are associated with the transmission of innocuous sensations^{23,24}. For instance, a woman with selective loss of large diameter, myelinated sensory fibres provided an opportunity to study C-

fibres in isolation²⁴. In this particular case, light touch to the back of the hand was felt as very diffuse and faint, yet pleasant. Thus, some C-fibres may be associated with sensations resulting from innocuous stimuli.

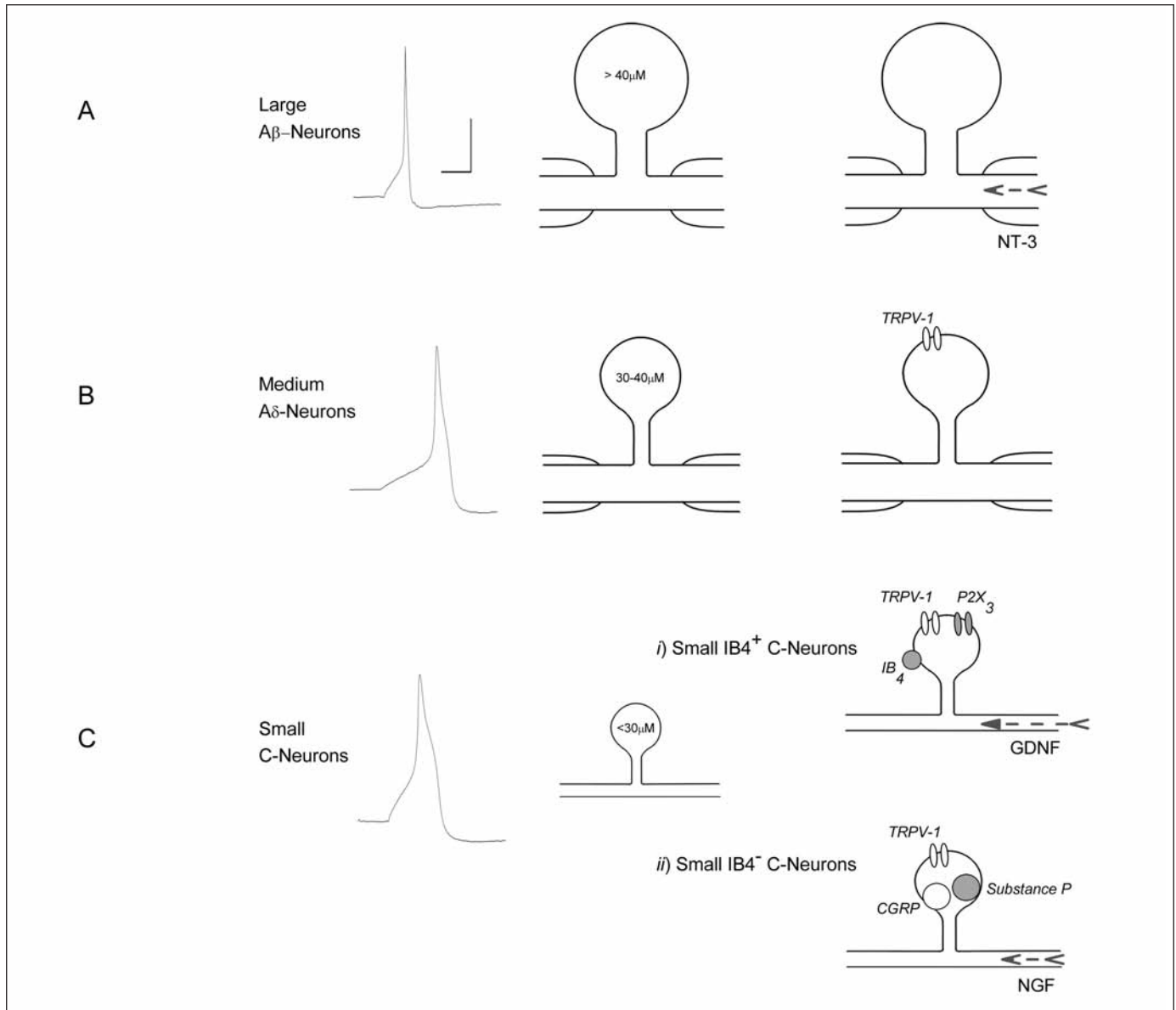


Figure: Diagram illustrating cutaneous sensory neuron subpopulations identified according to AP waveform, morphology, neurochemistry and target-derived neurotrophic support. A. Large A β -neurons have short AP durations with no shoulder on the repolarization phase, large cell body diameters (> 40 μ m) with thickly myelinated axons and are dependent on target-derived NT-3. B. Medium A δ -neurons have intermediate AP durations with the presence of a shoulder on the repolarization phase, intermediate cell body diameters (30-40 μ m) with thinly myelinated axons and express TRPV1 channels. C. Small C-neurons have long AP durations with the presence of a shoulder on the repolarization phase and small cell body diameters (< 30 μ m) with unmyelinated axons. Small neurons can be further subdivided into i) IB $_4$ -positive which express the P2X $_3$ receptor and are dependent target-derived GDNF; and ii) IB $_4$ -negative which express neuropeptides, such as SP and CGRP and are dependent on target-derived NGF. Both IB $_4$ -positive and -negative small neurons express TRPV1 channels. All AP waveforms were elicited in response to 2 ms depolarizing current pulses of appropriate suprathreshold magnitude²⁶. Calibration in A. (40 mV, 5 ms) refers to all AP waveforms. AP = Action potential; SP = Substance P; GDNF = Glial-Derived Neurotrophic Factor; NGF = Nerve Growth Factor; TRPV1 = Vanilloid Receptor Type 1

Morphology and Neurochemistry

Fibre diameter is also positively correlated to cell body diameter and, therefore, A β -fibres are associated with the largest cell body diameters, typically greater than 40 μ m, whereas, A δ - and C-fibres are associated with medium (30–40 μ m) and small (< 30 μ m) sized cell bodies, respectively²⁵ [Figure].

Small, cutaneous dorsal root ganglion (DRG) neurons can be divided into peptidergic and non-peptidergic subpopulations²⁷. The peptidergic neurons express substance P (SP) and calcitonin gene-related peptide (CGRP), while the non-peptidergic C-neurons express a binding site for the α -D-galactose-specific *Griffonia* (or *Bandeiraea*) *simplicifolia* IB₄ (GSA-IB₄) plant lectin²⁸. These IB₄-positive rat DRG neurons frequently express adenosine triphosphate-activated purinergic P2X₃ receptors²⁹ [Figure]. This serves as another defining neurochemical and, perhaps, functional feature of non-peptidergic small DRG neurons. On the other hand, immunoreactivity for CGRP is commonly used to define peptidergic, small sensory neurons since CGRP reactivity (40% of all sensory neurons) includes SP populations (20% of all sensory neurons), as well as other, SP exclusive, sensory neurons populations, such as somatostatin (SOM) expressing DRG neurons^{30–32}. In DRG, vanilloid receptor 1 (TRPV1) immunoreactivity is restricted to small- and medium-sized neurons with reactivity in both peptidergic and non-peptidergic sensory neurons^{33,34} [Figure]. Since TRPV1 is a transducer of noxious thermal stimuli, its presence is commonly used to distinguish nociceptive sensory neurons from, presumed, non-nociceptive neurons.

It is also important to consider that, while many of these cell surface and cytoplasmic molecules are useful as markers of phenotypes associated with distinct sensory neuron subpopulations, their presence varies between species, between innervated tissues, with animal age and after tissue injury^{35–37}.

Neurotrophic support

Neurotrophic factors of the nerve growth factor (NGF) family exert biological responses by binding to their respective high affinity receptors: tropomyosin receptor kinase A (TrkA), TrkB and TrkC³⁸. Additionally, all neurotrophins are capable of binding the p75 receptor which belongs to the tumor necrosis factor receptor (TNFR) family. In the adult nervous system, sensory neurons remain responsive to neurotrophic factors and the presence of several target-derived neurotrophins has been associated with the maintenance of differentiated sensory neuron phenotypes³⁹. For instance, peptidergic and non-peptidergic adult DRG neurons differ in neurotrophic support: the nerve growth factor (NGF) dependent and the glial cell line-derived neurotrophic factor (GDNF) dependent neurons, respectively^{36,40,41} [Figure]. Consistent with neurotrophin dependence, peptidergic neurons express TrkA, whereas the non-peptidergic express receptor components for GDNF signalling, including the GDNF family receptor alpha-1 (GFR α -1) and the transmembrane tyrosine kinase receptor, rearranged during transfection (RET) receptor^{36,41,42}. Upon removal of NGF or GDNF, respective cultured sensory neuron subpopulations are diminished or phenotypically switched⁴³. The NGF-dependent population (CGRP-positive) represents roughly 40% of DRG neurons, whereas the GDNF-dependent population (IB₄-positive) represents roughly 30%^{28,40} with minimal overlap

between the two populations. Further, the lack of behavioural responses to nociceptive stimuli in NGF and *trkA* null mutant mice is in agreement with the hypothesis that most TrkA neurons convey nociceptive information⁴⁴. More recently, the role of Ret signalling in the postnatal development of sensory neurons has been analyzed in mice carrying a specific deletion of the Ret gene in DRG neurons^{45,46}. The phenotype of this conditional knockout has indicated that Ret signalling is not required for DRG neuron survival, but is necessary for the proper differentiation of the non-peptidergic nociceptive subtype, as it appears to promote the expression of several markers that are characteristic of these sensory neurons and to support the normal extent of their peripheral projections in the skin⁴⁵.

Another member of the neurotrophin family, neurotrophin-3 (NT-3), also interacts with mature sensory neurons. In cutaneous afferents, mRNA for the NT-3 receptor, TrkC, is detected in larger sized adult sensory neurons which show little size overlap with neurons expressing TrkA⁴⁷. Further, *in situ* hybridization reveals that TrkC has minimal co-localization with TrkA expressing adult rat DRG⁴⁸. Thus, NT-3 responsive neurons may be functionally distinct from NGF responsive neurons. In support, single unit recordings from mice with null mutations of the NT-3 gene indicated that two mechanoreceptive subsets of cutaneous afferents require this factor: D-hair receptors and slowly adapting mechanoreceptors, while other cutaneous receptors were unaffected⁴⁹. In adult NT-3 heterozygous animals, slowly adapting afferents had the greatest reduction in incidence with corresponding morphological losses of A β -fibre axons. Merkel cells, which are the end organs of slowly adapting mechanoreceptors, were severely reduced. This loss of Merkel cells, together with their innervating A β -fibres, happens in the first postnatal weeks of life, in contrast to muscle spindles and afferents which are never formed in the absence of NT-3. Thus, NT-3 is essential for the maintenance, but not the establishment, of specific cutaneous afferents known to sub serve fine tactile discrimination in humans.

In summary, the dependence of sensory neurons on neurotrophic factors is dynamic, altering with nervous system development and after tissue injury. In addition, sensory neuron responsiveness may be dependent on target tissue, sensory ganglia (DRG or trigeminal ganglia), species and the presence of one or multiple neurotrophic factor receptors, including p75 co-expression. Despite these complexities, it can be suggested that mechanoreceptive populations tend to depend on NT-3, whereas, nociceptive populations tend to depend on NGF and GDNF. In addition, the TrkB ligand, brain derived neurotrophic factor, is likely involved in the maintenance of several sensory neuron populations.

Mechanisms of sensory transduction

Cutaneous sensory neurons can be further classified according to sensory modality. For example, thermoreceptors respond to warming or cooling, whereas mechanoreceptors respond to stretch, pressure and hair movement. In addition, many nociceptors are polymodal and respond to a variety of harmful stimuli. The transduction of stimulus modality into action potentials involves a variety of complex cellular and molecular processes⁵⁰.

Over the past decade, the molecular correlates of sensory transduction, whose activities depend on specific stimuli in the

surrounding environment, have been identified⁵¹. Cation channels, known as transient receptor potential (TRP) channels, represent the first illustration that sensory ion channels can be gated by a physical stimulus^{33,52}. These channels are divided into seven subfamilies, have six transmembrane domains, a pore region, cytoplasmic amino and carboxy termini and arrange as functional tetramers⁵³. Members of three families, the vanilloid TRP channels (TRPV), the melastatin or long TRP channels (TRPM), and the ankyrin transmembrane protein channels (TRPA) are of particular interest as thermoreceptors⁵⁴. In mammals, temperature sensitive TRPs are each tuned to distinct temperature ranges and, collectively, permit discrimination of temperature ranging from noxious cold to noxious heat⁵⁰. Four TRP channels belonging to the TRPV subfamily are activated by heating, with characteristic activation temperatures ranging from warm temperatures ($> 25^{\circ}\text{C}$ for TRPV4; $>31^{\circ}\text{C}$ for TRPV3), to heat ($> 43^{\circ}\text{C}$ for TRPV1) and noxious heat ($> 52^{\circ}\text{C}$ for TRPV2)⁵¹. In contrast, TRPM8 and TRPA1 are activated by cooling, ($< 28^{\circ}\text{C}$ for TRPM8; $<18^{\circ}\text{C}$ for TRPA1).

Many thermal TRPs are also chemo-, mechano- and/or osmo-sensitive. For instance, TRPV1 responds to protons and capsaicin, the pungent component of spicy peppers^{33,55}. In contrast, TRPM8 is activated by menthol, while TRPA1 responds to a variety of pungent compounds, including cinnamaldehyde (cinnamon), allicin (garlic) and isothiocyanates (wasabi)^{56,57}. TRPA1 and TRPV4 have been associated with mechanosensitivity^{58,59}. In addition, TRPV4 mediates animal behaviours in response to hypotonic stimuli and maybe of particular interest in hypersensitive states⁶⁰.

There are many other possible molecular correlates for sensory neuron transduction. For example, two pore potassium channels, such as TREK-1, close upon cooling and may allow depolarization of cold-sensitive neurons^{61,62}. Additionally, TREK-1 channels are stretch-sensitive and TREK-1 knockout mice have increased sensitivity to low-threshold mechanical stimuli^{61,63}. Acid sensing ion channels (ASICs) respond to protons and membrane stretch, however, a role in mechanoreception has been unsubstantiated with animal models⁶⁴. Several other molecules may be involved in sensory neuron transduction, including canonical-1 TRP channels (TRPC1), P2X₃ ATP-gated cation channels, protease activated receptors (PAR 1, 2 and 4) and voltage-gated channels (VGCs), including voltage-gated sodium channels (VGSCs)^{50,52,65,66}.

Taken together, the diverse sensory modalities of primary afferents may be associated with the expression pattern of transduction molecules, such as TRP channels, in cutaneous tissues. For instance, noxious heat sensations could be explained by the high expression of TRPV1 and TRPV2 in nociceptive A δ - and C-fibres^{50,55}. Though TRPM8 and TRPA1 are both expressed in small diameter sensory neurons, only TRPA1 expression co-localizes with putative nociceptive neuron markers, such as SP and CGRP, thereby, rationalizing noxious cold as distinct from cool thermo-sensations⁵². Consistent with polymodal nociceptors, TRPA1 is expressed in a subset of TRPV1 expressing nociceptive neurons responding to noxious mechanical and thermal stimuli⁵². The close apposition between free nerve endings (FNEs) and keratinocytes, as well as the synaptic junctions between Merkel cells and A β -fibres suggest skin cells can act as first-line transducers of physical stimuli⁵⁰. In support, Merkel cells express TRPV4 and keratinocytes express

TRPV3 and TRPV4^{50,52}. In a manner analogous to auditory and taste transduction, skin cells could respond to innocuous heat and mechanical stimuli and, then, chemically transmit the signal to sensory neurons, such as A β -fibres⁵⁰.

Peripheral connections

Sensory neuron connectivity, both peripherally and centrally, is vital to the discriminative task of the somatosensory system. Within the skin, there are several morphologically distinct nerve endings which can be classified according to afferent threshold, adaptation and, ultimately, sensory modality. For instance, physiological and cytological examination of the hairy skin in cats showed that free nerve endings (FNEs) are the nerve terminals of A δ - and C-fibres, sub serving the modalities of pain and temperature⁶⁷. The A δ -fibres pass through the papillary dermis and penetrate the basement epidermal layer where they lose their myelin sheath, while peptidergic and non-peptidergic C-fibres terminate in different epidermal layers of glabrous and hairy skin^{50,67}. Many FNEs are considered nociceptors. Certain nociceptors, whose afferent conduction velocities correspond to A δ - and C-fibres, are high threshold mechanoreceptors and respond vigorously to only intense (tissue damaging) mechanical stimuli^{68,69}. Other nociceptors are polymodal, whose afferent conduction velocities are reflective of C-fibres, and respond vigorously to noxious mechanical and thermal stimuli and to chemical substances relevant to tissue damage^{68,69}. Still, some nociceptors, in deep subcutaneous tissue, have such high thresholds that they are unresponsive or "silent" in acute events, but become active after chronic tissue damage⁶⁹⁻⁷¹. FNEs of A δ - and C-fibres have also been associated with innocuous stimuli where some are considered thermoreceptors or low threshold mechanoreceptors^{69,72-74}. As noted above, evidence is accumulating that many of the FNEs form *en passant* synapses with epidermal cells, such as keratinocytes, which express molecular correlates for stimulus transduction^{50,75}. Thus, FNEs may not always be the first-line transducers of pain and temperature.

Sensory corpuscles are capsulated low threshold mechanoreceptors formed by a central axon surrounded by variably arranged differentiated Schwann cell-related and perineurial-derived cells^{72,76}. Two main subtypes of sensory corpuscles can be considered: the extensively capsulated, Pacinian corpuscles and the poorly capsulated, Meissner corpuscles. Meissner corpuscles lie just beneath the epidermis, while Pacinian corpuscles reside in the dermis and deeper tissues and, in both cases, the capsulated arrangements appears to act as a filter that protects the sensory endings from irrelevant stimuli⁷⁷. Corpuscles can have A α -, A β - or A δ -sensory nerve fibres and upon static, mechanical skin displacement, only a few action potentials are recorded from sensory units and, therefore, corpuscle are defined as rapidly adapting⁷⁷. However, adaptation terminology is misleading since static displacement does not account for derivatives of displacement, such as velocity and direction²¹. Thus, many rapidly adapting mechanoreceptors do not adapt rapidly when presented their most effective stimulus, such as dynamic mechanical displacements. Therefore, Pacinian and Meissner corpuscles are vibration detectors which are ideally tuned to detect high (> 100 Hz) and low (< 100 Hz) dynamic displacements, respectively⁷⁷.

The Ruffini endings and Merkel cell–neurite complexes are slow adapting, low threshold mechanoreceptors of glabrous- and hairy skin^{21,77}. Ruffini endings are located in the connective tissue of the dermis and are relatively large spindle shaped structure tied into the local collagen matrix⁷⁷. Ruffini ending structure is considered analogous to that of the Golgi tendon organ in muscle and, therefore, suggests that Ruffini endings function as stretch receptors⁷². A α / β sensory axons branch between the fibrils and stretching of the skin tightens the fibrils which, in turn, leads to deformation and depolarization of the axonal ramifications^{21,78}. The Merkel cell is a special cell type in the basal layer of the epidermis that enfolds the unmyelinated ending of the A α / β afferent fibre^{21,77}. The Merkel cell–neurite complex is selectively sensitive to a particular component of local displacement which makes it sensitive to edges, corners and curvature. However, it is not known whether this selectivity is due to the Merkel cell or to the transducer mechanism within the afferent terminal⁷⁹.

Hairs are additional low threshold mechanoreceptors of hairy skin and have two main types of follicles⁷²: 1) Guard hairs have thick diameter shafts, such as that found on the human scalp or outer coat of furred mammals. 2) Vellus hairs have thin diameter shafts, such as that found on the human eyelid or the down hairs of furred animals. A α –, A β – or A δ –nerve fibre endings wrap around the hair follicle, collectively termed the piloneural complex and become activated upon the slightest bending of the hair^{50,72,74}. Ultrastructural analysis has revealed that axons innervate from the base of guard- and vellus-hair follicles and split into fork-like projections known as lanceolate terminals. Further, lanceolate terminals have been associated with rapidly adapting afferents and, thereby, enable hairs with movement detecting capabilities^{21,72}.

Electrophysiology

The electrical properties of DRG neurons correlate to receptor- and afferent-type and, ultimately, to sensory function. Intracellular recordings from somata in intact, but isolated, adult mouse DRG led to discovery of three distinct cell types on the basis of action potential (AP) shape, sensitivity to tetrodotoxin (TTX) and ionic dependence: 1) ‘fast’ (F) -neurons, exhibiting brief APs which were mediated by TTX-sensitive (TTX-S) Na⁺ channels; 2) ‘afterhyperpolarization’ (A) -neurons, exhibiting large and prolonged afterhyperpolarizations (AHPs) which were dependent on TTX-resistant (TTX-R) Na⁺ channels; and 3) ‘hump’ (H) -neurons, exhibiting broader spikes that were mediated by TTX-R Na⁺ and Ca²⁺ channels with the latter producing a characteristic ‘hump’ or shoulder on the descending limb of the AP^{80–82}. Similar AP shapes were reported in other studies where broad somatic APs tend to have slow C–, as well as some fast A β –conducting fibres, while narrow APs tend to have faster conducting fibres, including A α –, A β – and A δ –fibres [Figure]^{26,83,84}. In addition, broad somatic APs from C– and some A δ –neurons are often associated with a shoulder on the repolarization phase [Figure]. Studies in intact preparations, where the peripheral receptor could also be characterized, have associated sensory function with the heterogeneous electrical properties of DRG somatic membranes^{82,85–87}. The major trend is that somatic spikes in nociceptors are characterized by broad APs with shoulders on the descending limb, while receptors

responding to innocuous stimuli are characterized by narrower spikes. It was also discovered that A δ –fibres innervating high threshold mechanoreceptors (HTMRs) exhibit broader APs than A δ –fibres innervating D-hairs^{82,86}. A β HTMRs differ from A β low threshold mechanoreceptors (LTMRs) in the same general way, however, A β LTMRs supplying different receptor types (e.g., slowly adapting type I, Pacinian corpuscles, etc.) exhibit no correlation between receptor type and electrophysiology of the soma. The relationship between AP shape and receptor threshold in C-fibres appears to be species dependent. For instance, C-fibre AP duration is longer for high threshold (nociceptive) afferents than for low threshold afferents in rat and guinea pig^{88,89}, however, no such relationship was found in cats⁹⁰. Taken together, the variability of AP shapes in A-fibres and, possibly, C-fibres suggests AP duration is more related to receptor threshold than to conduction velocity.

The differential ionic dependencies and TTX sensitivities of APs reflect non-uniform ion channel expression among DRG cell types. Through several electrophysiological, pharmacological and molecular studies, it is now known that DRG neurons are host to a variety of voltage-gated ion channels⁹¹.

Voltage gated sodium channels (VGSC)s are hetero-multimeric proteins consisting of a large pore-forming α -subunit and small extracellular accessory β -subunits which modulate channel membrane insertion and channel gating⁹². Nine mammalian sodium channel isoforms have been identified and functionally expressed⁹³. TTX-S isoforms include Na_v1.1, Na_v1.2, Na_v1.3, Na_v1.4, Na_v1.6 and Na_v1.7, whereas, TTX-R isoforms include Na_v1.5, Na_v1.8 (formerly peripheral nerve sodium channel type 3 (PN3) and sensory neuron specific (SNS)) and Na_v1.9 (formerly novel voltage-gated Na⁺ channel (NaN))^{92,94,95}.

Variations in sodium current properties in DRG neurons are associated with a heterogeneous sodium channel population. For instance, electrophysiological studies in DRG somata have demonstrated the presence of kinetically slow TTX-R sodium current in small, but not large-sized neurons^{96,97}. Cloning studies identified sodium channel α -subunits Na_v1.8 and Na_v1.9 which produce TTX-R currents when heterologously expressed^{98–100}. Further, *in situ* hybridization has revealed that transcripts for the two TTX-R sodium channels are preferentially expressed in small diameter sensory neurons which include nociceptive (C-type) neurons. Waxman’s group demonstrated differential maintenance of the two DRG TTX-R isoforms, where NaV1.9 is preferentially expressed in small IB₄-positive DRG neurons while both small IB₄-positive and -negative neurons express Na_v1.8⁴³. The differential expression was also associated with alterations in sodium channel properties expected to influence excitability, such as a hyperpolarized voltage-dependence of activation and inactivation in IB₄-positive neurons. The persistent nature of currents mediated via Na_v1.9 was further predicted to underscore the longer AP durations observed in small IB₄-positive neurons⁸⁹. In a similar manner, many other isoforms have been characterized each with unique biophysical properties that influence AP generation, such as the ‘rapidly repriming’, resurgent Na_v1.6 sodium current. This current, by virtue of its rapid recovery from inactivation, can maintain high frequency firing¹⁰¹. Heterogeneous expression of VGSC isoforms thus tunes electrical behaviour in the various sensory

neuron subpopulations and their altered expression or modulation by mediators may be of consequence after tissue damage¹⁰¹⁻¹⁰³.

Immunocytochemical methods have also been used to determine the distribution of VGSC isoforms in sensory neurons and it is now known that large DRG neurons predominantly express TTX-S channels, such as Na_v1.1, Na_v1.6 and Na_v1.7, with some TTX-R Na_v1.8 expression, while small neurons express TTX-S channels, in conjunction with TTX-R Na_v1.8 and Na_v1.9 channels¹⁰¹. These findings have been further substantiated using intracellular recording, together with immunohistochemistry, to show the distribution of channels in DRG neurons that give rise to particular fibre types, such as the association of nociceptive C-fibres and broad APs with IB₄-positive cell bodies immunoreactive for the Na_v1.9 isoform^{89,101}. Last, it is known that expression of multiple sodium channels, including TTX-R VGSCs, is not limited to the cell body and extends along the fibres, thereby, demonstrating the likely importance of these channels in conduction and fibre characteristics¹⁰¹.

Voltage-gated calcium channels (VGCCs) are the critical link between membrane depolarization and calcium entry and represent of one of several ways calcium can influence membrane excitability and physiological processes, including neurotransmitter release^{104,105}. Biochemical characterization of VGCCs has revealed a complex protein structure composed of α 1 pore forming subunit, encoded by gene subfamilies: Ca_v1 to 3¹⁰⁴, as well as several auxiliary subunits^{105,106}.

Sensory neurons express several biophysically and pharmacologically distinct VGCCs¹⁰⁴. These include: 1) Low voltage activated (LVA) channels encoded by the gene subfamily Ca_v3 which have 'low' gating thresholds from -60 to -50 mV evoking rapidly inactivating 'transient' (T-type) currents, which are sensitive to changes in holding potential and which are non-selectively inhibited by amiloride and Ni²⁺^{104,105,107}. 2) High voltage activated (HVA) channels encoded by gene subfamilies Ca_v1 and 2 which have 'high' gating thresholds from -30 to -20 mV. Further, HVA Ca²⁺ currents include 'long-lasting' (L-type, Ca_v1) currents, 'Purkinje cell' (P/Q-type, Ca_v2.1) currents and 'neuronal' (N-type, Ca_v2.2) currents which are sensitive to blockade by dihydropyridines, omega-agatoxin IVA and by omega-conotoxin GVIA, respectively^{104,105,107-109}. 3) Last, there are the intermediate voltage activated calcium channels which have 'intermediate' gating thresholds, evoking currents that are 'resistant' (R-type, Ca_v2.3) to most toxins.

While N-type currents are equally proportionate in small, medium and large neurons, T-type currents have their greatest proportion in medium neurons and L-type currents have their greatest contribution in small neurons^{107,110-112}. The LVA and HVA channels are associated with different physiological roles. N-type VGCCs are highly expressed at presynaptic nerve terminals where they are involved in fast synaptic transmission¹¹³. In contrast, T-type VGCCs are expressed in cell bodies and nerve endings of afferent fibres where they partake in regulating neuronal excitability by contributing to the initiation of repetitive discharge^{113,114}. Further, the unique biophysical properties attributed to T-type VGCCs lower AP threshold, promote bursting activity and generate subthreshold membrane oscillations.

As well as their pore forming α subunits, VGCCs contain various accessory subunits. The α_2 - δ subunit, which has particular relevance to neuropathic pain mechanisms, is thought to be involved in the trafficking of channels to the plasma membrane¹¹⁵. It is upregulated by nerve injury¹¹⁶⁻¹¹⁹ and appears to be the primary site of action for the antiallodynic agents pregabalin¹²⁰ and gabapentin¹²¹⁻¹²³.

Potassium channels are the most diverse class of ion channels owing to numerous encoding genes, alternative mRNA splicing, α -subunit assemblies into dimeric or tetrameric channels, as well as associations with β -subunits¹²⁴. The variation in AHP duration, observed by Matsuda and colleagues, suggests that potassium conductances are another defining feature among sensory neurons⁸⁰. It is now known that DRG neurons contain a wide variety of potassium channels of all four families: voltage-gated (K_v), calcium-activated (K_{Ca}), inwardly-rectifying (K_{IR}) and two-pore (K_{2p}) channels. Although all four families have been associated with pain hypersensitivity¹²⁵, K_v and K_{Ca} channels and their relation to sensory neuron physiology will be the topic of further discussion. For more information on K_{IR} and K_{2p}, see reference numbers¹²⁵⁻¹²⁸.

K_v and K_{Ca} channels exist as tetramers composed of four pore forming α -subunits either alone or in association with regulatory β -subunits^{129,130}. The voltage-dependent delayed rectifier and fast transient potassium currents were first described in DRG neurons by Kostyuk's group¹³¹. The delayed rectifier current was characterized by its slow activation kinetics and lack of inactivation during maintained membrane depolarization. In contrast, the fast transient current or A-current (I_A) had, relatively, 'fast' activation kinetics and was almost completely inactivated at -50 mV. In addition, the fast transient current required a relatively small depolarization for activation when compared to the delayed rectifier current. Though both can be blocked by tetraethylammonium (TEA), fast transient potassium currents are also susceptible to block by 4-aminopyridine (4-AP)^{132,133}. The existence of delayed rectifier and fast transient potassium currents in DRG neurons was confirmed in subsequent studies. However, a third component was identified based on sensitivity to the Eastern green mamba venom, dendrotoxin, and greater susceptibility (<100 micromolar), relative to I_A (>100 micromolar), to 4-AP block^{134,135}. When isolated, the dendrotoxin-sensitive current (I_D) was calcium insensitive and displayed, relative to I_A , slow, incomplete inactivation^{135,136}. Biophysically, potassium currents could be divided into non-inactivating and transient A-currents with the later subdivided into fast (I_{Af}) and slow (I_{As}) components where I_{Af} had, relatively, faster kinetics and more negative voltage dependencies than I_{As} ¹³⁷. Thus, pharmacological and biophysical studies agree that at least three voltage-gated potassium currents can be readily identified in sensory neurons: sustained or non-inactivating, I_A or I_{Af} and I_D or I_{As} (but see also reference number¹³⁸).

There is a complex distribution of the voltage-gated potassium currents among sensory neurons. While most sensory cell types have a non-inactivating or sustained potassium current, the distribution of transient components is less understood. For instance, A-type currents have been identified in subpopulations of large and small sensory neurons, including those associated with mechanoreceptors and nociceptors,

respectively¹³⁸⁻¹⁴¹. In contrast, Villiere and colleagues reported that the proportion of neurons with A-type current is greatest in C-fibres and least in A α / β -fibres¹⁴². Under thorough pharmacological and biophysical analysis, Gold and colleagues characterized six voltage-gated potassium currents in rat DRG neurons, three transient and three sustained, and found that there is differential distribution among rat DRG neuron subpopulations¹³⁸. Thus, it remains possible that most sensory cell types have at least one transient and one sustained current. Regardless of the sensory cell type, most studies agree sustained potassium currents shape APs, while transient currents are involved in the latency of firing, low firing frequency, spike adaptation and that there may be an inverse relationship in the proportion of these two broad classes of potassium current in any given sensory cell^{137,139,141,143,144}.

Three classes of K_{Ca} channels can be distinguished on the basis of their biophysical and pharmacological properties¹⁰⁴. Large conductance BK_{Ca} channels are voltage-gated and sensitive to iberiotoxin, while small conductance (SK_{Ca}) and intermediate conductance (IK_{Ca}) channels are voltage-insensitive and can be blocked by apamin and clotrimazole, respectively. Modulation of K_{Ca} channels allows alterations in intracellular calcium concentration to regulate membrane excitability, whereby, the three K_{Ca} channels are thought to have distinct functional roles. For instance, BK_{Ca} channels are involved in AP repolarization and generation of the fast AHP (fast I_{AHP}), whereas, SK_{Ca} channels convey the I_{AHP} that mediates slow AHPs in small nociceptive DRG neurons^{104,145-147}. In contrast, the physiological role of IK_{Ca} in DRG neurons remains to be determined. Although the functions of SK_{Ca} and BK_{Ca} channels are well recognized in small diameter sensory neurons^{148,149}, the presence of SK_{Ca} channels in large diameter neurons¹⁵⁰ suggests that K_{Ca} channels have physiological importance in non-nociceptive neurons.

Hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channel subunits include four family members (HCN1-4) that share substantial homology^{151,152}. Individual HCN subunits assemble as homotetramers and, when expressed, homomers differ in two main respects: 1) rates of activation are in the order of HCN1>HCN2>HCN3>HCN4; and 2) HCN2 and 4 are strongly modulated by adenosine 3', 5'-cyclic monophosphate (cAMP) elevations which shifts the midpoint of activation in the positive direction by 12 to 20 mV. Heteromers may also form functional HCN channels and have intermediate properties which are related to subunit composition^{153,154}.

The HCN channels give rise to hyperpolarization-activated, non-selective cation current (I_H)¹⁵⁵. The slow activation of I_H in DRG neurons gives rise to a 'voltage sag' in response to hyperpolarizing current commands^{155,156}. Thus, the initial voltage attained by injection of hyperpolarizing current gradually abates during the course of the current command. I_H is also involved in setting resting membrane potential, participating in pacemaker activity and modulating synaptic activity¹⁵⁷. HCN channels 1 to 3, but likely not HCN4 (but see also reference number¹⁵⁸), are present in the somata and axons of DRG neurons¹⁵⁹⁻¹⁶¹. Further, HCN1 and fast I_H are predominately found in larger DRG neurons, while slower I_H currents are more variably expressed in small neurons¹⁶⁰⁻¹⁶². Last, knockout mutations or block with ZD7288 are associated with suppression

of sensory neuron hyperexcitability and pain-related behaviours and, therefore, IH has attracted recent interest in pain research¹⁶³.

Spinal projections

Once transduction has encoded information from various stimuli in an organism's environment, various levels of the central nervous system (CNS) become involved in the processing of this sensory information.

The initial stage for the central processing of pain occurs in the dorsal horn of the spinal cord and involves specific spatial terminations for primary afferents. For instance, the rostro-caudal and medio-lateral terminations of primary afferents encode the location of their individual peripheral receptive fields, thus, generating a somatotopic map of the body's surface onto the dorsal horn¹⁶⁴. On the other hand, primary afferent terminations in the dorso-ventral plane of the dorsal horn encode different functional classes of sensory neurons. The dorso-ventral plane of the dorsal horn is subdivided into six horizontal laminae (LI to LVI) where neurons of common morphological features define each lamina^{165,166}. The identities of primary afferents that terminate and release glutamate within the marginal layer (LI), the *substantia gelatinosa* (LII), the *nucleus proprius* (LIII to LIV) and LV have been determined. For instance, large myelinated fibres, innervating low threshold mechanoreceptors enter the cat spinal cord and send collaterals into the dorsal horn as they ascend and, sometimes, descend the dorsal column¹⁶⁷⁻¹⁷⁰. These collaterals terminate ipsilaterally in laminae ventral to the outer lamina II (LIIo) and include extensive terminations in laminae III and IV. Similar patterns of termination occur in the rat dorsal horn¹⁷¹. In contrast, identified C-fibres, including nociceptive and thermoreceptive fibres, terminate ipsilaterally in laminae I, II and V^{172,173}. Further, CGRP immunoreactivity revealed that peptidergic C-fibres, many of which co-localize SP or SOM, terminate in laminae I, IIo and V, whereas, IB₄ labelling and/or P2X₃ immunoreactivity has revealed that non-peptidergic C-fibres terminate dorsally within inner lamina II (LIIi)^{29,174,175}. Although both classes of C-fibres transmit nociceptive information to the spinal cord, the function of non-peptidergic C-fibres remains poorly understood³⁴. Nociceptive, high threshold mechanoreceptive A δ -fibres terminate ipsilaterally in laminae I and V, whereas innocuous A δ -fibres, innervating D-hairs, terminate ipsilaterally in the deeper part of lamina II and lamina III¹⁷⁶. Taken together, the superficial layers of the dorsal horn (LI and LIIo) receive heavy nociceptive input, while deeper laminae, with the exception of L5, receive non-nociceptive input.

Neuropathic pain

Neuropathic pain can be triggered by various insults including direct nerve and spinal cord trauma; viral infections including *Herpes zoster* and HIV; or metabolic diseases including diabetes¹⁸. Because the clinical presentation of neuropathic pain is often independent of any obvious signs of inflammation, it is sometimes described as 'non-inflammatory pain'.

In experimental animals, peripheral nerve damage, such as nerve sectioning (axotomy) or chronic constriction, induce pain-related behaviours that are widely accepted as a model for

human neuropathic pain. Such behaviours are associated with an enduring increase in the excitability of primary afferent neurons which, over a period of days or weeks, leads to the generation of increased excitability and synaptic activity in second order sensory neurons within the dorsal horn of the spinal cord. These events correspond to the phenomenon of 'central sensitization' which is a major component of many persistent pain states^{3,177}. The generation of ectopic activity, thus, provides a theoretical basis for the use of pre-emptive analgesia in surgery. However, traditional analgesics, such as morphine, have limited use in the treatment of neuropathic pain and, therefore, much effort has been devoted to the understanding of how peripheral nerve injury leads to increased excitability of the spinal dorsal horn.

Enduring increases in primary afferent activity as a trigger to central sensitization

Experiments using various nerve injury animal models suggest that the enduring increase in primary afferent activity originates both from the neuroma¹⁷⁸, that develops at the site of nerve injury, and from sensory cell bodies in the DRG^{179,180}.

DRG excitability increases two to seven weeks after sectioning (axotomy) of the sciatic nerve²⁶. Thus, the minimum current required to discharge an AP (rheobase) was reduced and sustained depolarizing current evoked a higher frequency of AP discharge. There were also increases in AP height and width. Voltage-clamp analysis supported these findings, as AP generating mechanisms, such as TTX-R and/or TTX-S Na⁺ currents (I_{Na}), were increased¹⁰². In contrast, AP regulatory mechanisms, such as steady state (delayed rectifier) K⁺ current (I_K) and HVA Ca²⁺-channel current (HVA- I_{Ba}) were attenuated¹¹². Further, alterations were most prevalent in the small-sized DRG neurons which are, presumably, nociceptive C- and A δ -fibres. However, with the onset of autotomy (self mutilation), which is believed to be a behavioral manifestation of human neuropathic pain, changes became most substantial in the large (A β) cell bodies^{26,102,112}.

Other studies also reported changes in electrical properties of DRG neurons after nerve injury that are consistent with increased primary afferent activity. For instance, intracellular recordings from neurons in intact DRG revealed that sacral 1 (S1) spinal nerve transection led to a significant reduction of the rheobase in A- and C-cell types¹⁸¹. The reduction of rheobase in A-cells was associated with a concomitant increase in apparent input resistance near the resting membrane potential (RMP). By contrast, the rheobase reduction in C-cells was associated with a depolarizing shift of the RMP. In addition, nerve injury produced significant action potential broadening in all cell types. In small-sized DRG neurons, axotomy reduced AP threshold, but was without alterations to RMP or AP shape¹⁸². After CCI, isolated small-, medium- and large-sized DRG neurons showed an increased incidence of spontaneous AP activity which was associated with a negative shift in AP threshold¹⁸³. Similar findings were reported after chronic compression of the DRG, however, injury-induced changes were more apparent in large- and medium-sized neurons than in small neurons¹⁸⁴. Despite differences in the experimental approach employed in these studies, similarities do exist, including a reduction in firing threshold or rheobase in nociceptive and, possibly, non-nociceptive sensory neurons. Further, distinct changes to AP and

AHP shape, as well as to passive membrane properties, suggest alterations in the underlying availability of particular ion channels may be associated with the sensory neuron subtype, as well as the nature of the nerve injury.

Nerve injury-induced changes to the availability of sodium channels. In contrast to the findings of Abdulla and Smith¹⁰² discussed above, Waxman and colleagues reported that axotomy injury resulted in the down regulation of TTX-R sodium current in small- and large-sized rat DRG neurons, leaving TTX-S sodium currents to make a greater proportion of the total sodium current in both cell populations^{185,186}. Similar observations were reported by Zhang and colleagues after an axotomy injury¹⁸². Further, the reduction in TTX-R sodium currents was paralleled by the emergence of a rapidly repriming TTX-S sodium current in small DRG neurons after axotomy. Rapidly repriming TTX-S current permits neuronal firing at higher than normal frequencies^{186,187} and upregulation of its corresponding transcript, Na_v1.3, as well as protein product have been observed in DRG neurons of adult rats after axotomy¹⁸⁸⁻¹⁹⁰, CCI¹⁹¹ and SNL¹⁹². In contrast, transcripts encoding TTX-R sodium currents are down regulated in small DRG neurons following axotomy^{100,189}. These alterations may, in part, be explained by a loss of target derived neurotrophic factors after peripheral nerve injuries. For instance, partial restoration of TTX-R currents, along with upregulation of α -SNS transcript, was reported after the administration of NGF to the proximal nerve stump¹⁹³. Further, intrathecal GDNF treatment prevented sensory hypersensitivity after SNL injury and was associated with block of A-fibre ectopic discharge and normalization of Na_v1.3 expression in injured DRG¹⁹⁴. In addition to the emergence of rapidly-repriming TTX-S sodium currents, loss of target derived GDNF is associated with a reduction in TTX-R currents in small IB4-positive DRG neurons, whereas loss of NGF is associated with a reduction in TTX-R currents in small peptidergic neurons⁴³. Taken together, it appears that nerve injury is commonly associated with a reduction in TTX-R sodium currents, allowing TTX-S currents to dominate as the major generators of AP upstroke and, possibly, spontaneous ectopic AP discharge in injured sensory neurons. However, Na_v1.8 upregulation, along with the presence of functional TTX-R sodium channels, have been associated with aberrant activity in uninjured C-fibres and neuropathic pain behaviours after partial nerve injury¹⁹⁵. Therefore, the relevance of particular sodium channel isoforms to neuropathic pain may depend on the extent of nerve injury and, possibly, the degree of associated inflammation. In broader terms, it is generally accepted that increased Na⁺ channel function is associated with the onset and maintenance of neuropathic pain and, in line with this, there has been an impetus for the development of novel, state-dependent ion channel modulators as potential therapeutic agents¹⁹⁶.

Nerve injury-induced changes to the availability of potassium channels. In large cutaneous afferent DRG neurons, a sustained potassium current component, as well as the transient current, I_A , but not I_D were reduced after axotomy¹⁹⁷. Compared to contralateral controls, mRNA expression for genes which encode delayed rectifier (K_v 1.1 and 1.2) and A-type (K_v 1.4, 2.2, 4.2, and 4.3) voltage-gated potassium channels were reduced in ipsilateral lumbar 4, 5 and 6 DRG one week following CCI¹⁹⁸. Unlike changes in $I_{K,Ca}$ after axotomy¹¹², L5 SNL decreased $I_{K,Ca}$

due to a direct effect on $I_{K,Ca}$ channels¹⁹⁹. Though all $I_{K,Ca}$ subtypes were decreased in small- and medium-sized DRG neurons from the injured nerve, medium-sized DRG neurons from the adjacent uninjured L4 nerve had increased iberitoxin sensitive (large conductance) and clotrimazole sensitive (intermediate conductance) $I_{K,Ca}$. In injured human peripheral nerves, there was a decrease in human intermediate conductance calcium-activated potassium channel 1 (hIK1)-like immunoreactivity predominately in large-, but also, in medium- and small-sized DRG neurons when compared to controls²⁰⁰. Like sodium channels, these changes were associated with a loss of target-derived neurotrophic support. Taken together, it appears that nerve injury promotes a reduction in sustained and/or A-type potassium currents which could account for broadening of APs, as well as contributing to sensory neuron hyperexcitability through a reduction in spike adaptation. Further, a reduction in $I_{K,Ca}$, whether directly or secondary to reductions in HVA calcium currents (see below) serves as an additional ionic mechanism for AP broadening and aberrant activity in sensory neurons after peripheral nerve injury.

Nerve injury-induced changes to the availability of calcium channels. After axotomy, the density of omega-conotoxin GVIA-sensitive (N-type) calcium current (I_{Ca}) is decreased and is concurrent with increased inactivation in large-sized DRG neurons associated with cutaneous afferents^{112,201}. LVA or T-type I_{Ca} is unaffected. By contrast, following CCI, T-type current density is increased in small-sized rat DRG neurons, but was without changes in voltage- and time-dependent parameters²⁰². Onset of diabetic neuropathy is also associated with increased T-type, but not HVA, current density in IB₄-positive, capsaicin responsive medium-sized rat DRG neurons, as well as a depolarizing shift in steady-state inactivation²⁰³.

Although the changes in I_{Ca} are variable and likely depend on the nerve injury model and DRG cell type studied, alterations are consistent with increased excitability. For instance, the reduction in HVA or N-type I_{Ca} could underlie decreased calcium-dependent potassium current after injury¹¹² which, in turn, would be expected to decrease AHP amplitude and shorten AHP duration, ultimately, increasing firing frequency²⁰⁴. However, the observed decrease in HVA I_{Ca} after peripheral nerve injury seems inconsistent with the effectiveness of drugs, such as the gabapentinoids²⁰⁵ and ziconotide^{113,206}, which directly or indirectly impede VGCC function. The likely explanation for this paradox is that, whilst nerve injury may reduce HVA VGCC expression on DRG cell bodies, expression in the central terminals of primary afferents may be increased. This may in turn lead to the increased release of neurotransmitters and other mediators from primary afferents and the onset of central sensitization. Thus, any consequence of VGCC blockade in DRG neurons would be overcome by drug action at nerve terminals. This possibility is illustrated by the actions of morphine which can increase DRG excitability by an action on Ca²⁺ channels²⁰⁷, yet opioids are known to produce analgesia, impart, by blocking presynaptic Ca²⁺ channels and, thereby, reducing neurotransmitter release from primary afferent terminals²⁰⁸.

By contrast, a CCI induced increase in T-type current has been correlated closely to more prominent afterdepolarizing potentials (ADP), as well as a lowered rheobase for AP firing in DRG neurons²⁰³. This has led to the identification of T-type

calcium channels as a potential therapeutic target in pain management^{196,209,210}.

Nerve injury-induced changes to the availability of HCN channels. Since the pacemaker current (I_H) acts to induce a depolarization after a hyperpolarizing event, any upregulation after nerve injury may contribute to enhanced neuronal excitability neuropathic pain. Chronic compression of the DRG (CCD) produces cutaneous hyperalgesia and enhanced excitability of neuronal somata^{184,211}. The CCD increased IH current density and rate of activation, without changing its reversal potential, voltage dependence of activation, or rate of deactivation in medium-sized rat DRG neurons associated with cutaneous afferents²¹¹. Further, SNL injury markedly increased pacemaker currents in large diameter DRG neurons²¹². Pharmacological blockade of I_H with ZD7288 decreased firing frequency of ectopic discharges originating in injured A β - and A δ -fibres and was concurrent with the reversal of mechanical allodynia. Changes may not be limited to the cell body, however, and can involve the axonal accumulation of the HCN channels at the site of sciatic nerve injury¹⁵⁹. Intriguingly, more recent reports suggest HCN channels are important in both the establishment and maintenance of neuropathic pain, where specific HCN channel isoforms could play important roles^{213,214}.

In addition to injury-induced changes in ion channel availability, extrinsic mechanisms also contribute to the generation of ectopic activity in sensory neurons. These include sprouting of perivascular sympathetic neurons within the DRG²¹⁵⁻²¹⁸. Although normal DRG neurons are insensitive to noradrenaline, injury-induced upregulation of α -adrenoceptors²¹⁸⁻²²⁰ and the formation of baskets of sympathetic axons around DRG neurons²¹⁵ enables excitation of neurons that may contribute to 'sympathetically maintained pain' (complex regional syndrome II). Additionally, neurogenic inflammation, involving the release of CGRP and SP from sensory nerve endings¹⁷, may contribute to altered ongoing activity in sensory neurons. Lastly, there is evidence that ectopic excitatory interactions may occur between neuronal cell bodies in the DRG following nerve injury²²¹.

Central sensitization

In 1983, Clifford Woolf's study of injury induced changes to the cutaneous receptive field properties of flexor motor neurons in rat provided the first pieces of evidence for spinal cord plasticity and central sensitization¹⁷⁷. Essentially, repeated noxious heat stimuli presented to the paw produced a sustained increase in the excitability of α -motor neuron axons which was characterized by: A β -fibre recruitment to the, normally, nociceptor specific reflex; expanse of the receptive field which was unresponsive to local anaesthetics applied to the site of injury; and all of which could be mimicked by C-fibre strength electrical stimulations of the sural nerve. Since these findings were difficult to explain within the context of a peripherally driven mechanism, a central process was implicated. It is now appreciated that central sensitization is a form of nervous system plasticity composed of increases in membrane excitability²²², synaptic facilitation^{223,224}, loss of inhibition²²⁵, reversal of inhibition (disinhibition)²²⁶ and enhancement of excitation²²⁷ of central circuitry which promotes spontaneous pain, allodynia and hyperalgesia after inflammation or lesions to the nervous

system³. The net effect is the recruitment of previously subthreshold inputs from low-threshold receptors and high-threshold receptors from outside a given receptive field to the output of central nociceptive neurons. These inputs can be experimentally revealed in the spinal cord after the administration of synaptic blockers of inhibitory transmission, such as GABA_A receptor antagonists, which enable A β -fibre input into the superficial dorsal horn²²⁵ and pain-like responses elicited by the movement of hairs²²⁸. This heightened pain response is protective when maintained by peripheral mechanisms responding to inflammatory cues which subside over the course of healing. A major feature of neuropathic pain, however, is that it is a manifestation of maladaptive plasticity in the nervous system where changes to the nociceptive pathway enabling central sensitization do not return to pre-injury status²²⁹. Therefore, the somatosensory system is left in a persistent state where it can no longer distinguish innocuous information from nociceptive information.

In the context of neuropathic pain, the mechanisms responsible for the establishment and maintenance of central sensitization remain poorly understood. What seems clear is that multiple mechanisms are involved after nerve injury to increase excitability and reduce inhibition³. It has been shown that 13–25 days of sciatic nerve CCI produces changes in the synaptic excitation of LII neurons, where there is a decrease in excitatory synaptic drive to inhibitory, tonic cells and an increase in excitatory synaptic drive to putative excitatory neurons²²⁷. Further, these changes are concurrent with the onset of mechanical allodynia and hyperalgesia. One of the early consequences of CCI is the activation of spinal microglia and the release of BDNF²³⁰. Since nervous system injury is associated with elevated levels of BDNF²³¹ and neuropathic pain-related behaviours are attenuated by sequestering BDNF²³⁰, BDNF has become a molecule of interest in nerve injury related central sensitization. We recently found that spinal cord cultures exposed to five to six days of BDNF produced a similar ‘electrophysiological signature’ to that seen with CCI²³² and that activated microglia enhanced overall dorsal horn excitability through the release of BDNF²³³. Microglia-derived BDNF has also been shown to mediate nerve injury induced disinhibition through causing the collapse of the transmembrane anion gradient and compromising control over firing rate in LI neurons^{230,234}. In addition to reducing inhibitory tone in the dorsal horn, BDNF release has been associated with the enhancement of N-methyl-D-aspartate (NMDA) receptor mediated depolarizations in the rat spinal cord²³⁵, as well as the enhancement of NMDA receptor mediated excitatory post synaptic currents (epscs) in LII dorsal horn neurons²²⁴. It was further demonstrated that ATP and its release from damaged cells can activate microglia and, through P2X₄ purinergic receptor (P2X₄R) stimulation, promotes the release of BDNF²³⁶.

One might, therefore, suggest that peripheral nerve injury and the resultant chronic increase in primary afferent excitability increases the release of ATP from primary afferent terminals. This activates microglia, triggering the release of BDNF which promotes an increase in superficial dorsal horn excitability through a functional loss of inhibitory circuits and enhancement of synaptic strength at excitatory synapses. BDNF is, however, only one piece to a very complicated puzzle and several studies

have described contributions of other molecules, cell types and processes to the establishment and maintenance of central sensitization after nerve injury^{3,229,237}.

Nerve injury

Ectopic discharge in primary afferents is secondary to direct axonal damage and disruption of the myelin sheath that surrounds many axons²³⁸. Cutting an axon, results in degeneration of the distal segment as a consequence of interruption in axonal flow and transport which deprives the distal axon and nerve ending of its normal metabolic interaction with the cell body^{239,240}. Wallerian degeneration leads to loss of the distal axonal segment and involves responses from glial cells, immune cells, in addition to peripheral nerves. The proximal portion of damaged primary afferents can undergo phenotypic switch in response to retrograde loss of target-derived trophic factors. However, the electrical behaviour of both injured and uninjured nerve fibres is altered in response to injury^{195,241}. In parallel, the chemical environment is changed and several mediators are known to interact with sensory neurons²³⁹. Alterations in sensory neuron phenotype and electrical activity likely contribute to central sensitization and neuropathic pain.

Nerve degeneration

Nerve degeneration is not limited to nerve transections, but exists in other models of nerve injury²⁴², as well as in disease states and infections^{243,244}. In the case of axotomy, the proximal axonal segment and attached cell body becomes isolated from the distal segment²⁴⁰. Cytoplasmic materials build up as the ends of both segments become sealed, forming swollen retraction bulbs. In a process known as Wallerian degeneration, the distal axon swells and becomes a series of beaded fragments. Cellular debris from the terminal and distal axon is then cleared by phagocytic cells, such as macrophages. Although variable among species, Wallerian degeneration in rodent models of peripheral nerve injury occurs within a few days following injury^{245–247}. In contrast, the proximal segment is spared since it is still physically and metabolically coupled to the surviving cell body. During degeneration, retrograde signals produce changes in the cell body and include swelling, eccentric positioning of the nucleus and the breaking apart of rough endoplasmic reticulum (chromatolysis)²⁴⁸. These changes are associated with the production of protein required for nerve regeneration and cease once connections are restored. If the cell body dies, the degenerative process spreads to the remaining proximal segment. Thus, nerve degeneration occurs over several days, affects the entire neuron and involves several cell types.

Inflammation

Injury to peripheral nerves results in a local inflammatory response characterized by the activation of resident mast cells and macrophages, supportive Schwann cells along the axon and satellite cells in the DRG^{237,239,249}. In addition, the inflammatory response is augmented by the infiltration of circulating phagocytes (macrophages and neutrophils), T-lymphocytes and natural killer cells which contribute to the removal of cellular debris, neutralization of pathogens, regeneration of axons and

formation of a neuroma^{237,250}. Corresponding to these events, an 'inflammatory soup' of bradykinins, SP, hydrogen ions, NGF, prostaglandins, histamine, ATP and proinflammatory cytokines is produced¹⁸.

Several lines of evidence underline the importance of the local inflammatory response in the generation of centralized pain. For instance, cardinal signs of inflammation, such as the presence of edema, correlate more strongly with nociceptive behaviours than the extent of fibre loss after fixed-diameter polyethylene cuff nerve injury²⁴². Encasing the nerve stump after sciatic nerve transection, in order to minimize contact with infiltrating immune cells and inflammatory mediators, attenuates pain-related behaviours, such as autotomy²⁵¹. The local response of early inflammatory cellular mediators, including the degranulation of mast cells and the accumulation of neutrophils, is important in the generation of hyperalgesia after partial nerve injuries^{252,253}. Cui and colleagues demonstrated a strong correlation between degree of local macrophage/monocyte infiltration among three nerve injury models and the presence of mechanical allodynia²⁵⁴. Lastly, peripheral nerve injuries such as CCI are associated with an inflammatory response of higher magnitude than sciatic nerve transections²⁵⁴ and invoke downregulation of GABAergic functions in the superficial dorsal horn while axotomy is ineffective²⁵⁵. Similarly, CCI produces greater augmentation of excitatory synaptic transmission than sciatic nerve axotomy²⁵⁶.

Several inflammatory mediators released from damaged tissue alter the electrical properties of sensory neurons. For instance, hydrogen ions and ATP act through the non-selective cation channels TRPV1, ASICs and P2X to depolarize sensory neurons towards AP threshold²⁵⁷⁻²⁵⁹. *In vivo* administration of SP to glabrous skin produces hyperalgesia and allodynia^{260,261}. Further, SP release depolarizes and excites small nociceptive sensory neurons^{262,263} which express the neurokinin-1 (NK1) receptor²⁶⁴. Excitation of peptidergic fibres, not only leads to neuropeptide release centrally, but antidromic propagation of APs can also result in peripheral release and further exacerbate inflammation (neurogenic inflammation)^{17,265}. In support, NK-1 receptor antagonists applied either centrally^{266,267} or peripherally²⁶⁸, attenuate or delay the onset of pain-related behaviours in response to nerve injury.

The involvement of NGF in inflammatory pain is well documented, however, NGF expression is upregulated in several cell types, including DRG neurons²⁶⁹, Schwann cells²³⁷ and satellite cells²⁷⁰ after nerve injury. Consistent with a role in nerve injury and, perhaps, neuropathic pain, NGF antagonism, with anti-serum application at the site of nerve injury, attenuated or delayed the onset of hyperalgesia after CCI^{269,271}. NGF release can enhance excitability of primary afferents in several ways, including an increase in TRPV1 activity²⁷², sensitivity²⁷³ and expression²⁷⁴. In addition to the maintenance of TTX-R sodium currents (Na_v1.8) through Trk receptors⁴³, NGF signalling through the p75 neurotrophin receptor²⁷⁵, can increase AP firing and is concurrent with an enhancement of TTX-R sodium current and a suppression of delayed rectifier-like potassium current²⁷⁶.

Endogenous proteases, such as trypsin, activate tethered ligand protease-activated receptors 2 (PAR-2), a novel class of G-protein coupled receptors (GPCR), expressed on small-sized

nociceptive DRG neurons²⁷⁷. PAR-2 activation reduces M-current which results in membrane depolarization and the generation of APs^{278,279}. In addition and similar to the activation of metabotropic P2Y purinergic receptors^{280,281} and bradykinin-2 (B2) receptors²⁸², PAR-2 activation sensitizes TRP channels which are associated with hyperalgesia^{277,283}. Consistent with these observations, injection of PAR-2 agonists *in vivo* induces pain-related behaviours⁶⁶, whereas, antagonists produce antinociceptive effects²⁸⁴.

The association of eicosanoids and their synthesizing enzymes, cyclo-oxygenase-1 (COX-1) and particularly the inducible COX-2, with inflammation and pain is well documented^{285,286}. For instance, prostaglandin E₂ (PGE₂) or prostaglandin I₂ (PGI₂; prostacyclin) administration induces hyperalgesia^{287,288}. Further, PGI₂ and PGE₂ enhance the sensitivity of primary afferents to either mechanical or chemical stimulation²⁸⁹⁻²⁹¹. PGE₂ and PGI₂ receptors (EP and IP, respectively) are expressed in sensory neurons²⁹²⁻²⁹⁴ and *in vitro* application of PGE₂ or PGI₂ on DRG neurons lowers firing threshold²⁹⁵ and increases AP firing upon current injection, elevated potassium or bradykinin application^{296,297}. Consistent with hyperexcitability, PGE₂ and a PGI₂ analogue suppress a sustained-type potassium current²⁹⁷ and, similar to serotonin, prostaglandins upregulate TTX-R sodium currents²⁹⁸⁻³⁰¹. In addition, I_H is positively modulated by PGE₂ in trigeminal ganglion cells³⁰². Through a receptor mediated mechanism of action, PGE₂ and PGI₂ also sensitize TRPV1 channels, producing hyperalgesia in mice³⁰³. Last, it has also been suggested that hyperalgesia induced by bradykinin, norepinephrine and cytokines is secondary to the production of prostaglandins³⁰⁴.

The actions of several major pro-inflammatory cytokines, such as TNF- α and IL-6, are also associated with pain hypersensitivity and, perhaps, neuropathic pain^{305,306}. However, accumulating evidence implicates direct involvement of IL-1 β in the enduring increase in sensory neuron excitability commonly observed after peripheral nerve injury. For instance, mRNA³⁰⁷ and protein^{308,309} expression of the IL-1 β receptor, IL-1RI, in sensory neurons implies that IL-1 β can directly affect primary afferents. In agreement, changes in the electrical properties of sensory neurons consistent with increased excitability occur within minutes of IL-1 β application^{309,310}, as well as the onset of pain-related behaviours after intraplantar injections of IL-1 β ³⁰⁹. Importantly, IL-1 β expression³¹¹, secretion³¹² and processing³¹³ are upregulated for several days following peripheral nerve injury and we have recently reported that such long-term (five to six days) exposure to IL-1 β alters the excitability of DRG neurons³¹⁴. For instance, the effects of long-term IL-1 β exposure in medium and small, IB₄-positive DRG neurons parallel changes observed after nerve injury, such as a reduction in rheobase and increased repetitive discharge. Since changes in injured sensory neurons may lead to central sensitization, IL-1 β may be of particular importance in the establishment of neuropathic pain.

At this point, another issue relevant to inflammation and peripheral nerve should be mentioned. It is generally believed that inflammation not only protects injured tissue from infection, but that it may itself initiate the healing process³¹⁵⁻³¹⁷. For example, it has recently been suggested that injury-induced increase in interleukin-1 β and TNF- α protein levels is required

for functional nerve recovery³¹¹. This implies that attempts to attenuate neuropathic pain by preventing the actions of interleukin-1 β and TNF- α , may lead to impaired restoration of function in nerve injury situations.

Phenotypic switch

Although CGRP and SP expression appears to be downregulated in injured sensory neurons^{318,319}, respective receptor antagonists applied either centrally^{266,267,320} or peripherally²⁶⁸, attenuate or delay the onset of pain-related behaviours in response to nerve injury. This apparent paradox may be explained by phenotypic shifts and / or contributions from uninjured sensory neurons after nerve injury. For instance, some nerve injuries³²¹ result in a phenotypic shift where large, rather than small, sensory neurons begin to express^{322,323} and become responsive to SP²⁶³. Another phenotypic switch pertains to BDNF. Under normal circumstances, BDNF mRNA and protein is expressed in nociceptive sensory neurons³²⁴, however, BDNF becomes upregulated in large diameter DRG neurons and is concurrent with increased anterograde transport to the dorsal horn after sciatic axotomy³²⁵. It has, therefore, been suggested that an injury induced shift in the phenotype of sensory neurons may alter the efficacy of synaptic input into the spinal cord²³⁷.

CONCLUSIONS

There is now strong evidence linking the inflammatory milieu after nerve injury to changes in the electrical activity of sensory neurons and pain-related behaviours. The net effect is that primary afferents send volleys of spontaneous, ectopic discharge to the dorsal horn which can then trigger central sensitization. The association between this enduring increase in primary afferent activity after peripheral nerve injury and neuropathic pain is now well established.

These findings do not contradict the description of neuropathic pain as 'non-inflammatory pain' as the initial inflammatory trigger normally subsides by the time the patient presents. Although evidence is mounting to support the role of interleukin-1 β and other cytokines in the onset of neuropathic pain, drugs which antagonize or prevent the actions of these mediators may be of limited value in the clinic as the use of such agents would be analogous to 'closing the barn door after the horse has bolted'. It is already well known that nonsteroidal anti-inflammatory drugs are largely ineffective in the treatment of neuropathic pain. However, it is possible that attenuation of the actions of inflammatory mediators may impact the transition from acute to chronic pain. Alternatively, the identification of various types of voltage gated ion channels in the aetiology of neuropathic pain may be more relevant to the development of therapy, because their participation may outlast that of inflammatory mediators.

ACKNOWLEDGMENTS

The authors thank Dr. Nataliya Bukhanova-Schultz for help with the figure.

GRANTS

Supported by Canadian Institutes of Health Research. PLS was supported by an Alberta Heritage Foundation for Medical

Research (AHFMR) studentship and an AHFMR Dr. Lionel E. McLeod Health Research Scholarship.

REFERENCES

1. Merskey H, Bogduk N. Part III: Pain terms, a current list with definitions and notes on usage. In: Merskey H, Bogduk N, editors. Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms. Seattle: IASP Press; 1994. p. 209-14.
2. Wang H, Woolf CJ. Pain TRPs. *Neuron*. 2005;46(1):9-12.
3. Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. *J Pain*. 2009;10(9):895-926.
4. Cox JJ, Reimann F, Nicholas AK, et al. An SCN9A channelopathy causes congenital inability to experience pain. *Nature*. 2006;444(7121):894-8.
5. Lee MC, Mouraux A, Iannetti GD. Characterizing the cortical activity through which pain emerges from nociception. *J Neurosci*. 2009;29(24):7909-16.
6. Wall PD. Introduction to the fourth edition. In: Wall PD, Melzack R, editors. Textbook of pain. London: Harcourt Publishers Limited; 1999. p. 1-8.
7. Klit H, Finnerup NB, Jensen TS. Central post-stroke pain: clinical characteristics, pathophysiology, and management. *Lancet Neurol*. 2009;8(9):857-68.
8. Price DD. Psychological and neural mechanisms of the affective dimension of pain. *Science*. 2000;288(5472):1769-72.
9. McCaffery M, Pasero C. Assessment: underlying complexities, misconceptions, and practical tools. In: McCaffery M, Pasero C, editors. Pain: clinical manual. 2 ed. St. Louis: Mosby; 1999. p. 35-102.
10. Rainville P, Carrier B, Hofbauer RK, et al. Dissociation of sensory and affective dimensions of pain using hypnotic modulation. *Pain*. 1999;82(2):159-71.
11. Rainville P, Duncan GH, Price DD, et al. Pain affect encoded in human anterior cingulate but not somatosensory cortex. *Science*. 1997;277(5328):968-71.
12. Mogil JS. Animal models of pain: progress and challenges. *Nat Rev Neurosci*. 2009;10(4):283-94.
13. Hummel M, Lu P, Cummons TA, et al. The persistence of a long-term negative affective state following the induction of either acute or chronic pain. *Pain*. 2008;140(3):436-45.
14. Sandkuhler J. Models and mechanisms of hyperalgesia and allodynia. *Physiol Rev*. 2009;89(2):707-58.
15. Backonja MM, Stacey B. Neuropathic pain symptoms relative to overall pain rating. *J Pain*. 2004;5(9):491-7.
16. Mogil JS, Crager SE. What should we be measuring in behavioral studies of chronic pain in animals? *Pain*. 2004;112(1-2):12-15.
17. Richardson JD, Vasko MR. Cellular mechanisms of neurogenic inflammation. *J Pharmacol Exp Ther*. 2002;302(3):839-45.
18. Irving GA. Contemporary assessment and management of neuropathic pain. *Neurology*. 2005;64(12 Suppl 3):S21-7.
19. Aley KO, Messing RO, Mochly-Rosen D, et al. Chronic hypersensitivity for inflammatory nociceptor sensitization mediated by the epsilon isoform of protein kinase C. *J Neurosci*. 2000;20(12):4680-5.
20. Honore P, Rogers SD, Schwei MJ, et al. Murine models of inflammatory, neuropathic and cancer pain each generates a unique set of neurochemical changes in the spinal cord and sensory neurons. *Neuroscience*. 2000;98(3):585-98.
21. Perl ER. Function of dorsal root ganglion neurons: an overview. In: Sheryl A. Scott, editor. Sensory neurons diversity, development, and plasticity. New York: Oxford University Press; 1992. p. 3-23.
22. Gasser HS. The control of excitation in the nervous system. *Bull N Y Acad Med*. 1937;13(6):324-48.
23. Light AR, Perl ER. Unmyelinated afferent fibers are not only for pain anymore. *J Comp Neurol*. 2003;461(2):137-9.
24. Olausson H, Lamarre Y, Backlund H, et al. Unmyelinated tactile afferents signal touch and project to insular cortex. *Nat Neurosci*. 2002;5(9):900-4.

25. Harper AA, Lawson SN. Conduction velocity is related to morphological cell type in rat dorsal root ganglion neurones. *J Physiol.* 1985;359:31-46.
26. Abdulla FA, Smith PA. Axotomy- and autotomy-induced changes in the excitability of rat dorsal root ganglion neurons. *J Neurophysiol.* 2001;85(2):630-43.
27. Aoki Y, Takahashi Y, Ohtori S, et al. Distribution and immunocytochemical characterization of dorsal root ganglion neurons innervating the lumbar intervertebral disc in rats: a review. *Life Sci.* 2004;74(21):2627-42.
28. Silverman JD, Kruger L. Selective neuronal glycoconjugate expression in sensory and autonomic ganglia: relation of lectin reactivity to peptide and enzyme markers. *J Neurocytol.* 1990;19(5):789-801.
29. Bradbury EJ, Burnstock G, McMahon SB. The expression of P2X3 purinoreceptors in sensory neurons: effects of axotomy and glial-derived neurotrophic factor. *Mol Cell Neurosci.* 1998;12(4-5):256-68.
30. Lee Y, Takami K, Kawai Y, et al. Distribution of calcitonin gene-related peptide in the rat peripheral nervous system with reference to its coexistence with substance P. *Neuroscience.* 1985;15(4):1227-37.
31. Lee Y, Kawai Y, Shiosaka S, et al. Coexistence of calcitonin gene-related peptide and substance P-like peptide in single cells of the trigeminal ganglion of the rat: immunohistochemical analysis. *Brain Res.* 1985;330(1):194-6.
32. Hokfelt T, Elde R, Johansson O, et al. Immunohistochemical evidence for separate populations of somatostatin-containing and substance P-containing primary afferent neurons in the rat. *Neuroscience.* 1976;1(2):131-6.
33. Caterina MJ, Schumacher MA, Tominaga M, et al. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature.* 1997;389(6653):816-24.
34. Guo A, Vulchanova L, Wang J, et al. Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the P2X3 purinoreceptor and IB4 binding sites. *Eur J Neurosci.* 1999;11(3):946-58.
35. Aoki YM, Ohtori SM, Takahashi KM, et al. Expression and co-expression of VR1, CGRP, and IB4-binding glycoprotein in dorsal root ganglion neurons in rats: differences between the disc afferents and the cutaneous afferents. *Spine.* 2005;30(13):1496-500.
36. Bennett DLH, Michael GJ, Ramachandran N, et al. A distinct subgroup of small DRG cells express GDNF receptor components and GDNF is protective for these neurons after nerve injury. *J Neurosci.* 1998;18(8):3059-72.
37. Bennett DL, Averill S, Clary DO, et al. Postnatal changes in the expression of the trkA high-affinity NGF receptor in primary sensory neurons. *Eur J Neurosci.* 1996;8(10):2204-8.
38. Stephens HE, Belliveau AC, Gupta JS, et al. The role of neurotrophins in the maintenance of the spinal cord motor neurons and the dorsal root ganglia proprioceptive sensory neurons. *Int J Dev Neurosci.* 2005;23(7):613-20.
39. Lindsay RM. The role of neurotrophic factors in functional maintenance of mature sensory neurons. In: Scott SA, editor. *Sensory neurons diversity, development, and plasticity.* New York; 1992. p. 404-20.
40. Averill S, McMahon SB, Clary DO, et al. Immunocytochemical localization of trkA receptors in chemically identified subgroups of adult rat sensory neurons. *Eur J Neurosci.* 1995;7(7):1484-94.
41. Molliver DC, Wright DE, Leitner ML, et al. IB4-binding DRG neurons switch from NGF to GDNF dependence in early postnatal life. *Neuron.* 1997;19(4):849-61.
42. Airaksinen MS, Saarma M. The GDNF family: signalling, biological functions and therapeutic value. *Nat Rev Neurosci.* 2002;3(5):383-94.
43. Fjell J, Cummins TR, Dib-Hajj SD, et al. Differential role of GDNF and NGF in the maintenance of two TTX-resistant sodium channels in adult DRG neurons. *Brain Res Mol Brain Res.* 1999; 67(2):267-82.
44. Snider WD, Wright DE. Neurotrophins cause a new sensation. *Neuron.* 1996;16(2):229-32.
45. Luo W, Wickramasinghe SR, Savitt JM, et al. A hierarchical NGF signaling cascade controls Ret-dependent and Ret-independent events during development of nonpeptidergic DRG neurons. *Neuron.* 2007;54(5):739-54.
46. Valdés-Sánchez KM, Pérez-Villalba A, Vega JA, et al. BDNF is essentially required for the early postnatal survival of nociceptors. *Dev Biol.* 2010;339(2):465-76.
47. McMahon SB, Armanini MP, Ling LH, et al. Expression and coexpression of Trk receptors in subpopulations of adult primary sensory neurons projecting to identified peripheral targets. *Neuron.* 1994;12(5):1161-71.
48. Wright DE, Snider WD. Neurotrophin receptor mRNA expression defines distinct populations of neurons in rat dorsal root ganglia. *J Comp Neurol.* 1995;351(3):329-38.
49. Airaksinen MS, Koltzenburg M, Lewin GR, et al. Specific subtypes of cutaneous mechanoreceptors require neurotrophin-3 following peripheral target innervation. *Neuron.* 1996;16(2): 287-95.
50. Lumpkin EA, Caterina MJ. Mechanisms of sensory transduction in the skin. *Nature.* 2007;445(7130):858-65.
51. Voets T, Droogmans G, Wissenbach U, et al. The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. *Nature.* 2004;430(7001):748-54.
52. Dhaka A, Viswanath V, Patapoutian A. TRP ion channels and temperature sensation. *Annu Rev Neurosci.* 2006;29(1):135-61.
53. Ramsey IS, Delling M, Clapham DE. An introduction to TRP channels. *Annu Rev Physiol.* 2006;68:619-47.
54. Schepers RJ, Ringkamp M. Thermoreceptors and thermosensitive afferents. *Neurosci Biobehav Rev.* 2009;33(3):205-12.
55. Caterina MJ, Julius D. The vanilloid receptor: a molecular gateway to the pain pathway. *Annu Rev Neurosci.* 2001;24:487-517.
56. Bandell M, Story GM, Hwang SW, et al. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron.* 2004;41(6):849-57.
57. Peier AM, Moqrich A, Hergarden AC, et al. A TRP channel that senses cold stimuli and menthol. *Cell.* 2002;108(5):705-15.
58. Kwan KY, Allchorne AJ, Vollrath MA, et al. TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron.* 2006;50(2):277-89.
59. Suzuki M, Mizuno A, Kodaira K, et al. Impaired pressure sensation in mice lacking TRPV4. *J Biol Chem.* 2003;278(25):22664-8.
60. Alessandri-Haber N, Joseph E, Dina OA, et al. TRPV4 mediates pain-related behavior induced by mild hypertonic stimuli in the presence of inflammatory mediator. *Pain.* 2005;118(1-2):70-9.
61. Alloui A, Zimmermann K, Mamet J, et al. TREK-1, a K⁺ channel involved in polymodal pain perception. *EMBO J.* 2006;25(11): 2368-76.
62. Maingret F, Lauritzen I, Patel AJ, et al. TREK-1 is a heat-activated background K⁺ channel. *EMBO J.* 2000;19(11):2483-91.
63. Patel AJ, Honore E, Maingret F, et al. A mammalian two pore domain mechano-gated S-like K⁺ channel. *EMBO J.* 1998;17(15):4283-90.
64. Lumpkin EA, Bautista DM. Feeling the pressure in mammalian somatosensation. *Curr Opin Neurobiol.* 2005;15(4):382-8.
65. Morris CE, Juranka PF. Nav channel mechanosensitivity: activation and inactivation accelerate reversibly with stretch. *Biophys J.* 2007;93(3):822-33.
66. Vergnolle N, Bunnett NW, Sharkey KA, et al. Proteinase-activated receptor-2 and hyperalgesia: a novel pain pathway. *Nat Med.* 2001;7(7):821-6.
67. Kruger L, Perl ER, Sedvec MJ. Fine structure of myelinated mechanical nociceptor endings in cat hairy skin. *J Comp Neurol.* 1981;198(1):137-54.
68. Burgess PR, Perl ER. Myelinated afferent fibres responding specifically to noxious stimulation of the skin. *J Physiol.* 1967; 190(3):541-62.
69. Bessou P, Perl ER. Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli. *J Neurophysiol.* 1969;32(6):1025-43.
70. Handwerker HO, Kilo S, Reeh PW. Unresponsive afferent nerve fibres in the sural nerve of the rat. *J Physiol.* 1991;435:229-42.
71. Michaelis M, Habler HJ, Jaenig W. Silent afferents: a separate class of primary afferents? *Clin Exp Pharmacol Physiol.* 1996;23(2): 99-105.

72. Munger BL, Ide C. The structure and function of cutaneous sensory receptors. *Arch Histol Cytol.* 1988;51(1):1-34.
73. Suzuki M, Watanabe Y, Oyama Y, et al. Localization of mechanosensitive channel TRPV4 in mouse skin. *Neurosci Lett.* 2003;353(3):189-92.
74. Alvarez FJ, Fyffe RE. Nociceptors for the 21st century. *Curr Rev Pain.* 2000;4(6):451-8.
75. Denda M, Nakatani M, Ikeyama K, et al. Epidermal keratinocytes as the forefront of the sensory system. *Exp Dermatol.* 2007;16(3):157-61.
76. González-Martínez T, Germanà, P, Monjil DF, et al. Absence of Meissner corpuscles in the digital pads of mice lacking functional TrkB. *Brain Res.* 2004;1002(1-2):120-8.
77. Johnson KO. The roles and functions of cutaneous mechanoreceptors. *Curr Opin Neurobiol.* 2001;11(4):455-61.
78. Brodal P. Peripheral parts of the somatosensory system. In: Brodal P, editor. *The central nervous system structure and function.* New York: Oxford University Press; 2010. p. 165-89.
79. Boulais N, Misery L. Merkel cells. *J Am Acad Dermatol.* 2007;57(1):147-65.
80. Matsuda Y, Yoshida S, Yonezawa T. Tetrodotoxin sensitivity and Ca component of action potentials of mouse dorsal root ganglion cells cultured in vitro. *Brain Res.* 1978;154(1):69-82.
81. Yoshida S, Matsuda Y, Samejima A. Tetrodotoxin-resistant sodium and calcium components of action potentials in dorsal root ganglion cells of the adult mouse. *J Neurophysiol.* 1978;41(5):1096-106.
82. Koerber HR, Druzinsky RE, Mendell LM. Properties of somata of spinal dorsal root ganglion cells differ according to peripheral receptor innervated. *J Neurophysiol.* 1988;60(5):1584-96.
83. Harper AA, Lawson SN. Electrical properties of rat dorsal root ganglion neurones with different peripheral nerve conduction velocities. *J Physiol.* 1985;359:47-63.
84. Gurtu S, Smith PA. Electrophysiological characteristics of hamster dorsal root ganglion cells and their response to axotomy. *J Neurophysiol.* 1988;59(2):408-23.
85. Nicholls JG, Baylor DA. Specific modalities and receptive fields of sensory neurons in CNS of the leech. *J Neurophysiol.* 1968;31(5):740-56.
86. Rose RD, Koerber HR, Sedivec MJ, et al. Somal action potential duration differs in identified primary afferents. *Neurosci Lett.* 1986;63(3):259-64.
87. Koerber HR, Mendell LM. Functional heterogeneity of dorsal root ganglion cells. In: Scott SA, editor. *Sensory neurons diversity, development, and plasticity.* New York: Oxford University Press; 1992. p. 77-96.
88. Djouhri L, Bleazard L, Lawson SN. Association of somatic action potential shape with sensory receptive properties in guinea-pig dorsal root ganglion neurones. *J Physiol.* 1998;513(Pt 3):857-72.
89. Fang X, Djouhri L, McMullan S, et al. Intense isolectin-B4 binding in rat dorsal root ganglion neurons distinguishes C-fiber nociceptors with broad action potentials and high Nav1.9 expression. *J Neurosci.* 2006;26(27):7281-92.
90. Traub RJ, Mendell LM. The spinal projection of individual identified A-delta- and C-fibers. *J Neurophysiol.* 1988;59(1):41-55.
91. Nowycky MC. Voltage-gated ion channels in dorsal root ganglion neurons. In: Scott SA, editor. *Sensory neurons diversity, development, and plasticity.* New York: Oxford University Press; 1992. p. 97-115.
92. Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron.* 2000;26(1):13-25.
93. Beneski DA, Catterall WA. Covalent labeling of protein components of the sodium channel with a photoactivable derivative of scorpion toxin. *PNAS.* 1980;77(1):639-43.
94. Catterall WA, Goldin AL, Waxman SG. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. *Pharmacol Rev.* 2005;57(4):397-409.
95. Terlau H, Heinemann SH, Stühmer W, et al. Mapping the site of block by tetrodotoxin and saxitoxin of sodium channel II. *FEBS Lett.* 1991;293(1-2):93-6.
96. Caffrey JM, Eng DL, Black JA, et al. Three types of sodium channels in adult rat dorsal root ganglion neurons. *Brain Res.* 1992;592(1-2):283-97.
97. Rizzo MA, Kocsis JD, Waxman SG. Slow sodium conductances of dorsal root ganglion neurons: intraneuronal homogeneity and interneuronal heterogeneity. *J Neurophysiol.* 1994;72(6):2796-815.
98. Akopian AN, Sivilotti L, Wood JN. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. *Nature.* 1996;379(6562):257-62.
99. Sangameswaran L, Delgado SG, Fish LM, et al. Structure and function of a novel voltage-gated, tetrodotoxin-resistant sodium channel specific to sensory neurons. *J Biol Chem.* 1996;271(11):5953-6.
100. Dib-Hajj SD, Tyrrell L, Black JA, et al. Na_v1.8, a novel voltage-gated Na channel, is expressed preferentially in peripheral sensory neurons and down-regulated after axotomy. *PNAS.* 1998;95(15):8963-8.
101. Rush AM, Cummins TR, Waxman SG. Multiple sodium channels and their roles in electrogenesis within dorsal root ganglion neurons. *J Physiol.* 2007;579(Pt 1):1-14.
102. Abdulla FA, Smith PA. Changes in Na⁺ channel currents of rat dorsal root ganglion neurons following axotomy and axotomy-induced autotomy. *J Neurophysiol.* 2002;88(5):2518-29.
103. Cummins TR, Dib-Hajj SD, Black JA, et al. A novel persistent tetrodotoxin-resistant sodium current in SNS-null and wild-type small primary sensory neurons. *J Neurosci.* 1999;19(24):43RC.
104. Gover TD, Moreira TH, Weinreich D. Role of calcium in regulating primary sensory neuronal excitability. In: Canning BJ, Spina D, editors. *Sensory nerves: handbook of experimental pharmacology.* Berlin Heidelberg: Springer; 2009. p. 563-87.
105. Catterall WA, Perez-Reyes E, Snutch TP, et al. International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. *Pharmacol Rev.* 2005;57(4):411-25.
106. Catterall WA. Structure and regulation of voltage-gated Ca²⁺ channels. *Annu Rev Cell Dev Biol.* 2000;16(1):521-55.
107. Scroggs RS, Fox AP. Calcium current variation between acutely isolated adult rat dorsal root ganglion neurons of different size. *J Physiol.* 1992;445:639-58.
108. Fuchs A, Rigaud M, Sarantopoulos CD, et al. Contribution of calcium channel subtypes to the intracellular calcium signal in sensory neurons: the effect of injury. *Anesthesiology.* 2007;107(1):117-27.
109. Acosta CG, Lopez HS. δ Opioid receptor modulation of several voltage-dependent Ca²⁺ currents in rat sensory neurons. *J Neurosci.* 1999;19(19):8337-48.
110. Schroeder JE, Fischbach PS, McCleskey EW. T-type calcium channels: heterogeneous expression in rat sensory neurons and selective modulation by phorbol esters. *J Neurosci.* 1990;10(3):947-51.
111. Lu SG, Zhang X, Gold MS. Intracellular calcium regulation among subpopulations of rat dorsal root ganglion neurons. *J Physiol.* 2006;577(1):169-90.
112. Abdulla FA, Smith PA. Axotomy- and autotomy-induced changes in Ca²⁺ and K⁺ channel currents of rat dorsal root ganglion neurons. *J Neurophysiol.* 2001;85(2):644-58.
113. Zamponi GW, Lewis RJ, Todorovic SM, et al. Role of voltage-gated calcium channels in ascending pain pathways. *Brain Res Rev.* 2009;60(1):84-9.
114. Todorovic SM, Jevtovic-Todorovic V. The role of T-type calcium channels in peripheral and central pain processing. *CNS Neurol Disord Drug Targets.* 2006;5(6):639-53.
115. Felix R, Gurnett CA, De Waard M, et al. Dissection of functional domains of the voltage-dependent Ca²⁺ channel $\alpha_2\delta$ subunit. *J Neurosci.* 1997;17(18):6884-91.
116. Newton RA, Bingham S, Case PC, et al. Dorsal root ganglion neurons show increased expression of the calcium channel [alpha]2[delta]-1 subunit following partial sciatic nerve injury. *Mol Brain Res.* 2001;95(1-2):1-8.
117. Luo ZD, Chaplan SR, Higuera ES, et al. Upregulation of dorsal root ganglion [alpha]2[delta] calcium channel subunit and its correlation with allodynia in spinal nerve-injured rats. *J Neurosci.* 2001;21(6):1868-75.

118. Boroujerdi A, Kim HK, Lyu YS, et al. Injury discharges regulate calcium channel α -2-delta-1 subunit upregulation in the dorsal horn that contributes to initiation of neuropathic pain. *Pain*. 2008;139(2):358-66.
119. Luo ZD, Calcutt NA, Higuera ES, et al. Injury type-specific calcium channel α 2 δ -1 subunit up-regulation in rat neuropathic pain models correlates with antiallodynic effects of gabapentin. *J Pharmacol Exp Ther*. 2002;303(3):1199-205.
120. Field MJ, Cox PJ, Stott E, et al. Identification of the α 2- δ -1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of pregabalin. *PNAS*. 2006;103(46):17537-42.
121. Marais E, Klugbauer N, Hofmann F. Calcium channel α 2 δ subunits—structure and gabapentin binding. *Mol Pharmacol*. 2001;59(5):1243-8.
122. Stefani A, Spadoni F, Bernardi G. Gabapentin inhibits calcium currents in isolated rat brain neurons. *Neuropharmacology*. 1998;37(1):83-91.
123. Gee NS, Brown JP, Dissanayake VUK, et al. The novel anticonvulsant drug, gabapentin (Neurontin), binds to the subunit of a calcium channel. *J Biol Chem*. 1996;271(10):5768-76.
124. Gutman GA, Chandy KG, Grissmer S, et al. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol Rev*. 2005;57(4):473-508.
125. Ocaña M, Cendán CM, Cobos EJ, et al. Potassium channels and pain: present realities and future opportunities. *Eur J Pharmacol*. 2004;500(1-3):203-19.
126. Bayliss DA, Barrett PQ. Emerging roles for two-pore-domain potassium channels and their potential therapeutic impact. *Trends Pharmacol Sci*. 2008;29(11):566-75.
127. Lesage F, Lazdunski M. Molecular and functional properties of two-pore-domain potassium channels. *Am J Physiol Renal Physiol*. 2000;279(5):F793-801.
128. Hibino H, Inanobe A, Furutani K, et al. Inwardly rectifying potassium channels: their structure, function, and physiological roles. *Physiol Rev*. 2010;90(1):291-366.
129. Pischalnikova A, Sokolova O. The domain and conformational organization in potassium voltage-gated ion channels. *J Neuroimmune Pharmacol*. 2009;4(1):71-82.
130. Ghatta S, Nimmagadda D, Xu X, et al. Large-conductance, calcium-activated potassium channels: structural and functional implications. *Pharmacol Ther*. 2006;110(1):103-16.
131. Kostyuk PG, Veselovsky NS, Fedulova SA, et al. Ionic currents in the somatic membrane of rat dorsal root ganglion neurons-III. Potassium currents. *Neuroscience*. 1981;6(12):2439-44.
132. Kameyama M. Ionic currents in cultured dorsal root ganglion cells from adult guinea pigs. *J Membr Biol*. 1983;72(3):195-203.
133. Kasai H, Kameyama M, Yamaguchi K, et al. Single transient K channels in mammalian sensory neurons. *Biophys J*. 1986;49(6):1243-7.
134. Penner R, Petersen M, Pierau FK, et al. Dendrotoxin: a selective blocker of a non-inactivating potassium current in guinea-pig dorsal root ganglion neurones. *Pflugers Arch*. 1986;407(4):365-9.
135. Stansfeld CE, Marsh SJ, Halliwell JV, et al. 4-Aminopyridine and dendrotoxin induce repetitive firing in rat visceral sensory neurones by blocking a slowly inactivating outward current. *Neurosci Lett*. 1986;64(3):299-304.
136. Stansfeld C, Feltz A. Dendrotoxin-sensitive K⁺ channels in dorsal root ganglion cells. *Neurosci Lett*. 1988;93(1):49-55.
137. McFarlane S, Cooper E. Kinetics and voltage dependence of A-type currents on neonatal rat sensory neurons. *J Neurophysiol*. 1991;66(4):1380-91.
138. Gold MS, Shuster MJ, Levine JD. Characterization of six voltage-gated K⁺ currents in adult rat sensory neurons. *J Neurophysiol*. 1996;75(6):2629-46.
139. Yoshida S, Matsumoto S. Effects of α -Dendrotoxin on K⁺ currents and action potentials in tetrodotoxin-resistant adult rat trigeminal ganglion neurons. *J Pharmacol Exp Ther*. 2005;314(1):437-45.
140. Everill B, Rizzo MA, Kocsis JD. Morphologically identified cutaneous afferent DRG neurons express three different potassium currents in varying proportions. *J Neurophysiol*. 1998;79(4):1814-24.
141. Vydyanathan A, Wu ZZ, Chen SR, et al. A-type voltage-gated K⁺ currents influence firing properties of isolectin B4-positive but not isolectin B4-negative primary sensory neurons. *J Neurophysiol*. 2005;93(6):3401-9.
142. Villiere V, McLachlan EM. Electrophysiological properties of neurons in intact rat dorsal root ganglia classified by conduction velocity and action potential duration. *J Neurophysiol*. 1996;76(3):1924-41.
143. Connor JA, Stevens CF. Voltage clamp studies of a transient outward membrane current in gastropod neural somata. *J Physiol*. 1971;213(1):21-30.
144. Catacuzzeno L, Fioretti B, Pietrobon D, et al. The differential expression of low-threshold K⁺ currents generates distinct firing patterns in different subtypes of adult mouse trigeminal ganglion neurones. *J Physiol*. 2008;586(Pt 21):5101-18.
145. Gold MS, Shuster MJ, Levine JD. Role of a Ca(2⁺)-dependent slow afterhyperpolarization in prostaglandin E2-induced sensitization of cultured rat sensory neurons. *Neurosci Lett*. 1996;205(3):161-4.
146. Scholz A, Gruss M, Vogel W. Properties and functions of calcium-activated K⁺ channels in small neurones of rat dorsal root ganglion studied in a thin slice preparation. *J Physiol*. 1998;513(Pt 1):55-69.
147. Zhang XL, Mok LP, Katz EJ, et al. BKCa currents are enriched in a subpopulation of adult rat cutaneous nociceptive dorsal root ganglion neurons. *Eur J Neurosci*. 2010;31(3):450-62.
148. Christian EP, Togo J, Naper KE. Guinea pig visceral C-fiber neurons are diverse with respect to the K⁺ currents involved in action-potential repolarization. *J Neurophysiol*. 1994;71(2):561-74.
149. Morita K, Katayama Y. Calcium-dependent slow outward current in visceral primary afferent neurones of the rabbit. *Pflugers Arch*. 1989;414(2):171-7.
150. Bahia PK, Suzuki R, Benton DC, et al. A functional role for small-conductance calcium-activated potassium channels in sensory pathways including nociceptive processes. *J Neurosci*. 2005;25(14):3489-98.
151. Kaupp UB, Seifert R. Molecular diversity of pacemaker ion channels. *Annu Rev Physiol*. 2001;63:235-57.
152. Santoro B, Liu DT, Yao H, et al. Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain. *Cell*. 1998;93(5):717-29.
153. Ulens C, Tytgat J. Functional heteromerization of HCN1 and HCN2 pacemaker channels. *J Biol Chem*. 2001;276(9):6069-72.
154. Orio P, Madrid R, de la Peña E, et al. Characteristics and physiological role of hyperpolarization activated currents in mouse cold thermoreceptors. *J Physiol*. 2009;587(9):1961-76.
155. Mayer ML, Westbrook GL. A voltage-clamp analysis of inward (anomalous) rectification in mouse spinal sensory ganglion neurones. *J Physiol*. 1983;340:19-45.
156. Scroggs RS, Todorovic SM, Anderson EG, et al. Variation in IH, IIR, and ILEAK between acutely isolated adult rat dorsal root ganglion neurons of different size. *J Neurophysiol*. 1994;71(1):271-9.
157. Pape HC. Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Annu Rev Physiol*. 1996;58:299-327.
158. Cho HJ, Staikopoulos V, Ivanusic J, et al. Hyperpolarization-activated cyclic-nucleotide gated 4 (HCN4) protein is expressed in a subset of rat dorsal root and trigeminal ganglion neurons. *Cell Tissue Res*. 2009;338(2):171-7.
159. Jiang YQ, Xing GG, Wang SL, et al. Axonal accumulation of hyperpolarization-activated cyclic nucleotide-gated cation channels contributes to mechanical allodynia after peripheral nerve injury in rat. *Pain*. 2008;137(3):495-506.
160. Tu H, Deng L, Sun Q, et al. Hyperpolarization-activated, cyclic nucleotide-gated cation channels: roles in the differential electrophysiological properties of rat primary afferent neurons. *J Neurosci Res*. 2004;76(5):713-22.

161. Kouranova EV, Strassle BW, Ring RH, et al. Hyperpolarization-activated cyclic nucleotide-gated channel mRNA and protein expression in large versus small diameter dorsal root ganglion neurons: correlation with hyperpolarization-activated current gating. *Neuroscience*. 2008;153(4):1008-19.
162. Momin A, Cadiou H, Mason A, et al. Role of the hyperpolarization-activated current Ih in somatosensory neurons. *J Physiol*. 2008;586(Pt 24):5911-29.
163. Viana F, Belmonte C. Funny currents are becoming serious players in nociceptor's sensitization. *J Physiol*. 2008;586(Pt 24):5841-2.
164. Doubell TP, Mannion JR, Woolf CJ. The dorsal horn: state-dependent sensory processing, plasticity and the generation of pain. In: Wall PD, Melzack R, editors. *Textbook of pain*. London: Harcourt Publishers Limited; 1999. p. 165-81.
165. Rexed B. A cytoarchitectonic atlas of the spinal cord in the cat. *J Comp Neurol*. 1954;100(2):297-379.
166. Rexed B. The cytoarchitectonic organization of the spinal cord in the cat. *J Comp Neurol*. 1952;96(3):414-95.
167. Brown AG, Rose PK, Snow PJ. The morphology of hair follicle afferent fibre collaterals in the spinal cord of the cat. *J Physiol*. 1977;272(3):779-97.
168. Brown AG, Rose PK, Snow PJ. Morphology and organization of axon collaterals from afferent fibres of slowly adapting type I units in cat spinal cord. *J Physiol*. 1978;277:15-27.
169. Brown AG, Fyffe RE, Noble R. Projections from Pacinian corpuscles and rapidly adapting mechanoreceptors of glabrous skin to the cat's spinal cord. *J Physiol*. 1980;307:385-400.
170. Brown AG, Fyffe RE, Rose PK, et al. Spinal cord collaterals from axons of type II slowly adapting units in the cat. *J Physiol*. 1981;316:469-80.
171. Woolf CJ. Central terminations of cutaneous mechanoreceptive afferents in the rat lumbar spinal cord. *J Comp Neurol*. 1987;261(1):105-19.
172. Light AR, Trevino DL, Perl ER. Morphological features of functionally defined neurons in the marginal zone and substantia gelatinosa of the spinal dorsal horn. *J Comp Neurol*. 1979;186(2):151-71.
173. Sugiura Y, Terui N, Hosoya Y. Difference in distribution of central terminals between visceral and somatic unmyelinated (C) primary afferent fibers. *J Neurophysiol*. 1989;62(4):834-40.
174. Traub RJ, Solodkin A, Ruda MA. Calcitonin gene-related peptide immunoreactivity in the cat lumbosacral spinal cord and the effects of multiple dorsal rhizotomies. *J Comp Neurol*. 1989;287(2):225-37.
175. Ribeiro-da-Silva A, Cuello AC. Organization of peptidergic neurons in the dorsal horn of the spinal cord: anatomical and functional correlates. *Prog Brain Res*. 1995;104:41-59.
176. Light AR, Perl ER. Spinal termination of functionally identified primary afferent neurons with slowly conducting myelinated fibers. *J Comp Neurol*. 1979;186(2):133-50.
177. Woolf CJ. Evidence for a central component of post-injury pain hypersensitivity. *Nature*. 1983;306(5944):686-8.
178. Govrin-Lippmann R, Devor M. Ongoing activity in severed nerves: source and variation with time. *Brain Res*. 1978;159(2):406-10.
179. Wall PD, Devor M. Sensory afferent impulses originate from dorsal root ganglia as well as from the periphery in normal and nerve injured rats. *Pain*. 1983;17(4):321-39.
180. Kajander KC, Wakisaka S, Bennett GJ. Spontaneous discharge originates in the dorsal root ganglion at the onset of a painful peripheral neuropathy in the rat. *Neurosci Lett*. 1992;138(2):225-8.
181. Kim YI, Na HS, Kim SH, et al. Cell type-specific changes of the membrane properties of peripherally-axotomized dorsal root ganglion neurons in a rat model of neuropathic pain. *Neuroscience*. 1998;86(1):301-9.
182. Zhang JM, Donnelly DF, Song XJ, et al. Axotomy increases the excitability of dorsal root ganglion cells with unmyelinated axons. *J Neurophysiol*. 1997;78(5):2790-4.
183. Study RE, Kral MG. Spontaneous action potential activity in isolated dorsal root ganglion neurons from rats with a painful neuropathy. *Pain*. 1996;65(2-3):235-42.
184. Ma C, LaMotte RH. Enhanced excitability of dissociated primary sensory neurons after chronic compression of the dorsal root ganglion in the rat. *Pain*. 2005;113(1-2):106-12.
185. Everill B, Cummins TR, Waxman SG, et al. Sodium currents of large (A β -type) adult cutaneous afferent dorsal root ganglion neurons display rapid recovery from inactivation before and after axotomy. *Neuroscience*. 2001;106(1):161-9.
186. Cummins TR, Waxman SG. Downregulation of tetrodotoxin-resistant sodium currents and upregulation of a rapidly repriming tetrodotoxin-sensitive sodium current in small spinal sensory neurons after nerve injury. *J Neurosci*. 1997;17(10):3503-14.
187. Cummins TR, Aglieco F, Renganathan M, et al. Nav1.3 sodium channels: rapid repriming and slow closed-state inactivation display quantitative differences after expression in a mammalian cell line and in spinal sensory neurons. *J Neurosci*. 2001;21(16):5952-61.
188. Waxman SG, Kocsis JD, Black JA. Type III sodium channel mRNA is expressed in embryonic but not adult spinal sensory neurons, and is reexpressed following axotomy. *J Neurophysiol*. 1994;72(1):466-70.
189. Dib-Hajj S, Black JA, Felts P, et al. Down-regulation of transcripts for Na channel α -SNS in spinal sensory neurons following axotomy. *PNAS*. 1996;93(25):14950-4.
190. Black JA, Cummins TR, Plumpton C, et al. Upregulation of a silent sodium channel after peripheral, but not central, nerve injury in DRG neurons. *J Neurophysiol*. 1999;82(5):2776-85.
191. Dib-Hajj SD, Fjell J, Cummins TR, et al. Plasticity of sodium channel expression in DRG neurons in the chronic constriction injury model of neuropathic pain. *Pain*. 1999;83(3):591-600.
192. Kim CH, Oh Y, Chung JM, et al. The changes in expression of three subtypes of TTX sensitive sodium channels in sensory neurons after spinal nerve ligation. *Brain Res Mol Brain Res*. 2001;95(1-2):153-61.
193. Dib-Hajj SD, Black JA, Cummins TR, et al. Rescue of alpha -SNS sodium channel expression in small dorsal root ganglion neurons after axotomy by nerve growth factor in vivo. *J Neurophysiol*. 1998;79(5):2668-76.
194. Boucher TJ, Okuse K, Bennett DL, et al. Potent analgesic effects of GDNF in neuropathic pain states. *Science*. 2000;290(5489):124-7.
195. Gold MS, Weinreich D, Kim CS, et al. Redistribution of Na(V)1.8 in uninjured axons enables neuropathic pain. *J Neurosci*. 2003;23(1):158-66.
196. Hildebrand ME, Smith PL, Bladen C, et al. A novel slow-inactivation-specific ion channel modulator attenuates neuropathic pain. *Pain*. 2011;152(4):833-43.
197. Everill B, Kocsis JD. Reduction in potassium currents in identified cutaneous afferent dorsal root ganglion neurons after axotomy. *J Neurophysiol*. 1999;82(2):700-8.
198. Kim DS, Choi JO, Rim HD, et al. Downregulation of voltage-gated potassium channel [alpha] gene expression in dorsal root ganglia following chronic constriction injury of the rat sciatic nerve. *Mol Brain Res*. 2002;105(1-2):146-52.
199. Sarantopoulos CD, McCallum JB, Rigaud M, et al. Opposing effects of spinal nerve ligation on calcium-activated potassium currents in axotomized and adjacent mammalian primary afferent neurons. *Brain Res*. 2007;1132(1):84-99.
200. Boettger MK, Till S, Chen MX, et al. Calcium-activated potassium channel SK1- and IK1-like immunoreactivity in injured human sensory neurons and its regulation by neurotrophic factors. *Brain*. 2002;125(2):252-63.
201. Baccei ML, Kocsis JD. Voltage-gated calcium currents in axotomized adult rat cutaneous afferent neurons. *J Neurophysiol*. 2000;83(4):2227-38.
202. Jagodic MM, Pathirathna S, Jokovic PM, et al. Upregulation of the T-type calcium current in small rat sensory neurons after chronic constrictive injury of the sciatic nerve. *J Neurophysiol*. 2008;99(6):3151-6.
203. Jagodic MM, Pathirathna S, Nelson MT, et al. Cell-specific alterations of T-type calcium current in painful diabetic neuropathy enhance excitability of sensory neurons. *J Neurosci*. 2007;27(12):3305-16.

204. Titmus MJ, Faber DS. Axotomy-induced alterations in the electrophysiological characteristics of neurons. *Prog Neurobiol.* 1990;35(1):1-51.
205. Charles P. Mechanisms of analgesia by gabapentin and pregabalin – calcium channel $\alpha_2\text{-}\delta$ [Cav $\alpha_2\text{-}\delta$] ligands. *Pain.* 2009;142(1-2):13-16.
206. Schmidtke A, Lötsch J, Freynhagen R, et al. Ziconotide for treatment of severe chronic pain. *Lancet.* 2010;375(9725):1569-77.
207. Abdulla FA, Smith PA. Axotomy reduces the effect of analgesic opioids yet increases the effect of nociceptin on dorsal root ganglion neurons. *J Neurosci.* 1998;18(23):9685-94.
208. Hori Y, Endo K, Takahashi T. Presynaptic inhibitory action of enkephalin on excitatory transmission in superficial dorsal horn of rat spinal cord. *J Physiol.* 1992;450(1):673-85.
209. Choe W, Messinger RB, Leach E, et al. TTA-P2 is a potent and selective blocker of T-type calcium channels in rat sensory neurons and a novel antinociceptive agent. *Mol Pharmacol.* 2011;80(5):900-10.
210. Todorovic SM, Jevtovic-Todorovic V. T-type voltage-gated calcium channels as targets for the development of novel pain therapies. *Br J Pharmacol.* 2011;163(3):484-95.
211. Yao H, Donnelly DF, Ma C, et al. Upregulation of the hyperpolarization-activated cation current after chronic compression of the dorsal root ganglion. *J Neurosci.* 2003;23(6):2069-74.
212. Chaplan SR, Guo HQ, Lee DH, et al. Neuronal hyperpolarization-activated pacemaker channels drive neuropathic pain. *J Neurosci.* 2003;23(4):1169-78.
213. Emery EC, Young GT, Berrocso EM, et al. HCN2 ion channels play a central role in inflammatory and neuropathic pain. *Science.* 2011;333(6048):1462-6.
214. Takasu K, Ono H, Tanabe M. Spinal hyperpolarization-activated cyclic nucleotide-gated cation channels at primary afferent terminals contribute to chronic pain. *Pain.* 2010;151(1):87-96.
215. McLachlan EM, Janig W, Devor M, et al. Peripheral nerve injury triggers noradrenergic sprouting within dorsal root ganglia. *Nature.* 1993;363(6429):543-6.
216. Devor M, Janig W, Michaelis M. Modulation of activity in dorsal root ganglion neurons by sympathetic activation in nerve-injured rats. *J Neurophysiol.* 1994;71(1):38-47.
217. Sato J, Perl ER. Adrenergic excitation of cutaneous pain receptors induced by peripheral nerve injury. *Science.* 1991;251(5001):1608-10.
218. Petersen M, Zhang J, Zhang JM, et al. Abnormal spontaneous activity and responses to norepinephrine in dissociated dorsal root ganglion cells after chronic nerve constriction. *Pain.* 1996;67(2-3):391-7.
219. Birder LA, Perl ER. Expression of α_2 -adrenergic receptors in rat primary afferent neurones after peripheral nerve injury or inflammation. *J Physiol.* 1999;515(2):533-42.
220. Abdulla FA, Smith PA. Ectopic α_2 -adrenoceptors couple to N-type Ca^{2+} channels in axotomized rat sensory neurons. *J Neurosci.* 1997;17(5):1633-41.
221. Amir R, Devor M. Functional cross-excitation between afferent A- and C-neurons in dorsal root ganglia. *Neuroscience.* 1999;95(1):189-95.
222. Dalal A, Tata M, Allegre G, et al. Spontaneous activity of rat dorsal horn cells in spinal segments of sciatic projection following transection of sciatic nerve or of corresponding dorsal roots. *Neuroscience.* 1999;94(1):217-28.
223. Woolf CJ, Thompson SW. The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. *Pain.* 1991;44(3):293-9.
224. Garraway SM, Petruska JC, Mendell LM. BDNF sensitizes the response of lamina II neurons to high threshold primary afferent inputs. *Eur J Neurosci.* 2003;18(9):2467-76.
225. Baba H, Ji RR, Kohno T, et al. Removal of GABAergic inhibition facilitates polysynaptic A fiber-mediated excitatory transmission to the superficial spinal dorsal horn. *Mol Cell Neurosci.* 2003;24(3):818-30.
226. Coull JA, Boudreau D, Bachand K, et al. Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature.* 2003;424(6951):938-42.
227. Balasubramanian S, Stemkowski PL, Stebbing MJ, et al. Sciatic chronic constriction injury produces cell-type-specific changes in the electrophysiological properties of rat substantia gelatinosa neurons. *J Neurophysiol.* 2006;96(2):579-90.
228. Sivilotti L, Woolf CJ. The contribution of GABAA and glycine receptors to central sensitization: disinhibition and touch-evoked allodynia in the spinal cord. *J Neurophysiol.* 1994;72(1):169-79.
229. Costigan M, Scholz J, Woolf CJ. Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu Rev Neurosci.* 2009;32:1-32.
230. Coull JA, Beggs S, Boudreau D, et al. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature.* 2005;438(7070):1017-21.
231. Dougherty KD, Dreyfus CF, Black IB. Brain-derived neurotrophic factor in astrocytes, oligodendrocytes, and microglia/macrophages after spinal cord injury. *Neurobiol Dis.* 2000;7(6 Pt B):574-85.
232. Lu VB, Ballanyi K, Colmers WF, et al. Neuron type-specific effects of brain-derived neurotrophic factor in rat superficial dorsal horn and their relevance to 'central sensitization'. *J Physiol.* 2007;584(Pt 2):543-63.
233. Lu VB, Biggs JE, Stebbing MJ, et al. Brain-derived neurotrophic factor drives the changes in excitatory synaptic transmission in the rat superficial dorsal horn that follow sciatic nerve injury. *J Physiol.* 2009;587(Pt 5):1013-32.
234. Prescott SA, Sejnowski TJ, De Koninck Y. Reduction of anion reversal potential subverts the inhibitory control of firing rate in spinal lamina I neurons: towards a biophysical basis for neuropathic pain. *Mol Pain.* 2006;2:32.
235. Kerr BJ, Bradbury EJ, Bennett DL, et al. Brain-derived neurotrophic factor modulates nociceptive sensory inputs and NMDA-evoked responses in the rat spinal cord. *J Neurosci.* 1999;19(12):5138-48.
236. Ulmann L, Hatcher JP, Hughes JP, et al. Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. *J Neurosci.* 2008;28(44):11263-8.
237. Scholz J, Woolf CJ. The neuropathic pain triad: neurons, immune cells and glia. *Nat Neurosci.* 2007;10(11):1361-8.
238. Devor M. Ectopic discharge in A beta afferents as a source of neuropathic pain. *Exp Brain Res.* 2009;196(1):115-28.
239. Gaudet A, Popovich P, Ramer M. Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. *J Neuroinflammation.* 2011;8(1):110.
240. Kelley J. Reactions of neurons to injury. In: Kandel ER, Schwartz JH, editors. *Principles of neural science.* New York: Elsevier Science Publishing Co., Inc. 1985. p. 187-95.
241. Ma C, Shu Y, Zheng Z, et al. Similar electrophysiological changes in axotomized and neighboring intact dorsal root ganglion neurons. *J Neurophysiol.* 2003;89(3):1588-602.
242. Mosconi T, Kruger L. Fixed-diameter polyethylene cuffs applied to the rat sciatic nerve induce a painful neuropathy: ultrastructural morphometric analysis of axonal alterations. *Pain.* 1996;64(1):37-57.
243. Jones GJ, Barsby NL, Cohen EA, et al. HIV-1 Vpr causes neuronal apoptosis and in vivo neurodegeneration. *J Neurosci.* 2007;27(14):3703-11.
244. Acharjee S, Noorbakhsh F, Stemkowski PL, et al. HIV-1 viral protein R causes peripheral nervous system injury associated with in vivo neuropathic pain. *FASEB J.* 2010;24(11):4343-53.
245. Tsao JW, George EB, Griffin JW. Temperature modulation reveals three distinct stages of Wallerian degeneration. *J Neurosci.* 1999;19(12):4718-26.
246. Luttges MW, Kelly PT, Gerren RA. Degenerative changes in mouse sciatic nerves: electrophoretic and electrophysiologic characterizations. *Exp Neurol.* 1976;50(3):706-33.
247. Perrin FE, Lacroix S, Aviles-Trigueros M, et al. Involvement of monocyte chemoattractant protein-1, macrophage inflammatory protein-1 α and interleukin-1 β in Wallerian degeneration. *Brain.* 2005;128(Pt 4):854-66.

248. Wells MR, Vaidya U. Morphological alterations in dorsal root ganglion neurons after peripheral axon injury: association with changes in metabolism. *Exp Neurol*. 1989;104(1):32-8.
249. Sorkin LS, Schafers M. Immune cells in peripheral nerve. In: DeLeo JA, Sorkin LS, Watkins LR, editors. *Immune and glia regulation of pain*. Seattle: IASP Press; 2007. p. 3-19.
250. Xie WR, Deng H, Li H, et al. Robust increase of cutaneous sensitivity, cytokine production and sympathetic sprouting in rats with localized inflammatory irritation of the spinal ganglia. *Neuroscience*. 2006;142(3):809-22.
251. Okuda T, Ishida O, Fujimoto Y, et al. The autotomy relief effect of a silicone tube covering the proximal nerve stump. *J Orthop Res*. 2006;24(7):1427-37.
252. Zuo Y, Perkins NM, Tracey DJ, et al. Inflammation and hyperalgesia induced by nerve injury in the rat: a key role of mast cells. *Pain*. 2003;105(3):467-79.
253. Perkins NM, Tracey DJ. Hyperalgesia due to nerve injury: role of neutrophils. *Neuroscience*. 2000;101(3):745-57.
254. Cui JG, Holmin S, Mathiesen T, et al. Possible role of inflammatory mediators in tactile hypersensitivity in rat models of mononeuropathy. *Pain*. 2000;88(3):239-48.
255. Moore KA, Kohno T, Karchewski LA, et al. Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord. *J Neurosci*. 2002;22(15):6724-31.
256. Chen Y, Balasubramanyan S, Lai AY, et al. Effects of sciatic nerve axotomy on excitatory synaptic transmission in rat substantia gelatinosa. *J Neurophysiol*. 2009;102(6):3203-15.
257. Tominaga M, Caterina MJ, Malmberg AB, et al. The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron*. 1998;21(3):531-43.
258. Chen CC, Akopian AN, Sivilotti L, et al. A P2X purinoceptor expressed by a subset of sensory neurons. *Nature*. 1995;377(6548):428-31.
259. Chen CC, England S, Akopian AN, et al. A sensory neuron-specific, proton-gated ion channel. *PNAS*. 1998;95(17):10240-5.
260. Nakamura-Craig M, Gill BK. Effect of neurokinin A, substance P and calcitonin gene related peptide in peripheral hyperalgesia in the rat paw. *Neurosci Lett*. 1991;124(1):49-51.
261. Carlton SM, Zhou S, Coggeshall RE. Localization and activation of substance P receptors in unmyelinated axons of rat glabrous skin. *Brain Res*. 1996;734(1-2):103-8.
262. Dray A, Pinnock RD. Effects of substance P on adult rat sensory ganglion neurones in vitro. *Neurosci Lett*. 1982;33(1):61-6.
263. Abdulla FA, Stebbing MJ, Smith PA. Effects of substance P on excitability and ionic currents of normal and axotomized rat dorsal root ganglion neurons. *Eur J Neurosci*. 2001;13(3):545-52.
264. Li HS, Zhao ZQ. Small sensory neurons in the rat dorsal root ganglia express functional NK-1 tachykinin receptor. *Eur J Neurosci*. 1998;10(4):1292-9.
265. Yonehara N, Yoshimura M. Influence of painful chronic neuropathy on neurogenic inflammation. *Pain*. 2001;92(1-2):259-65.
266. Cahill CM, Coderre TJ. Attenuation of hyperalgesia in a rat model of neuropathic pain after intrathecal pre- or post-treatment with a neurokinin-1 antagonist. *Pain*. 2002;95(3):277-85.
267. Lee SE, Kim JH. Involvement of substance P and calcitonin gene-related peptide in development and maintenance of neuropathic pain from spinal nerve injury model of rat. *Neurosci Res*. 2007;58(3):245-9.
268. Jang JH, Nam TS, Paik KS, et al. Involvement of peripherally released substance P and calcitonin gene-related peptide in mediating mechanical hyperalgesia in a traumatic neuropathy model of the rat. *Neurosci Lett*. 2004;360(3):129-32.
269. Herzberg U, Eliav E, Dorsey JM, et al. NGF involvement in pain induced by chronic constriction injury of the rat sciatic nerve. *Neuroreport*. 1997;8(7):1613-18.
270. Zhou XF, Deng YS, Chie E, et al. Satellite-cell-derived nerve growth factor and neurotrophin-3 are involved in noradrenergic sprouting in the dorsal root ganglia following peripheral nerve injury in the rat. *Eur J Neurosci*. 1999;11(5):1711-22.
271. Ro LS, Chen ST, Tang LM, et al. Effect of NGF and anti-NGF on neuropathic pain in rats following chronic constriction injury of the sciatic nerve. *Pain*. 1999;79(2-3):265-74.
272. Chuang HH, Prescott ED, Kong H, et al. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P₂-mediated inhibition. *Nature*. 2001;411(6840):957-62.
273. Shu X, Mendell LM. Acute sensitization by NGF of the response of small-diameter sensory neurons to capsaicin. *J Neurophysiol*. 2001;86(6):2931-8.
274. Ji RR, Samad TA, Jin SX, et al. p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. *Neuron*. 2002;36(1):57-68.
275. Zhang YH, Nicol GD. NGF-mediated sensitization of the excitability of rat sensory neurons is prevented by a blocking antibody to the p75 neurotrophin receptor. *Neurosci Lett*. 2004;366(2):187-92.
276. Zhang YH, Vasko MR, Nicol GD. Ceramide, a putative second messenger for nerve growth factor, modulates the TTX-resistant Na⁺ current and delayed rectifier K⁺ current in rat sensory neurons. *J Physiol*. 2002;544(2):385-402.
277. Amadesi S, Nie J, Vergnolle N, et al. Protease-activated receptor 2 sensitizes the capsaicin receptor transient receptor potential vanilloid receptor 1 to induce hyperalgesia. *J Neurosci*. 2004;24(18):4300-12.
278. Linley JE, Rose K, Patil M, et al. Inhibition of M current in sensory neurons by exogenous proteases: a signaling pathway mediating inflammatory nociception. *J Neurosci*. 2008;28(44):11240-9.
279. Alier KA, Endicott JA, Stemkowski PL, et al. Intrathecal administration of proteinase-activated receptor-2 agonists produces hyperalgesia by exciting the cell bodies of primary sensory neurons. *J Pharmacol Exp Ther*. 2008;324(1):224-33.
280. Kress M, Guenther S. Role of [Ca²⁺]_i in the ATP-induced heat sensitization process of rat nociceptive neurons. *J Neurophysiol*. 1999;81(6):2612-19.
281. Tominaga M, Wada M, Masu M. Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. *PNAS*. 2001;98(12):6951-6.
282. Cesare P, McNaughton P. A novel heat-activated current in nociceptive neurons and its sensitization by bradykinin. *PNAS*. 1996;93(26):15435-9.
283. Dai Y, Wang S, Tominaga M, et al. Sensitization of TRPA1 by PAR2 contributes to the sensation of inflammatory pain. *J Clin Invest*. 2007;117(7):1979-87.
284. Cenac N, Andrews CN, Holzhausen M, et al. Role for protease activity in visceral pain in irritable bowel syndrome. *J Clin Invest*. 2007;117(3):636-47.
285. Salmon JA, Higgs GA. Prostaglandins and leukotrienes as inflammatory mediators. *Br Med Bull*. 1987;43(2):285-96.
286. Kiefer W, Dannhardt G. COX-2 inhibition and pain management: a review summary. *Expert Rev Clin Immunol*. 2005;1(3):431-42.
287. Ferreira SH, Nakamura M, de Abreu Castro MS. The hyperalgesic effects of prostacyclin and prostaglandin E₂. *Prostaglandins*. 1978;16(1):31-7.
288. Higgs EA, Moncada S, Vane JR. Inflammatory effects of prostacyclin (PGI₂) and 6-oxo-PGF₁[α] in the rat paw. *Prostaglandins*. 1978;16(2):153-62.
289. Schepelmann K, Linger K, Schaible HG, et al. Inflammatory mediators and nociception in the joint: Excitation and sensitization of slowly conducting afferent fibers of cat's knee by prostaglandin I₂. *Neuroscience*. 1992;50(1):237-47.
290. Birrell GJ, McQueen DS, Iggo A, et al. PGI₂-induced activation and sensitization of articular mechanonociceptors. *Neurosci Lett*. 1991;124(1):5-8.
291. Devor M, White DM, Goetzel EJ, et al. Eicosanoids, but not tachykinins, excite C-fiber endings in rat sciatic nerve-end neuromas. *Neuroreport*. 1992;3(1):21-4.
292. Oida H, Namba T, Sugimoto Y, et al. In situ hybridization studies of prostacyclin receptor mRNA expression in various mouse organs. *Br J Pharmacol*. 1995;116(7):2828-37.

293. Sugimoto Y, Shigemoto R, Namba T, et al. Distribution of the messenger RNA for the prostaglandin e receptor subtype ep3 in the mouse nervous system. *Neuroscience*. 1994;62(3):919-28.
294. Matsumura K, Watanabe Y, Onoe H, et al. Prostacyclin receptor in the brain and central terminals of the primary sensory neurons: an autoradiographic study using a stable prostacyclin analogue [3H]iloprost. *Neuroscience*. 1995;65(2):493-503.
295. Schaible HG, Schmidt RF. Excitation and sensitization of fine articular afferents from cat's knee joint by prostaglandin E2. *J Physiol*. 1988;403:91-104.
296. Nicol GD, Cui M. Enhancement by prostaglandin E2 of bradykinin activation of embryonic rat sensory neurones. *J Physiol*. 1994;480(Pt 3):485-92.
297. Nicol GD, Vasko MR, Evans AR. Prostaglandins suppress an outward potassium current in embryonic rat sensory neurons. *J Neurophysiol*. 1997;77(1):167-76.
298. Devor M, Govrin-Lippmann R, Angelides K. Na⁺ channel immunolocalization in peripheral mammalian axons and changes following nerve injury and neuroma formation. *J Neurosci*. 1993;13(5):1976-92.
299. Gold MS, Reichling DB, Shuster MJ, et al. Hyperalgesic agents increase a tetrodotoxin-resistant Na⁺ current in nociceptors. *PNAS*. 1996;93(3):1108-12.
300. Gold MS, Levine JD. DAMGO inhibits prostaglandin E2-induced potentiation of a TTX-resistant Na⁺ current in rat sensory neurons in vitro. *Neurosci Lett*. 1996;212(2):83-6.
301. Khasar SG, Gold MS, Levine JD. A tetrodotoxin-resistant sodium current mediates inflammatory pain in the rat. *Neurosci Lett*. 1998;256(1):17-20.
302. Ingram SL, Williams JT. Modulation of the hyperpolarization-activated current (I_h) by cyclic nucleotides in guinea-pig primary afferent neurons. *J Physiol*. 1996;492 (Pt 1):97-106.
303. Moriyama T, Higashi T, Togashi K, et al. Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. *Mol Pain*. 2005;1(1):3.
304. Taiwo YO, Levine JD. Characterization of the arachidonic acid metabolites mediating bradykinin and noradrenaline hyperalgesia. *Brain Res*. 1988;458(2):402-6.
305. Sommer C, Kress M. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. *Neurosci Lett*. 2004;361(1-3):184-7.
306. Leung L, Cahill CM. TNF-alpha and neuropathic pain--a review. *J Neuroinflammation*. 2010;7:27.
307. Copray JC, Mantingh I, Brouwer N, et al. Expression of interleukin-1 beta in rat dorsal root ganglia. *J Neuroimmunol*. 2001;118(2):203-11.
308. Liu L, Yang TM, Liedtke W, et al. Chronic IL-1beta signaling potentiates voltage-dependent sodium currents in trigeminal nociceptive neurons. *J Neurophysiol*. 2006;95(3):1478-90.
309. Binshtok AM, Wang H, Zimmermann K, et al. Nociceptors are interleukin-1beta sensors. *J Neurosci*. 2008;28(52):14062-73.
310. Takeda M, Tanimoto T, Kadoi J, et al. Enhanced excitability of nociceptive trigeminal ganglion neurons by satellite glial cytokine following peripheral inflammation. *Pain*. 2007;129(1-2):155-66.
311. Nadeau S, Filali M, Zhang J, et al. Functional recovery after peripheral nerve injury is dependent on the pro-inflammatory cytokines IL-1 β and TNF: implications for neuropathic pain. *J Neurosci*. 2011;31(35):12533-42.
312. Rotshenker S, Amar S, Barak V. Interleukin-1 activity in lesioned peripheral nerve. *J Neuroimmunol*. 1992;39(1-2):75-80.
313. Kawasaki Y, Xu ZZ, Wang X, et al. Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. *Nat Med*. 2008;14(3):331-6.
314. Stemkowski PL, Smith PA. Long-term IL-1 β exposure causes subpopulation dependent alterations in rat dorsal root ganglion neuron excitability. *J Neurophysiol*. 2012;107:1586-97.
315. Temporin K, Tanaka H, Kuroda Y, et al. Interleukin-1 beta promotes sensory nerve regeneration after sciatic nerve injury. *Neurosci Lett*. 2008;440(2):130-3.
316. Temporin K, Tanaka H, Kuroda Y, et al. IL-1[beta] promotes neurite outgrowth by deactivating RhoA via p38 MAPK pathway. *Biochem Biophys Res Commun*. 2008;365(2):375-80.
317. Horie H, Sakai I, Akahori Y, et al. IL-1 beta enhances neurite regeneration from transected-nerve terminals of adult rat DRG. *Neuroreport*. 1997;8(8):1955-9.
318. Sommer C, Myers RR. Neurotransmitters in the spinal cord dorsal horn in a model of painful neuropathy and in nerve crush. *Acta Neuropathol*. 1995;90(5):478-85.
319. Marchand JE, Wurm WH, Kato T, et al. Altered tachykinin expression by dorsal root ganglion neurons in a rat model of neuropathic pain. *Pain*. 1994;58(2):219-31.
320. Bennett AD, Chastain KM, Hulsebosch CE. Alleviation of mechanical and thermal allodynia by CGRP(8-37) in a rodent model of chronic central pain. *Pain*. 2000;86(1-2):163-75.
321. Baranowski AP, Priestley JV, McMahon S. Substance P in cutaneous primary sensory neurons--a comparison of models of nerve injury that allow varying degrees of regeneration. *Neuroscience*. 1993;55(4):1025-36.
322. Noguchi K, Dubner R, De Leon M, et al. Axotomy induces preprotachykinin gene expression in a subpopulation of dorsal root ganglion neurons. *J Neurosci Res*. 1994;37(5):596-603.
323. Noguchi K, Kawai Y, Fukuoka T, et al. Substance P induced by peripheral nerve injury in primary afferent sensory neurons and its effect on dorsal column nucleus neurons. *J Neurosci*. 1995;15(11):7633-43.
324. Michael GJ, Averill S, Nitkunan A, et al. Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord. *J Neurosci*. 1997;17(21):8476-90.
325. Tonra JR, Curtis R, Wong V, et al. Axotomy upregulates the anterograde transport and expression of brain-derived neurotrophic factor by sensory neurons. *J Neurosci*. 1998;18(11):4374-83.