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’. Pain minimizes contact with the injurious stimulus; thus, promoting a protective response. This evokes both a reflex withdrawal from the stimulus and complex behavioural strategies to avoid further contact with such stimuli. 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.” 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 stimuli. Through this process, the nature (chemical, mechanical or thermal), location, intensity and temporal aspects of the noxious stimulus are transmitted to the thalamus and subsequently to the somatosensory cortex where they are processed and translated into subjective experience. The affective dimension, on the other hand, involves the emotional response to pain, which is mediated by the limbic system, particularly the amygdala and anterior cingulate cortex. This emotional response plays a critical role in the perception and modulation of pain.

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-1β, 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.
of the stimulus are communicated to the higher centres. 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 nociception. For example, poststroke pain can be independent of peripheral nociceptor activation. The affective dimension is the moment-by-moment unpleasantness of pain, made up of emotional feelings associated with future implications, including distress, fear and suffering. These concerns are the result of pain-related activity in limbic structures, such as the amygdala which elicits complex behaviours leading to escape and avoidance.

The affective dimension suggests psychosocial aspects can influence the perception of pain such that ’pain is whatever the person says it is’. 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 subjects. 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.

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 behaviour. 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 experience. Until recently, measures which include the monitoring of pain affect, such as conditioned place aversion (CPA), have been uncommon. The focus on nociception in pain research has been associated with the clinical failure of several potential pain medicines. 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 painful. In contrast, allodynia is defined as ‘pain due to a stimulus which does not normally provoke pain’. 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 nociceptors. Chronic pain resulting from tissue injury is often associated with paroxysmal spontaneous pain. Unlike allodynia and hyperalgesia, spontaneous pain is non-evoked and is the most universal clinical symptom in chronic pain states, such as neuropathic pain. In addition, spontaneous pain appears to be a much better predictor of ‘average’ and ‘worst’ pain ratings than evoked pain hypersensitivities. 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 measurements. This reluctance has been attributed to the uncertainty of animal behaviours corresponding to spontaneous pain and, therefore, represents another important challenge in translational pain research.

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 hyperalgesia. Inflammation is critical to this process, whereby, a complex cascade of events (discussed below) leads to the activation and sensitization of sensory nerve fibres. This heightened state of sensitivity subsides in the absence of further tissue damage and once the wound has healed.

Pain symptoms persisting long after an initial insult suggest that the pain response has become maladaptive and, thus, can be considered a disease. These chronic pain states can be neuropathic or inflammatory in aetiology. 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 diabetes. Chronic inflammatory pain is thought to be the consequence of an underlying inflammatory disorder related to tissue pathology such as arthritis, gastritis, colitis or dermatitis. 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 analgesics, both pain states are thought to involve nervous system plasticity. 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 diameter. 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 species. However, it is known that mammalian A-fibres can conduct up to 100 m/s while C-fibres conduct at <1m/s. The majority of afferents that transmit painful information are Aδ or C, while the
majority of afferents that convey innocuous thermal or mechanical information are Aβ-fibres. However, thinly myelinated and, possibly, some unmyelinated small afferents are associated with the transmission of innocuous sensations. For instance, a woman with selective loss of large diameter, myelinated sensory fibres provided an opportunity to study C-fibres in isolation. 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.

![Diagram illustrating cutaneous sensory neuron subpopulations identified according to AP waveform, morphology, neurochemistry and target-derived neurotrophic support. A. Large Aβ-neurons have short AP durations with no shoulder on the repolarization phase, large cell body diameters (> 40 μm) with thickly myelinated axons and are dependent on target-derived NT-3. B. Medium Aδ-neurons have intermediate AP durations with the presence of a shoulder on the repolarization phase, intermediate cell body diameters (30-40 μm) 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 μm) with unmyelinated axons. Small neurons can be further subdivided into i) IB4-positive which express the P2X3 receptor and are dependent target-derived GDNF; and ii) IB4-negative which express neuropeptides, such as SP and CGRP and are dependent on target-derived NGF. Both IB4-positive and –negative small neurons express TRPV1 channels. All AP waveforms were elicited in response to 2 ms depolarizing current pulses of appropriate suprathreshold magnitude. 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]
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Fibre diameter is also positively correlated to cell body diameter and, therefore, Aβ-fibres are associated with the largest cell body diameters, typically greater than 40 μm, whereas, Aδ- and C-fibres are associated with medium (30-40 μm) and small (< 30 μm) 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 (SO) expressing DRG neurons30-32. In DRG, vanilloid receptor 1 (TRPV1) immunoreactivity is restricted to small- and medium-sized neurons with reactivity in both peptidergic and non-peptidergic sensory neurons33,34 [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 sub-populations, their presence varies between species, between innervated tissues, with animal age and after tissue injury35-37.

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, respectively36,40,41 [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 (GFRα-1) and the transmembrane tyrosine kinase receptor, rearranged during tranfacement (RET) receptor36,41,42. 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%-35% 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 neurons45,46. 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 mecanoreceptors, 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 Aβ-fibre axons. Merkel cells, which are the end organs of slowly adapting mecanoreceptors, were severely reduced. This loss of Merkel cells, together with their innervating Aβ-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 mecanoreceptive 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 neurotrophic 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 mecanoreceptors 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
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surrounding environment, have been identified\textsuperscript{51}. Cation channels, known as transient receptor potential (TRP) channels, represent the first illustration that sensory ion channels can be gated by a physical stimulus\textsuperscript{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 tetramers\textsuperscript{53}. 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 thermoreceptors\textsuperscript{60}. In mammals, temperature sensitive TRPs are each tuned to distinct temperature ranges and, collectively, permit discrimination of temperature ranging from noxious cold to noxious heat\textsuperscript{50}. 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)\textsuperscript{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-, mechan- and/or osmo-sensitive. For instance, TRPV1 responds to protons and capsaicin, the pungent component of spicy peppers\textsuperscript{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)\textsuperscript{56,57}. TRPA1 and TRPV4 have been associated with mechanosensitivity\textsuperscript{58,59}. In addition, TRPV4 mediates animal behaviours in response to hypotonic stimuli and may be of particular interest in hypersensitive states\textsuperscript{60}.

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\textsuperscript{61,62}. Additionally, TREK-1 channels are stretch-sensitive and TREK-1 knockout mice have increased sensitivity to low-threshold mechanical stimuli\textsuperscript{61,63}. Acid sensing ion channels (ASICs) respond to protons and membrane stretch, however, a role in mechanocognition has been unsubstantiated with animal models\textsuperscript{64}. Several other molecules may be involved in sensory neuron transduction, including canonical-1 TRP channels (TRPC1), P2X, ATP-gated cation channels, protease activated receptors (PAR 1, 2 and 4) and voltage-gated channels (VGCs), including voltage-gated sodium channels (VGSCs)\textsuperscript{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\(\delta\)- and C-fibres\textsuperscript{30,55}. 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-sensations\textsuperscript{52}. Consistent with polymodal nociceptors, TRPA1 is expressed in a subset of TRPV1 expressing nociceptive neurons responding to noxious mechanical and thermal stimuli\textsuperscript{52}. The close apposition between free nerve endings (FNEs) and keratinocytes, as well as the synaptic junctions between Merkel cells and A\(\beta\)-fibres suggest skin cells can act as first-line transducers of physical stimuli\textsuperscript{50}. In support, Merkel cells express TRPV4 and keratinocytes express TRPV3 and TRPV4\textsuperscript{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\(\beta\)-fibres\textsuperscript{50}.

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\(\delta\)- and C-fibres, subserving the modalities of pain and temperature\textsuperscript{67}. The A\(\delta\)-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\textsuperscript{50,67}. Many FNEs are considered nociceptors. Certain nociceptors, whose afferent conduction velocities correspond to A\(\delta\)- and C-fibres, are high threshold mechanoreceptors and respond vigorously to only intense (tissue damaging) mechanical stimuli\textsuperscript{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\textsuperscript{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 damage\textsuperscript{69,71}. FNEs of A\(\delta\)- and C-fibres have also been associated with innocuous stimuli where some are considered thermoreceptors or low threshold mechanoreceptors\textsuperscript{59,72,74}. 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\textsuperscript{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 differentiated Schwann cell-related and perineurial-derived cells\textsuperscript{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 appear to act as a filter that protects the sensory endings from irrelevant stimuli\textsuperscript{77}. Corpuscles can have A\(\alpha\)-, A\(\beta\)- or A\(\delta\)-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 adapting\textsuperscript{77}. However, adaptation terminology is misleading since static displacement does not account for derivatives of displacement, such as velocity and direction\textsuperscript{21}. 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, respectively\textsuperscript{77}. 
The Ruffini endings and Merkel cell–neurite complexes are slow adapting, low threshold mechanoreceptors of glabrous- and hairy skin\(^{21,71}\). Ruffini endings are located in the connective tissue of the dermis and are relatively large spindle shaped structure tied into the local collagen matrix\(^7\). Ruffini ending structure is considered analogous to that of the Golgi tendon organ in muscle and, therefore, suggests that Ruffini endings function as stretch receptors\(^7\). A\(\alpha/\beta\) 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\(\alpha/\beta\) 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 terminal\(^{79}\).

Hairs are additional low threshold mechanoreceptors of hairy skin and have two main types of follicles\(^7\): 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\(\alpha\)-, A\(\beta\)- or A\(\delta\)-nerve 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 Ca\(^{2+}\) 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\(\beta\)-conducting fibres, while narrow APs tend to have faster conducting fibres, including A\(\alpha\)-, A\(\beta\)- and A\(\delta\)-fibres [Figure].\(^{26,63,84}\) In addition, broad somatic APs from C- and some A\(\delta\)-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\(\delta\)-fibres innervating high threshold mechanoreceptors (HTMRs) exhibit broader APs than A\(\beta\)-fibres innervating D-hairs\(^{82,86}\). A\(\beta\) HTMRs differ from A\(\beta\) low threshold mechanoreceptors (LTMRs) in the same general way, however, A\(\beta\) 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 cats\(^{90}\). 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 channels\(^{91}\).

Voltage gated sodium channels (VGSCs) are heteromultimeric proteins consisting of a large pore-forming \(\alpha\)-subunit and small extracellular accessory \(\beta\)-subunits which modulate channel membrane insertion and channel gating\(^9\). Nine mammalian sodium channel isoforms have been identified and functionally expressed\(^{93}\). TTX-S isoforms include Na\(_\alpha_1.1\), Na\(_\alpha_1.2\), Na\(_\alpha_1.3\), Na\(_\alpha_1.4\), Na\(_\alpha_1.6\) and Na\(_\alpha_1.7\), whereas, TTX-R isoforms include Na\(_\alpha_1.5\), Na\(_\alpha_1.8\) (formerly peripheral nerve sodium channel type 3 (PN3) and sensory neuron specific (SNS)) and Na\(_\alpha_1.9\) (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 \(\alpha\)-subunits Na\(_\alpha_1.8\) and Na\(_\alpha_1.9\) 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 IB\(\beta\)-positive DRG neurons while both small IB\(\beta\)-positive and -negative neurons express Na\(_\alpha_1.8\).\(^{83}\) 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 IB\(\beta\)-positive neurons. The persistent nature of currents mediated via Na\(_\alpha_1.9\) was further predicted to underscore the longer AP durations observed in small IB\(\beta\)-positive neurons\(^{89}\). 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\(_\alpha_1.6\) sodium current. This current, by virtue of its rapid recovery from inactivation, can maintain high frequency firing\(^91\). 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 damage\textsuperscript{101-103}.

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\textsubscript{v}1.1, Na\textsubscript{v}1.6 and Na\textsubscript{v}1.7, with some TTX-R Na\textsubscript{v}1.8 expression, while small neurons express TTX-S channels, in conjunction with TTX-R Na\textsubscript{v}1.8 and Na\textsubscript{v}1.9 channels\textsuperscript{101}. 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 A\textsuperscript{δ}PAPs with IB\textsubscript{P} positive cell bodies immunoreactive for the Na\textsubscript{v}1.9 isoform\textsuperscript{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 characteristics\textsuperscript{101}.

Voltage-gated calcium channels (VGCCs) are the critical link between membrane depolarization and calcium entry and represent one of several ways calcium can influence membrane excitability and physiological processes, including neurotransmitter release\textsuperscript{104,105}. Biochemical characterization of VGCCs has revealed a complex protein structure composed of α1 pore forming subunit, encoded by gene subfamilies: Ca\textsubscript{v}1 to 3\textsuperscript{104}, as well as several auxiliary subunits\textsuperscript{105,106}.

Sensory neurons express several biophysically and pharmacologically distinct VGCCs\textsuperscript{104}. These include: 1) Low voltage activated (LVA) channels encoded by the gene subfamily Ca\textsubscript{v}3 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 Ni\textsuperscript{2+}\textsuperscript{104,105,107}. 2) High voltage activated (HVA) channels encoded by gene subfamilies Ca\textsubscript{v}1 and 2 which have ‘high’ gating thresholds from -30 to -20 mV. Further, HVA Ca\textsubscript{v}2\textsuperscript{α} currents include ‘long-lasting’ (L-type, Ca\textsubscript{v}1) currents, ‘Purkinje cell’ (P/Q-type, Ca\textsubscript{v}2.1) currents and ‘neuronal’ (N-type, Ca\textsubscript{v}2.2) currents which are sensitive to blockade by dihydropyridines, omega-agatoxin IVA and by omega-conotoxin GVIA, respectively\textsuperscript{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\textsubscript{v}2.3) 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\textsuperscript{107,108-112}. 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 transmission\textsuperscript{113}. 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\textsuperscript{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\textsubscript{1-δ} subunit, which has particular relevance to neuropathic pain mechanisms, is thought to be involved in the trafficking of channels to the plasma membrane\textsuperscript{115}. It is upregulated by nerve injury\textsuperscript{116-119} and appears to be the primary site of action for the antiallodynic agents pregabalin\textsuperscript{120} and gabapentin\textsuperscript{121-123}.

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-subunits\textsuperscript{124}. The variation in AHP duration, observed by Matsuda and colleagues, suggests that potassium conductances are another defining feature among sensory neurons\textsuperscript{80}. It is now known that DRG neurons contain a wide variety of potassium channels of all four families: voltage-gated (K\textsubscript{v}), calcium-activated (K\textsubscript{Ca}), inwardly-rectifying (K\textsubscript{iR}) and two-pore (K\textsubscript{2P}) channels. Although all four families have been associated with pain hypersensitivity\textsuperscript{125}, K\textsubscript{iR} and K\textsubscript{2P} channels and their relation to sensory neuron physiology will be the topic of further discussion. For more information on K\textsubscript{iR} and K\textsubscript{2P}, see reference numbers\textsuperscript{125-128}.

K\textsubscript{iR} and K\textsubscript{2P} channels exist as tetramers composed of four pore forming α-subunits either alone or in association with regulatory β-subunits\textsuperscript{129,130}. The voltage-dependent delayed rectifier and fast transient potassium currents were first described in DRG neurons by Kostyuk’s group\textsuperscript{131}. 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\textsubscript{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)\textsuperscript{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\textsubscript{A} (>100 micromolar), to 4-AP block\textsuperscript{134,135}. When isolated, the dendrotoxin-sensitive current (I\textsubscript{d}) was calcium insensitive and displayed, relative to I\textsubscript{A}, slow, incomplete inactivation\textsuperscript{135,136}. Biophysically, potassium currents could be divided into non-inactivating and transient A-currents with the later subdivided divided into fast (I\textsubscript{A}f) and slow (I\textsubscript{As}) components where I\textsubscript{Af} had, relatively, faster kinetics and more negative voltage dependencies than I\textsubscript{As}\textsuperscript{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\textsubscript{A} or I\textsubscript{Af} and I\textsubscript{As} or I\textsubscript{As} (but see also reference number\textsuperscript{138}).

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,
respectively\textsuperscript{138-141}. In contrast, Villiere and colleagues reported that the proportion of neurons with A-type current is greatest in C-fibres and least in Aδ/β-fibres\textsuperscript{142}. 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 subpopulations\textsuperscript{138}. 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\textsuperscript{137,139,141,143,144}.

Three classes of K\textsubscript{Ca} channels can be distinguished on the basis of their biophysical and pharmacological properties\textsuperscript{104}. Large conductance BK\textsubscript{Ca} channels are voltage-gated and sensitive to iberiotoxin, while small conductance (SK\textsubscript{Ca}) and intermediate conductance (IK\textsubscript{Ca}) channels are voltage-insensitive and can be blocked by apamin and chlormazole, respectively. Modulation of K\textsubscript{Ca} channels allows alterations in intracellular calcium concentration to regulate membrane excitability, whereby, the three K\textsubscript{Ca} channels are thought to have distinct functional roles. For instance, BK\textsubscript{Ca} channels are involved in AP repolarization and generation of the fast AH\textsubscript{P} (fast I\textsubscript{AHP}), whereas, SK\textsubscript{Ca} channels convey the I\textsubscript{AH} that mediates slow AH\textsubscript{P}s in small nociceptive DRG neurons\textsuperscript{104,145,147}. In contrast, the physiological role of IK\textsubscript{Ca} in DRG neurons remains to be determined. Although the functions of SK\textsubscript{Ca} and BK\textsubscript{Ca} channels are well recognized in small diameter sensory neurons\textsuperscript{148,149}, the presence of SK\textsubscript{Ca} channels in large diameter neurons\textsuperscript{150} suggests that K\textsubscript{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\textsuperscript{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\textsuperscript{153,154}.

The HCN channels give rise to hyperpolarization-activated, non-selective cation current (I\textsubscript{h})\textsuperscript{155}. The slow activation of I\textsubscript{h} in DRG neurons gives rise to a ‘voltage sag’ in response to hyperpolarizing current commands\textsuperscript{155,156}. Thus, the initial voltage attained by injection of hyperpolarizing current gradually abates during the course of the current command. I\textsubscript{h} is also involved in setting resting membrane potential, participating in pacemaker activity and modulating synaptic activity\textsuperscript{157}. HCN channels 1 to 3, but likely not HCN4 (but see also reference number\textsuperscript{158}), are present in the somata and axons of DRG neurons\textsuperscript{159-161}. Further, HCN1 and fast I\textsubscript{h} are predominately found in larger DRG neurons, while slower I\textsubscript{h} Currents are more variably expressed in small neurons\textsuperscript{160-162}. 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 research\textsuperscript{163}.

**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 horn\textsuperscript{164}. 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\textsuperscript{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 column\textsuperscript{167-170}. 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 horn\textsuperscript{171}. In contrast, identified C-fibres, including nociceptive and thermoreceptive fibres, terminate ipsilaterally in laminae I, II and V\textsuperscript{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, IB\textsubscript{4} labelling and/or P2X\textsubscript{2} immunoreactivity has revealed that non-peptidergic C-fibres terminate dorsally within inner lamina II (LIIid)\textsuperscript{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 understood\textsuperscript{14}. Nociceptive, high threshold mechanoreceptive Aδ-fibres terminate ipsilaterally in laminae I and V, whereas innocuous Aδ-fibres, innervating D-hairs, terminate ipsilaterally in the deeper part of lamina II and lamina III\textsuperscript{176}. Taken together, the superficial layers of the dorsal horn (L1 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 diabetes\textsuperscript{18}. 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 persistant pain states. 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 neuroma that develops at the site of nerve injury, and from sensory cell bodies in the DRG. DRG excitability increases two to seven weeks after sectioning (axotomy) of the sciatic nerve. 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, were increased. In contrast, AP regulatory mechanisms, such as steady state (delayed rectifier) K+ current (Ih) and HVA Ca2+-channel current (HVA-Ih) were attenuated. Further, alterations were most prevalent in the small-sized DRG neurons which are, presumably, nociceptive C- and Aδ-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 (Aβ) cell bodies.

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 types. 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 shape. 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 threshold. 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 neurons. 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 Smith 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. Similar observations were reported by Zhang and colleagues after an axotomy injury. 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 and upregulation of its corresponding transcript, Na1.3, as well as protein product have been observed in DRG neurons of adult rats after axotomy and SNL. In contrast, transcripts encoding TTX-R sodium currents are down regulated in small DRG neurons following axotomy. 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 stump. Further, intrathecal GDNF treatment prevented sensory hypersensitivity after SNL injury and was associated with block of A-fibre ectopic discharge and normalization of Na1.3 expression in injured DRG. 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 neurons. 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, Na1.8 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 injury. 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 agents.
due to a direct effect on $I_{\text{K,Ca}}$ channels. Though all $I_{\text{K,Ca}}$ 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 iberiotoxin sensitive (large conductance) and clotrimazole sensitive (intermediate conductance) $I_{\text{K,Ca}}$. 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 controls. 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 $I_{\text{K,Ca}}$ 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 ($I_{\text{N}}$) is decreased and is concurrent with increased inactivation in large-sized DRG neurons associated with cutaneous afferents. LVA or T-type $I_{\text{Ca}}$ 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 parameters. Onset of diabetic neuropathy is also associated with increased T-type, but not HVA, current density in IB$^+$-positive, capsaicin responsive medium-sized rat DRG neurons, as well as a depolarizing shift in steady-state inactivation. Although the changes in $I_{\text{Ca}}$ 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 $I_{\text{Ca}}$ could underlie decreased calcium-dependent potassium current after injury which, in turn, would be expected to decrease AHP amplitude and shorten AHP duration, ultimately, increasing firing frequency. However, the observed decrease in HVA $I_{\text{Ca}}$ after peripheral nerve injury seems inconsistent with the effectiveness of drugs, such as the gabapentinoids and ziconotide, 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 Ca$^{2+}$ channels, yet opioids are known to produce analgesia, impart, by blocking presynaptic Ca$^{2+}$ channels and, thereby, reducing neurotransmitter release from primary afferent terminals.

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 neurons. This has led to the identification of T-type calcium channels as a potential therapeutic target in pain management.

Nerve injury-induced changes to the availability of HCN channels. Since the pacemaker current ($I_{\text{h}}$) 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 produces cutaneous hyperalgesia and enhanced excitability of neuronal somata. The CCD increases 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 afferents. Further, SNL injury markedly increased pacemaker currents in large diameter DRG neurons. Pharmacological blockade of $I_{\text{h}}$ with ZD7288 decreased firing frequency of ectopic discharges originating in injured Aβ- and Aδ-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 injury. 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.

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 the sprouting of perivascular sympathetic nerves within the DRG. Although normal DRG neurons are insensitive to noradrenaline, injury-induced upregulation of $\alpha$-adrenoceptors and the formation of baskets of sympathetic axons around DRG neurons 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 endings, 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 injury.

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 sensitization. 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: Aβ-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 excitability, synaptic facilitation, loss of inhibition, reversal of inhibition (disinhibition) and enhancement of excitation of central circuitry which promotes spontaneous pain, allodynia and hyperalgesia after inflammation or lesions to the nervous system.

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system. 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 αβ-fibre input into the superficial dorsal horn and pain-like responses elicited by the movement of hairs. 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 status.

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 inhibition. 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 neurons. 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 BDNF. Since nervous system injury is associated with elevated levels of BDNF and neuropathic pain-related behaviours are attenuated by sequestering BDNF, 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 CCI and that activated microglia enhanced overall dorsal horn excitability through the release of BDNF. 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 LII neurons. 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 cord, as well as the enhancement of NMDA receptor mediated excitatory post synaptic currents (epsps) in LII dorsal horn neurons. It was further demonstrated that ATP and its release from damaged cells can activate microglia and, through P2X_4 purinergic receptor (P2X_R) stimulation, promotes the release of BDNF.

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.

Nerve injury

Ectopic discharge in primary afferents is secondary to direct axonal damage and disruption of the myelin sheath that surrounds many axons. 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. 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. In parallel, the chemical environment is changed and several mediators are known to interact with sensory neurons. 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 transactions, but exists in other models of nerve injury, as well as in disease states and infections. In the case of axotomy, the proximal axonal segment and attached cell body becomes isolated from the distal segment. 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. 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 (chondrotolysis). 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. 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. Corresponding to these events, an ‘inflammatory soup’ of bradykinins, SP, hydrogen ions, NGF, prostaglandins, histamine, ATP and proinflammatory cytokines is produced.

Several lines of evidence underline the importance of the local inflammatory response in the generation of centralised 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 injury. 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 autotomy. 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. Cui and colleagues demonstrated a strong correlation between degree of local macrophage/macocyte infiltration among three nerve injury models and the presence of mechanical allodynia. Lastly, peripheral nerve injuries such as CCI are associated with an increased magnitude than sciatic nerve transection and invoke downregulation of GABAergic functions in the superficial dorsal horn while axotomy is ineffective. Similarly, CCI produces greater augmentation of excitatory synaptic transmission than sciatic nerve axotomy.

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 threshold. In vivo administration of SP to glabrous skin produces hyperalgesia and allodynia. Further, SP release depolarizes and excites small nociceptive sensory neurons which express the neurokinin-1 (NK1) receptor. 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). In support, NK-1 receptor antagonists applied either centrally or peripherally 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 neurons, Schwann cells and satellite cells 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. NGF release can enhance excitability of primary afferents in several ways, including an increase in TRPV1 activity, sensitivity and expression. In addition to the maintenance of TTX-R sodium currents (Na\(^+\)1.8) through Trk receptors, NGF signalling through the p75 neurotrophin receptor can increase AP firing and is concurrent with an enhancement of TTX-R sodium current and a suppression of delayed rectifier-like potassium current.

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 neurons. PAR-2 activation reduces M-current which results in membrane depolarization and the generation of APs. In addition and similar to the activation of metabotropic P2Y purinergic receptors and bradykinin-2 (B2) receptors, PAR-2 activation sensitizes TRP channels which are associated with hyperalgesia. Consistent with these observations, injection of PAR-2 agonists in vivo induces pain-related behaviours, whereas, antagonists produce antinociceptive effects.

The association of eicosanoids and their synthesizing enzymes, cyclooxygenase-1 (COX-1) and particularly the inducible COX-2, with inflammation and pain is well documented. For instance, prostaglandin E\(_2\) (PGE\(_2\)) or prostaglandin I\(_2\) (PGI\(_2\); prostacyclin) administration induces hyperalgesia. Further, PGI\(_2\) and PGE\(_2\) enhance the sensitivity of primary afferents to either mechanical or chemical stimulation. PGE\(_2\) and PGI\(_2\) receptors (EP and IP, respectively) are expressed in sensory neurons and in vitro application of PGE\(_2\) or PGI\(_2\) on DRG neurons lowers firing threshold and increases AP firing upon current injection, elevated potassium or bradykinin application. Consistent with hyperexcitability, PGE\(_2\) and a PGI\(_2\) analogue suppress a sustained-type potassium current and, similar to serotonin, prostaglandins upregulate TTX-R sodium currents. In addition, \(I_H\) is positively modulated by PGE\(_2\), in trigeminal ganglion cells. Through a receptor mediated mechanism of action, PGE\(_2\) and PGI\(_2\) also sensitize TRPV1 channels, producing hyperalgesia in mice. Last, it has also been suggested that hyperalgesia induced by bradykinin, norepinephrine and cytokines is secondary to the production of prostaglandins.

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. However, accumulating evidence implicates direct involvement of IL-1β in the enduring increase in sensory neuron excitability commonly observed after peripheral nerve injury. For instance, mRNA and protein expression of the IL-1β receptor, IL-1R1, in sensory neurons implies that IL-1β can directly affect primary afferents. In agreement, changes in the electrical properties of sensory neurons consistent with increased excitability occur within minutes of IL-1β application, as well as the onset of pain-related behaviours after intraplantar injections of IL-1β. Importantly, IL-1β expression, secretion and processing 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-1β alters the excitability of DRG neurons. For instance, the effects of long-term IL-1β exposure in medium and small, IB\(_3\)-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-1β 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 process. For example, it has recently been suggested that injury-induced increase in interleukin-1β and TNF-α protein levels is required...
for functional nerve recovery. This implies that attempts to attenuate neuropathic pain by preventing the actions of interleukin-1β 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, respective receptor antagonists applied either centrally or peripherally, 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 injuries result in a phenotypic shift where large, rather than small, sensory neurons begin to express and become responsive to SP. Another phenotypic switch pertains to BDNF. Under normal circumstances, BDNF mRNA and protein is expressed in nociceptive sensory neurons, however, BDNF becomes upregulated in large diameter DRG neurons and is concurrent with increased anterograde transport to the dorsal horn after sciatic axotomy. 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 cord.

**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-1β and other cytokines in the onset of neuropathic pain, drugs which antagonize 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.

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