Introduction

Among all patients with neurological disorders, pain is probably the most common symptom experienced. Identifying, understanding, and treating pain is a responsibility of health care providers in all branches of neurology.

This text is about pain in neurological diseases, both "neuropathic" pain that is recognized in sensory neuron disorders but also other types of pain less commonly considered. These latter disorders include for example, pain in Parkinson's disease, ALS, and muscle diseases. The text includes specific chapters on mechanisms of neuropathic pain, updated with new ideas on its pathogenesis, and a summary of recent therapeutic guidelines. Most of the text however centers on actual patient encounters at the Neurology clinics of the Universities of Wurzburg and Calgary. This emphasis is to make the vignettes relevant to clinical practitioners but also to introduce researchers and workers in industry to the complex and challenging issues faced during direct patient encounters. Therapeutic failures are common among our vignettes, highlighting the fact that current therapeutic advances remain inadequate – a challenge for researchers to find better approaches.

In each section, we highlight a clinic patient case in a "vignette" emphasizing the patient's history, clinical findings, investigations, diagnosis, and treatment. Some vignettes indicate highly complex health care trajectories, another important lesson for non-clinicians. Despite the vignettes arising from widely separated health care environments in Canada and Germany, the similarities in these health care challenges are remarkable. Approximately half of the vignettes arise from each clinic. Keeping in mind that the literature is evolving and that some types of neurological pain have had limited attention, we review key literature points in each vignette and provide citations where possible. As might be expected, the citation density varies widely among these disorders indicating an uneven literature available to guide clinicians or instruct others. In many instances, illustrations are taken directly from the patients presented but other illustrations are also provided as general examples.

Medicine is a complex specialty that requires a tailored approach to care for individuals. While large cohorts of patients studied in clinical trials provide essential evidence on which to base practice, the reality is much more complex. We have hoped to convey these challenges, and some current insights into the associated neurological pain in this text.

Section A The biology of neuropathic pain

Chapter 1

Mechanisms of neuropathic pain

The clinical cases presented in this text highlight the variety of clinical neurological disorders associated with pain. As should be evident from the range of conditions presented and the parts of the nervous system they impact, pain can arise from any level of the neuraxis: sensory receptor to cortex. Similarly the disease processes that generate it are extensive from sensory neuropathies to thalamic cerebral infarction. The experience of pain is subjective, influenced by the context in which it occurs and the person in whom it develops. It may be painful to one person while it brings on a heightened, reduced or even absent response in another. The degree of pain is heavily influenced by the circumstances in which it occurs and the underlying psychological status of the sufferer. For example, patients with depression may have greater difficulty coping with pain and its intensity may be magnified by the concurrent problems they face. For these reasons, it is best described as a "pain experience," as distinguished from nociception, the transmission of potentially harmful stimuli through neuroanatomical "wiring" and neurochemical mediators.

"Neuropathic pain" is a subset of the pain experience specific to damage incurred in the nervous system. A current definition of neuropathic pain is provided by Treede and colleagues who describe it as "pain arising as a direct consequence of a lesion or disease affecting the somatosensory system" (1). In this description, neuropathic pain was graded on the following criteria: 1. pain with a distinct neuroanatomically plausible distribution; 2. a history suggestive of a relevent lesion or disease involving the peripheral or central somatosensory system; 3. demonstration of the distinct neuroanatomically plausible distribution by at least one confirmatory test; 4. demonstration of the relevant lesion or disease by at least one confirmatory test. With the above criteria, definite neuropathic pain met all 4, probable 1, 2 and 3 or 4, possible 1 and 2.

Strictly speaking, neuropathic pain has been used to refer to symptoms associated with neuropathy, a disorder of the peripheral nervous system. Using this qualification, its characteristics share common descriptive features: burning, prickling, tingling (paresthesiae), electrical sensations, and pain generated by otherwise innocuous stimuli (allodynia). More recently, neuropathic pain has also been used to describe pain arising anywhere along the neuraxis. This broader usage has generated more descriptors, as demonstrated in the cases presented in this text.

This introductory chapter presents a brief overview of the mechanisms and mediators of pain in neurological conditions. It is a body of knowledge that is rapidly expanding beyond ideas that can be conveyed at the time of writing. Much of this knowledge has evolved from investigations of specific peripheral neuropathic pain mechanisms. The pain process involves the nervous system widely; even pain of exclusive peripheral origin may lead to secondary changes within the sensory ganglia, dorsal horn of spinal cord, brainstem, thalamus, and cortex. Major aspects are illustrated in a summary scheme [Figure 1.1]. We begin by discussing mechanisms that generate pain in the periphery at either damaged or intact distal axons and nerve terminals, and then proceed to analyze its development higher along the neuraxis.

Ectopic impulses

The development of the pain experience from distal injuries begins with ectopic, or unexpected spontaneous discharges from peripheral axons and their terminals. The terminals of axons that normally subserve nociception are found in the epidermis of the skin but also in many other tissues such as the meninges, liver capsule, joint capsule, periosteum, mesentery, and muscle. A large complement of afferent axons, usually unmyelinated "C" fibers, are normally found in nerves to muscles and serve to transmit painful stimuli. This explains why muscles can become painful after prolonged exercise or during needle electromyography.

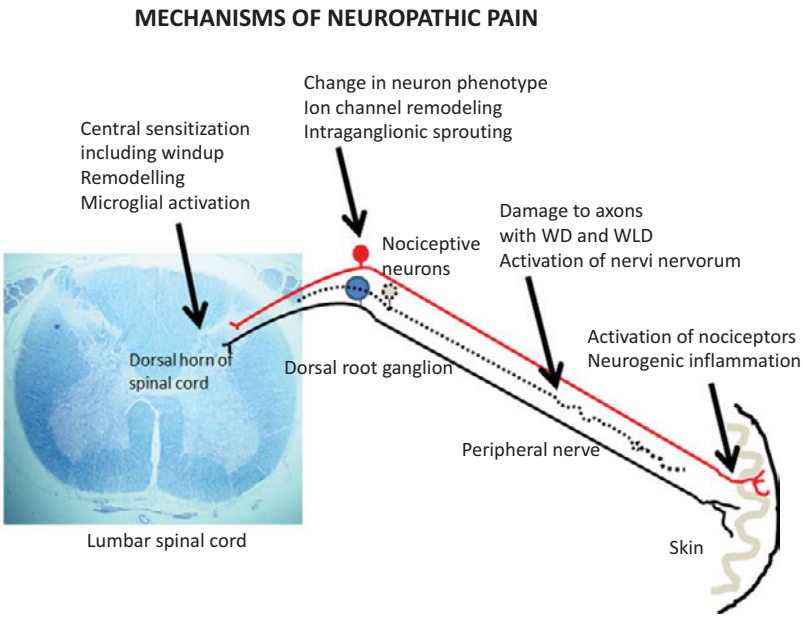

Figure 1.1. Simplified scheme identifying major events involving the neuraxis in the generation of neuropathic pain. These include (from right to left), activation of nociceptors with neurogenic inflammation, a cascade of molecular events associated with Wallerian (WD) and Wallerian-like degeneration (WLD) in the nerve trunk, alterations in neuronal phenotypes in the dorsal root ganglia with ion channel remodeling and intraganglionic sprouting, and changes in the dorsal horn of the spinal cord including remodeling, windup, and microglial activation.

At one time, it was also deliberately evoked by neurologists who tested patients for tabes dorsalis by identifying lack of sensation to deep pain while squeezing major proximal muscles. Tabes dorsalis, a tertiary complication of syphilis (neurosyphilis), inflames and damages the dorsal root entry zone of afferent fibers resulting in loss of deep pain sensitivity.

Normally nociception is transmitted by unmyelinated C axons and small myelinated Aδ axons with conduction velocities of 0.5–2.0 m/s and 12–30 m/s, respectively. It is not known whether specialized structures within these fibers are found at their terminals, but it is generally thought that they transmit nociception through "bare" or "free" nerve endings. They are stimulated by noxious mechanical, chemical, or thermal stimuli through specialized channels or receptors. A large complement of "silent" nociceptors, those only recruited during tissue damage and more significant injuries, may also exist.

In discussing neuropathic pain, our emphasis is on how damaged or diseased axons or neurons generate pain. Ectopic action potential discharges can be recorded from single axons proximal to a nerve trunk injury and their frequency has a strong direct correlation with the perceived intensity of pain. Ectopic discharges may be continuous or tonic, in phasic bursts, or completely irregular. How quiescent transmitting axons develop the capacity for generating ectopic potentials is uncertain but the process likely involves a redistribution or alteration in their ion channels. Devor and colleagues demonstrated that injured axons swell to form axonal endbulbs, enlarged proximal portions of severed axons where axoplasmic material can accumulate (2). Within these endbulbs, accumulations of sodium channels, some inserted into the membrane, may be capable of generating ectopic activity. Specific blockers of these channels, such as phenytoin or lidocaine, interrupt spontaneous discharges. Several mechanisms are capable of changing the electrical properties of sodium channel subtypes Na_v 1.1 to 1.9 and thus the sensitivity of the respective nerve fiber (3, 4) [see below]. Genetic alterations in the sodium channel subtype Na_v 1.7 may lead to hyperactive channels. This is the pathophysiological basis of disorders such as erythromelalgia and paroxysmal extreme pain disorder (5). On the other hand, mutations that silence the channel lead to congenital insensitivity to pain (6).

In addition to structural alterations of individual damaged axons, pain may also arise from afferent fibers that self-innervate the peripheral nerve trunk. Nervi nervorum are unmyelinated axons found within the epineurial nerve sheath (7–9). This fascinating mechanism is considered in more detail later.

The activation or sensitization of peripheral axons, especially after injury, involves the participation of a range of pain-generating and pain-attenuating

molecules, considered below. The sheer numbers of players involved has prompted diverse thoughts on therapy and intervention to treat neuropathic pain.

Algesic molecules

Within the vicinity of an injured peripheral nerve axon, a range of newly expressed molecules are capable of facilitating ectopic discharges that result in pain. These molecules are termed algesic. Extracellular potassium, released from dead and dying cells, helps to depolarize nearby axons. Similarly, there may be accumulation of hydrogen ions in ischemic acidotic injured tissues that activate acid-sensitive ion channels and TRP channels (see below). Several algesic molecules likely influence the excitability of damaged axons by synergistic interactions, or by changing the properties of the final common pathway mediated by ion channels expressed on axons. Specific ion channels are described below.

Combinations of algesic molecules released from local macrophages and leukocytes comprise an "inflammatory soup" that generates neuropathic pain: these include tumour necrosis factor α (TNFα), nitric oxide (NO), interleukin-1β (IL-1β), proteases that act on PAR2 receptors, and others. Algesic molecules are linked with generating pain behavior in experimental animal models. Histamine is associated with the development of closely related sensory symptoms including both pain and itch. Nerve growth factor (NGF) is a potent algesic substance causing sensations of deep aching pain when injected subcutaneously or intramuscularly. NGF may act through its high affinity TrkA receptors, known to be expressed in small caliber sensory axons that subserve nociception.

Neuropeptides, such as substance P (SP) and calcitonin gene-related peptide (CGRP), are released by sensory axons in tissues during injury. It is possible that they auto-reactivate the sensory axons that originally released them to amplify pain sensations. Better described however, is a pathway in which SP and CGRP act on mast cells to release histamine and serotonin as direct algesic molecules. Bradykinin is an algesic molecule that arises from the proteolytic cleavage product of kininogen during inflammation. Bradykinin acts on B1 and B2 receptors, both of which are upregulated in sensory ganglia after a peripheral injury. B2 receptors rise early, within 48 h of injury, whereas B1 receptors rise later, by 14 days. Both contribute toward pain behavior as demonstrated by pharmacological inhibitors to either bradykinin receptor that dampen neuropathic pain in rats (10).

ATP acts as an algesic molecule through specific purinergic receptors that include P2X3, P2X2/3, P2X4, P2X7, and P2Y. Alternatively, P2X3 antagonists are analgesic. P2X receptors are nonselective cation channels permeable to calcium, sodium, and potassium with downstream actions on the MAPK pathway. Whereas P2X3, P2X2/3, and P2Y activate neurons directly, P2X4 and P2Y may activate neurons indirectly through the activation of nearby microglia (11). P2Y may also modulate TRPV1 receptors and increase their sensitivity (see below). In a related pathway, P1 receptors activated by adenosine inhibit nociception.

During peripheral nerve injury, there is rapid expression of inflammatory mediators produced by intrinsic cells such as Schwann cells, endothelial cells, and fibroblasts in the early phase, within hours. These mediators are also expressed by invading macrophages and T-lymphocytes at later phases over several days. One interesting scenario involves a sequence of events that follows initial primary axon damage: activation of Schwann cells occurs as a result of calcium released from damaged axons (12), the activation of Toll-like receptors (13), and the subsequent production of pro-inflammatory cytokines, chemokines. Additional molecules involved in this process include calpain (14, 15) and later glutamate (16). Chemokines such as CCL-2, in addition to their release by inflammatory cells, may be packed into neuronal synaptic vesicles and serve as neurotransmitters in the spinal cord (17, 18). After injury, Schwann cells and hematogenous macrophages newly express iNOS (inducible nitric oxide synthase), a potent generator of NO, an additional inflammatory mediator (19).

Prostaglandins synthesized by cyclooxygenases (COX1 and COX2), especially PGE2, are also mediators of pain during inflammation. COX2 may be the more important of the two cyclooxygenase enzymes during neuropathic pain and it is mainly expressed by Schwann cells and inflammatory cells such as macrophages after nerve injury (20–22). PGE2 acting through its EP1 receptors activates sodium and potassium channels in neurons, inhibits potassium channels, releases SP and CGRP, and sensitizes neurons to bradykinin and capsaicin (23).

Proteinase-activated receptors (PARs) are a special class of cell surface receptors activated by proteinases such as thrombin, trypsin, mast cell tryptase, and others (24). Cleavage at their extracellular amino

terminus exposes a tethered ligand that in turn activates the receptor. PAR1 and PAR2 are expressed on sensory afferents, and PAR2 agonists can induce the release of SP and CGRP by sensory neurons to promote hyperalgesia (25). In contrast, PAR1 agonists are associated with analgesia (26).

Taken together, it is highly likely that the neuropathic phenotype arises from a combination of algesic molecules, in turn overcoming endogenous forms of analgesia, considered next.

Analgesic molecules

Injured peripheral nerves also express analgesic mediators that dampen pain sensations. The endogenous opioids are the most prominent. For example, β endorphin and met-enkephalin are expressed by inflammatory cells at injury sites (27, 28). This form of expression may attenuate pain by altering the release of algesic molecules from inflammatory cells that infiltrate the injured nerve. In addition however, endogenous opioids can act directly on local μ opioid receptors expressed by axons, including axonal endbulbs. For example, ligation of μ opioid receptors by small near nerve doses of agonists reversed features of experimental neuropathic pain including thermal hyperalgesia and mechanical allodynia (29).

Nociceptin (orphanin FQ) is an analgesic molecule that acts on opioid receptor-like 1 (ORL1) receptors. Both the ligand and receptor are expressed by DRG sensory neurons and their expression increases following peripheral inflammatory stimuli (30). Part of their analgesic action arises from suppression of inflammatory pain mediators.

Cannabinoids are analogs of Δ^9-tetrahydrocannabinol (THC), the active ingredient of marijuana. They operate as analgesics by acting on two separate receptors, labeled CB1 and CB2 (31–33). CB1 receptors are found in the central and peripheral nervous system where they inhibit calcium influx and enhance inward-rectifying potassium channels. In contrast, CB2 receptors are largely localized to immune cells (mast cells, T cells, B cells, NK cells, microglial cells, and macrophages in addition to monocytes and PMNs) and keratinocytes. Expression may also be found on microglial cells in the dorsal horn of the spinal cord that are activated following nerve injury. After nerve injury CB2 receptor expression is dramatically upregulated on primary sensory neurons. Thus, inhibition of CB1 receptors, CB2 receptors or both attenuate neuropathic pain but CB1 inhibition may cause undesirable side-effects because of its unrelated actions on neurons (31).

Anti-inflammatory cytokines such as IL-4 and IL-10 not only counteract pro-inflammatory cytokines but also appear to have an intrinsic analgesic action (34). Overall, these molecules are coordinated within an anti-inflammatory systemic cytokine profile that attenuates neuropathic pain in humans with peripheral neuropathy (35).

Galanin is a neuropeptide that can influence pain behavior. Activation of its GALR1 receptors inhibits nociception, whereas its GALR2 receptors are excitatory. By blocking SP and CGRP action and increasing the actions of morphine, its predominant action during peripheral nerve injury or inflammation is analgesic (36). Finally, the neurotrophic molecule NT-3 acts to reduce thermal hyperalgesia by downregulating the expression of TRPV1 channels (see below) (37).

We next consider how specific anatomical sites of the peripheral nervous system and spinal cord participate in the generation of neuropathic pain. We start by considering how axons participate in peripheral inflammation.

Neurogenic inflammation and nervi nervorum

Tissue damage that generates pain is closely associated with *neurogenic inflammation*. Neurogenic inflammation refers to the active participation of terminal sensory axons in expanding and intensifying an inflammatory reaction associated with tissue damage. Classically, activated sensory axons within inflamed tissues send discharges back toward the central nervous system. At branch points along the activated sensory axon tree, axons arising send backfired antidromic (the reverse direction of physiological axon activation) discharges down axons where they release neuropeptides (38, 39). Because these territories are adjacent to those originally associated with sensory terminal activation (branch territories), the release of these neuropeptides can thereby enlarge the inflammatory response. SP and CGRP are potent vasodilators, whereas SP also causes plasma extravasation from vessels. Both may act on mast cells, causing their degranulation and release of histamine, serotonin, and proteases (39).

Through neurogenic inflammation, local neuropeptides contribute to the cardinal signs of inflammation in tissues, as elegantly described by Lewis

(38, 40): dolor (pain), rubor (redness from rises in blood flow), and tumor (swelling from plasma extravasation). Peripheral nerve trunks, self-innervated by SP and CGRP nervi nervorum axons in their epineurial sheaths, also undergo this type of enhanced inflammation (7). It is possible that inflammatory neuropathies may generate neuropathic pain through this mechanism without the presence of overt axon damage (8, 9).

Overactive neurogenic inflammation may also contribute to pain syndromes such as complex regional pain syndromes I and II (CRPS I and II). CRPS I by definition does not include actual nerve damage, whereas CRPS II does. Reflex sympathetic dystrophy (RSD) is a term previously applied to CRPS; adrenergic overactivity was thought to be involved in its pathogenesis. The evidence for prominent adrenergic involvement however, has been limited and other mechanisms may account for its features. For example, chronic activation of SP and CGRP containing C nociceptors may better account for the redness and swelling that accompany CRPS (41, 42). Next we discuss how axon degeneration may generate inflammation and pain.

Inflammation, Wallerian degeneration, Wallerian-like degeneration

Following nerve injury, axons and neurons are exposed to inflammatory pain mediators, or algesic molecules as discussed above. Specific inflammatory disorders of peripheral nerves, such as vasculitis, CIDP or GBS, may be expected to attract large numbers of lymphocytes and macrophages to the endoneurium. After simple axon injuries however, such as transection or crush, an inflammatory cascade also occurs. Augustus Waller described a sequence of pathological changes that develop distal to sharp nerve transections since referred to as Wallerian degeneration (WD) (43). Wallerian-like degeneration (WLD) refers to a near identical sequence of events in distal axons that may follow a crush or occur in other neuropathies.

Within 1–2 days following injury, axonal swellings or endbulbs develop in proximal stump axons. Their potential role in elaborating neuropeptides and expressing sodium channels was considered above. Later, by days 3–5 following nerve injury, there is an influx of macrophages into the injured peripheral nerve that is accompanied by cytokines, chemokines, NGF, NO, and other molecules. Previous studies have outlined the timetable of cytokine and chemokine expression during WD with both early rises in some mediators and later rises in others. One working hypothesis suggests that neuropathic pain may be triggered and maintained as a function of the intensity of the nearby inflammatory milieu. In conditions such as diabetes, where the onset and clearance of inflammation is delayed, neuropathic pain may be prolonged (44). During regeneration and clearance of the products of WD, neuropathic pain diminishes. The only caveat to this concept is that regenerating sprouts can exhibit mechanosensitivity and may promote some types of neuropathic pain during recovery.

Next we discuss how DRG sensory neurons participate in pain generation.

Changes within sensory dorsal root ganglia and their terminals

Sensory dorsal root ganglia (DRGs) that house the cell bodies, or perikarya, of sensory neurons, participate in the development of neuropathic pain. Normally, DRG neurons can be segregated into various types depending on their size, peptide content, and other properties (45). Small sensory neurons are classically associated with nociception, express SP, CGRP, and also express TrkA receptors that are ligated by NGF. A second population of small sensory neurons are known as non-peptidergic, are responsive to GDNF, express the GDNF and neurturin receptors GFRα1 and GFRα2, respectively, and have a binding site for the *Griffonia simplicifolia* IB4 plant lectin. These neurons project to lamina IIi (inner portion of lamina II) and are referred to as NGF unresponsive small afferents. Thus, these neurons differ from TrkA neurons that project to laminae I and IIo. After axon injuries, DRG neurons change their phenotype in numerous ways, facilitating pain discharges and allowing them to act as independent generators of ectopic discharges. Thus, after a peripheral axon lesion, ectopic discharges can arise not only from damaged axons at the injury site, but also from their parent DRG neurons. DRG neurons assume pacemaker-like properties, generating spontaneous ectopic discharges that correlate with pain intensity (2).

The alterations in DRG neurons associated with axon injury and neuropathic pain involve expression of specific ion channel subtypes. Both rises and

declines in specific channels have been described: declines in Na_v 1.9 (NaN/SNS2; TTX resistant, found in small to medium sized neurons), Na_v 1.8 (SNS/PN3; TTX resistant; found in small neurons), I_{KIR} (inwardly rectifying), and K_v1.4 (fast transient potassium channel, small neurons) and rises in Na_v1.3 (sodium channel, Naβ3 subunit in small neurons) and KCNQ2,3,5 (related to potassium channels and associated with M currents). Still other changes are described in large DRG sensory neurons but their association with neuropathic pain is uncertain, e.g., declines in K_v 1.1,1.2, T Ca^{2+}, K_vβ2.1, and I_{KIR}. Some ion channel changes in both large and small neurons differ when the ganglia are inflamed or exposed to NGF (see review by Lawson (46)).

Alterations in Na_v1.7 (PN1 or hNE9), also expressed in DRG sensory neurons, are of considerable interest (47). For example, lack of functional Na_v1.7 is associated with insensitivity to pain but paradoxically a pain syndrome known as erythromelalgia can arise from mutations of Na_v1.7. The mutated channel has altered properties that promote hyperexcitability of neurons. Patients with erythromelalgia describe the cardinal features of neuropathic pain including chronic burning sensations of the limbs. An additional pain syndrome known as paroxysmal extreme pain disorder (PEPD) is associated with a separate distinct mutation of Na_v1.7. Unexpected expression of Na_v1.7 has been identified in the axons of chronic human neuromas where they may contribute toward ectopic discharges and neuropathic pain (48). Recently, Faber et al. (49) identified missense gain of function mutations of Na_v1.7 that result in neuronal hyperexcitability in 8 of 28 patients with idiopathic painful small fiber neuropathy. The collaboration with Na_v1.8, nearly exclusively expressed in DRG sensory neurons, is also involved in the pain syndromes linked to mutant Na_v1.7 channels. Na_v1.8 may also be a critical player in other types of neuropathic pain (47). The hyperpolarization-activated, cyclic nucleotide-gated cation channel (HCN2) in Na_v1.8 expressing nociceptors appears to be critical for the firing of these neurons after nerve injury and in inflammation (50). The roles of Na_v1.9 and Na_v1.3 are less clear.

Sensory neurons also express A-type K+ channels that attenuate pain. Two subtypes known as Kv3.4 and Kv4.3 have distinguished themselves; when these subtypes are suppressed by antisense oligodeoxynucleotides, rats develop mechanical hyperalgesia without thermal hyperalgesia (51).

There are changes in calcium channel expression and function, including their interaction with adrenergic receptors (52, 53) after axon injury. A notable example is the α2δ-1 calcium channel subunit, upregulated after axon injury, and now known to be the target of the analgesic compounds pregabalin and gabapentin (54–56). N-type calcium channels (Ca_v2.2) may be particularly important in the generation of neuropathic and chronic pain. Widely distributed in the nervous system, these channels exhibit high density expression in DRG neurons and in the synaptic terminals of the dorsal horn, laminae I and II. In the dorsal horn of the spinal cord, N-type channels control glutamate and SP release (57). Peptides isolated from cone snails, such as ω-conotoxin-MVIIA and ω-conotoxin-GVIA that block N-type calcium channels, are potent analgesic molecules (58, 59). Moreover, there are two calcium channel splice isoforms of N-type calcium channels labeled e37a and e37b that have differing properties (60). E37a is expressed in 55% of nociceptive capsaicin-sensitive neurons, and contributes to SP release. E37a knockdown blocks basal thermal and mechanical nociception, thermal and mechanical inflammatory hyperalgesia tested using the formalin paw test, and thermal hyperalgesia in the chronic constriction (CCI) neuropathic pain model. E37b has fewer effects overall but alters tactile allodynia. Inhibition of the binding of collapsin response mediator protein 2 (CRMP-2) to Ca_v2.2 decreased neuropeptide release from DRG neurons and reduced excitatory synaptic transmission in the spinal cord dorsal horn (61).

In addition to ion channels, there is a wide repertoire of molecular changes in DRG sensory neurons that have sustained an injury to their axons. Some are associated with the development of pain, whereas others prepare neurons for regenerative activity (RAGs, or regeneration associated genes). Morphological changes accompany alterations of RAGs, retrograde changes known as the "cell body reaction" (or the "axon reaction": central chromatolysis and displacement of nuclei to the periphery of the cell (62)). Medium diameter sensory afferents, newly expressing SP, initiate the transmission of pain information. After axotomy, small neurons upregulate galanin, a potential analgesic peptide, downregulate μ opioid receptors, and downregulate the peptides SP and CGRP, the TrkA NGF receptor, and P2X3 purinergic receptors. P2X3 receptors are prominent on IB4 non-peptidergic neurons. After injury, large neurons upregulate BDNF,

TNFα, NPY, and α2 adrenergic receptors. In contrast to the downregulation of opioid receptors after injury described above, a partial nerve injury model showed increases in μ-opioid receptors (MORs) expressed by sensory neurons ipsilateral to the injury (29). Peripheral inflammation also generates rises in CGRP, SP, and BDNF in DRG neurons that contrast to the declines observed after injury (63).

DRG neurons express κ and δ opioid receptors, sometimes colocalized, that influence pain transmission and that may change during inflammation (64). Small DRG neurons express δ-opioid receptors (DOR) that, like MORs, are analgesic (63). Nociceptin and its receptor ORL1 are expressed in larger numbers of DRG neurons after peripheral inflammatory lesions (30).

Sensory neurons that are not primarily injured but housed in the same DRG as injured neighbors may change their phenotype in ways that promote neuropathic pain. This interesting phenomenon may arise from interactions of intact "en passant" axons with inflammatory mediators associated with damaged neighbours, or through interactions within the DRG itself. Examples of neurons that change their DRG phenotype when not directly injured are those containing CGRP (65) and P2X3 (66). P2X3 receptors, discussed above, are cation channels activated by ATP, exclusively localized to DRG and trigeminal sensory neurons. They may facilitate pain neurotransmission. CGRP, as discussed above, arises from small and medium sized sensory neurons. It has a role in neurogenic inflammation as a vasodilator and potentiates SP nociceptive signaling in the dorsal horn of the spinal cord.

Another important family of molecules expressed by sensory neurons are the transient receptor potential proteins (TRPs), ion channels permeable to cations. The classical TRP channels include the capsaicin receptor (TRPV1) that mediates heat and acid stimuli and the TRPM8 or menthol channel that mediates cold sensations. Other TRPs include TRPV2,3,4 and TRPA1 (mild warming for TRPV4; moderate warming to 31°C for TRPV3, heat for TRPV1 and noxious heat for TRPV2 with mild cooling by TRPM8 and noxious cooling by TRPA1). Ipsilateral to a partial nerve injury (CCI, chronic constriction injury), increases in TRPA1, TRPV1, TRPV2, and TRPM8 were observed (37, 67). Rises in TRPV1 occur in small to medium sized neurons in the DRG and could be suppressed by NT-3 (37). TRPM8 (transient receptor potential melastatin 8), the cold- and menthol-sensitive receptor neurons comprise 5–10% of the DRG neuron population; this proportion increased ipsilateral to an experimental neuropathic lesion (chronic constriction injury) along with a rise in their sensitivity (68). This may be a mechanism for the process of cold allodynia, the perception of innocuous cold stimuli as painful.

Acid-sensing ion channels (ASICs) also participate in the generation of pain, with functions and expression patterns that overlap with TRPs (69). These are voltage-independent depolarizing cation channels largely used by sodium ions. The PNS expresses ASIC 1a,b, 2a,b, and ASIC3. ASIC3 are the most well characterized and serve as mediators of acid-induced cutaneous pain, integrators of thermal hypersensitivity during inflammation, and mediators of muscle pain. Bohlen et al. (70) examined the toxin from the venom of the Texus coral snake (*Micrurus tener tener*) and purified a toxin responsible for the intense pain generated by this venom. The toxin, MitTx, was highly selective for ASIC1a and 1b channels.

In addition to morphological and molecular changes in parent neuron perikarya after injury, there are also changes in the behavior of their neighbouring glial cells. Sensory neurons in DRG are closely surrounded by perineuronal satellite cells, cytoplasmic poor relatives of SCs that closely wrap them. Remarkably, these cells enlarge and proliferate after injury indicating a sensitivity to events involving the neuron some distance away. While it is known that satellite cells can elaborate growth factors, it is also possible that they participate in the neuronal remodeling that leads to neuropathic pain. This hypothesis remains unexplored. Macrophages influx into DRG after distal axotomy and can elaborate IL-6, and likely other inflammatory mediators (71–74). Thus, both changes in perikaryal properties and involvement in neighboring cells may allow DRGs to act as pacemakers as neuropathic pain develops.

Injuries to peripheral nerves also induce retrograde sprouting of sympathetic adrenergic axons from nearby perivascular innervation into ganglia (75–77). The axons form pericellular basket-like structures in which tyrosine hydroxylase (TH) varicosities contact sensory neuron perikarya. This interesting property depends on NGF availability in the ganglia as a result of retrograde transport of the growth factor from target tissues. Noradrenergic sympathetic sprouting varies in its latency of onset in experimental nerve injury and pain models. This intriguing phenomenon

may be related to "sympathetically mediated pain" as described in CRPS I and II (Chapter 18).

The possibility that neuropathic pain might also be generated by direct damage to DRGs is also a consideration. Relatively little work has examined the consequences of direct traumatic DRG injury. The possibility that common pain syndromes such as cervical or lumbar spondylosis may involve compression and damage of DRG neurons is addressed in Chapter 3 (cervical radiculopathy).

Alterations in DRG neuronal behavior "feed forward" into altered processing of pain discharges in the central nervous system, considered next.

Role of the dorsal horn of spinal cord

The dorsal horn of the spinal cord (and the nucleus of the spinal trigeminal tract) is the first central afferent center to receive nociceptive discharges. Their complex circuitry predictably influences the information that ascends to other CNS centers. Similarly, their output can be modified by descending systems that are largely anti-nociceptive but also pro-nociceptive. As one might expect, their connections also undergo important changes in the setting of peripheral nerve injury or inflammation.

The dorsal horn is divided into Rexed laminae, the outermost of which by definition is layer I. Unmyelinated C afferents largely synapse in layer II (IB4 neurons may also synapse in layer I), also known as the substantia gelatinosa. Small myelinated Aδ nociceptive afferents synapse in layers I and V. The most important neurotransmitter released by afferents is glutamate, in turn acting on NMDA and AMPA receptors of second order sensory fibers or interneurons (63). Other neurotransmitters that act in concert with glutamate include the neuropeptides SP and CGRP. Dorsal horn neurons can be classified as projection neurons with axons travelling to higher centers or interneurons that are capable of influencing pain transmission. The projection neurons are more likely to be found in lamina I and they cross the midline to ascend in the spinothalamic tract (78). Interneurons are classified as nociceptive-specific (majority), polymodal, or wide dynamic range, coding both innocuous and injurious stimuli and they synapse, among others, with projection neurons. Many interneurons also demonstrate the property of windup referring to an increase in their sensitivity with repeated stimuli. This property is mediated by glutamate through NMDA (N-methyl-D-aspartate) receptors. Predictably, infusion of NMDA intrathecally in rats generates a regional pain syndrome (79). Recently, zinc has been discovered to be an endogenous modulator of excitatory neurotransmission in the spinal cord through NMDA receptors containing a particular subunit named NR2A (80). The adipocytokine leptin is a molecule that enhances NMDA receptor function in neuropathic pain (81, 82).

Mapping the activation of the proto-oncogene c-fos protein Fos has allowed analysis of the neuroanatomic basis of pain pathway participation after injury or inflammation, and its modulation (e.g., by analgesics) (see review by Coggeshall (83)). Fos rapidly rises in neurons during activation of the nociceptive or pain pathways and its behavior is specific, induced by few stimuli beyond pain. Although its expression can persist in chronic pain states, its expression is typically short-term in a maximum range of 30 min to 2 h. This facilitates its analysis in acute pain studies. Laminae I and II develop intense expression of Fos after noxious stimuli followed by upregulation in lamina V and others. Higher centers that express Fos include the reticular formation, the nucleus of the solitary tract, the parabrachial nucleus, the periaqueductal gray, the locus coeruleus, the hypothalamus, the thalamus, and the cortex. Fos upregulation follows peripheral nerve lesions, spinal cord damage, dorsal root avulsion, and DRG irritation. Interestingly, simple dorsal root injury does not activate Fos. As expected from the earlier discussion, Fos expression can be attenuated by a range of analgesics including: opioids, NMDA antagonists, $GABA_B$ agonists, NK1 antagonists, cannabinoids, and nonsteroidal anti-inflammatory agents (83).

The remodeling of architecture in the dorsal horn of the spinal cord following nerve injury is not confined to neurons. Microglia, described as the resident inflammatory cells of the CNS, are activated in the dorsal horn within 24 h of a peripheral nerve injury. There are morphological changes, proliferation, and rises in expression of several critical molecules including CR3, toll-like receptor-4, CD14, CD4, and MC class II protein (84). Key microglial proteins such as P2X4 receptors and the second messenger p38 MAP kinase are likely critical in mediating pain signals. Microglia may facilitate pain signaling by dampening dorsal horn inhibitory mechanisms or facilitating excitation. BDNF released from microglia appears to be an important participant in central sensitization (85). Beggs and Salter (86) suggest that enhanced BDNF release acts on lamina I neurons of the doral horn

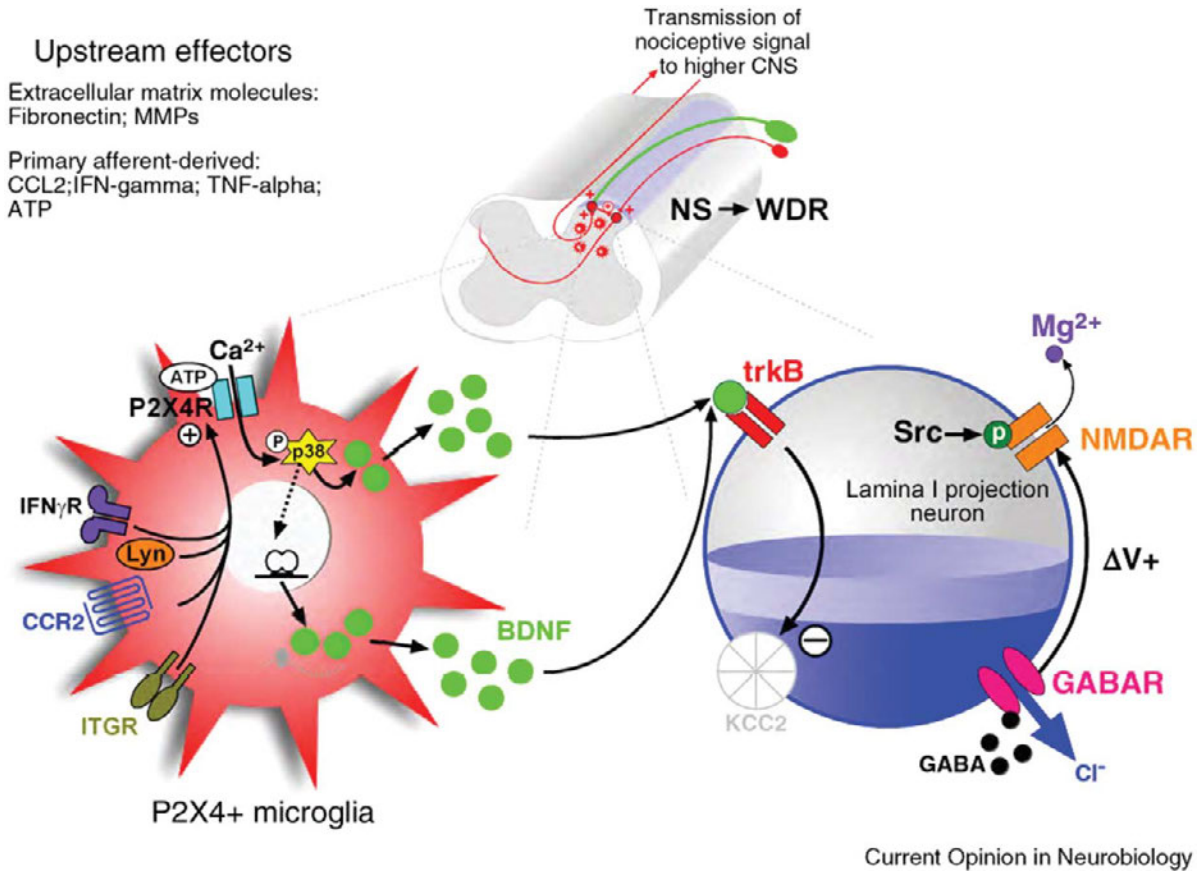

Figure 1.2. Hypothesis linking events in microglia with projection neurons in the dorsal horn of the spinal cord as proposed by Beggs and Salter (86). In this scheme, activation of P2X4 receptors increases BDNF synthesis and release through a p38MAPK-dependent mechanism. BDNF downregulates KCC2 channels in the membranes of dorsal horn lamina I neurons, reducing GABA inhibition and increasing neuronal excitability in response to NMDA receptor agonists. The overall result is to change neurons that are normally nociceptive-specific to become wide dynamic range in phenotype, increasing hyperalgesia, allodynia, and spontaneous pain. Reproduced with permission from Beggs and Salter (86).

through TrkB receptors to suppress KCC2 chloride transporters. This increases intraneuronal Cl^- levels, leading to a role of GABA receptors in rendering neuron hyperexcitability. Both diminished GABA inhibition and facilitated glutamatergic input contribute to a change in neurons from a nociceptive specific to wide dynamic range phenotype, contributing to hyperalgesia, allodynia, and spontaneous pain [Figure 1.2].

Diminished synaptic inhibition may also be caused by spinal endocannabinoids using CB1 receptors, which are activated upon strong nociceptive simulation and reduce the release of GABA and glycine (87). Overall, changes in the excitability of the dorsal horn involve several mechanisms that include specific changes in spontaneous excitability (88), windup enhancement of central sensitivity through NMDA (89, 90), and loss of GABAergic inhibition (91).

Brainstem relays and the descending modulation of pain

There are two main brainstem pain centers: the parabrachial area found in the midbrain and rostral pons and the periaqueductal gray area of the midbrain. Parabrachial neurons project to the amygdala and ventrolateral and medial hypothalamus (78). The periaqueductal gray area projects descending axons to the spinal cord. Closely associated with the periaqueductal gray neurons are those of the rostral ventromedial medulla.