Underlying Mechanisms of Neuropathic Pain

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Educational Objectives

1. Describe peripheral mechanisms of neuropathic pain.

2. Explain spinal and brain circuits involved in neuropathic pain.

3. Discuss translational aspects of neuropathic pain mechanisms.

Introduction

The International Association for the Study of Pain (IASP) defines neuropathic pain as pain that is caused by a lesion or disease of the somatosensory system (www.iasp-pain.org; see also [87]). Neuropathic pain may persist beyond the phase of the acute injury, turning into a chronic disease. The pathophysiology of neuropathic pain includes a complex and redundant system of neural generators, circuits, and mediators that we are only beginning to understand. The neuroanatomical changes in response to injury consist of a combination of peripheral and central adaptive and maladaptive mechanisms. The adaptive processes are varied and include changes in both pronociceptive and antinociceptive systems. This chapter will focus on neuronal properties, ion channels and receptors, synaptic physiology, and the regulation of inflammatory mediators after nerve injury. Since most progress has been made through research in animal models, we will carefully explore which findings have been successfully translated to the situation in human disease.

Neuropathic Pain Pathophysiology: The Peripheral Nervous System

Background

Normally, sensory experience arises when impulse discharges initiated by noxious stimuli applied to nociceptive sensory endings in skin, viscera, and other peripheral tissues reach a conscious brain. When tissues become inflamed (e.g., secondary to burns or infections), nociceptive endings may become hypersensitive, resulting in inflammatory pain. But when frank injury or disease has affected peripheral nerves (neuropathy), sensory ganglia (gangliopathy), or sensory roots (radiculopathy), pain signals may arise at ectopic locations at a distance from the sensory ending. The resulting abnormal impulse discharge gives rise to neuropathic pain. The pathophysiological changes responsible for the development of ectopic impulse discharge ("ectopia") can be precipitated by a wide variety of events including trauma (frequently iatrogenic), nerve entrapment, infection (bacterial or viral), sterile inflammation, metabolic abnormalities, malnutrition,

vascular abnormalities, neurotoxins (including many chemotherapeutic agents), radiation, inherited mutations, and autoimmune attack. Each event gives rise to one or more distinct clinical diagnoses, where the common denominator is the presence of neural pathology and resulting ectopia. The most common locations of ectopic impulse generation are the site of injury itself and associated dorsal root ganglia (DRGs). In addition to directly driving pain sensation (spontaneous or evoked), the abnormal impulse discharge in the periphery may induce and maintain various pain amplification processes in the central nervous system (CNS). These processes fall under the general headings of "central sensitization" and "endogenous pain modulation." In this section we will touch on the characteristics of ectopic impulse generation, some of the cellular mechanisms that underlie it, and how currently available drug treatments affect these mechanisms to provide a measure of pain relief. In sections below we will discuss how impulses generated in the periphery, in healthy or inflamed sensory endings, or ectopically in the event of neuropathy, amplify nociceptive signals at synaptic relays in the CNS.

Nerve Injury and Disease Trigger Ectopia

From Neural Pathology to Pain

All of the untoward events that underlie the various neuropathic pain diagnoses cause frank pathology in the axon or cell soma of sensory neurons and/or disruption of the myelin sheath that surrounds myelinated axons (dysmyelination and demyelination). Disruption of the sheath that bundles nonmyelinated axons (Remak bundles), which, like myelin, also derive from Schwann cells, may contribute as well. It is clear why damage that prevents axons from propagating nerve impulses causes hypoesthesia and numbness, so-called "negative" symptoms, given that nerve conduction is disrupted. It is much less obvious why such pathology may also cause "positive" sensory symptoms and signs such as ongoing and evoked paresthesias, dysesthesias, and pain.

The link between nerve pathology and abnormal sensation is ectopic impulse generation. If nerve injury happens suddenly, a brief, sharp sensation arises owing to "injury discharge" in the acutely severed axons. In general, however, ectopia results from secondary changes in the afferent neuron that develop over time [25]. It is important to note that ectopic

discharge and consequent neuropathic pain are not obligatory consequences of nerve injury. They do not always occur. Simple paper cuts, for example, always cut fine axonal branches but only rarely precipitate neuropathic pain. Likewise, it is very uncommon for tooth extraction or root canal treatment to provoke chronic pain. Even major nerve injury does so in only some individuals. There appear to be other predisposing factors that determine whether painful ectopia will arise after a particular type of nerve injury in a particular individual. One factor that has captured the imagination of investigators recently is genetic predisposition [5].

Spontaneous Ectopic Discharge

When conditions are right, a significant fraction of sensory neurons begin to fire spontaneously in the hours and days following nerve injury, as many as one third in some experimental preparations in animals. Microneurographic recordings in patients with nerve injury have revealed that similar changes occur in humans diagnosed with a variety of chronic pain conditions, including some conditions such as fibromyalgia and phantom limb pain that were previously assumed to originate in the CNS [25,63,76]. This information has led some investigators to speculate that chronic pain after nerve injury always has its origins in the periphery, and that secondary CNS changes serve to amplify and distort primary peripheral nervous system (PNS) signals but do not drive pain directly. This possibility could have important consequences for the targeting of therapeutic interventions.

There are a number of alternative cellular origins of spontaneous ectopic impulse activity, which include the swollen endbulbs that form in the neuroma just proximal to sites of axonal transection [39], outgrowing sprouts, patches of demyelination, and the cell soma in the DRG. In animal experiments, sustained ectopic firing usually begins earliest in myelinated axons (A fibers), sometimes only hours after axotomy, with a preference for muscle over cutaneous afferents. Activity in unmyelinated (C) fibers tends to develop in earnest later, after a few weeks. Specific parameters vary with the type of nerve, the type of injury, the impulse source (e.g., axon or soma), and the strain and species of the animal studied [25]. Firing pattern is usually tonic-rhythmic (i.e., continuous), bursty, or irregular, with the occasional neuron showing complex, often cyclic, variations in firing pattern. Firing rates vary from very slow (<1 impulse/second)

to very high (>100 impulses/second, especially within impulse bursts). The process that underlies each of these patterns is reasonably well understood [1]. Interestingly, observations in nerves in which only some of the axons have been severed show that spontaneous ectopic activity may also develop in neighboring "uninjured" axons, particularly in C fibers [79,92]. The complex and idiosyncratic mix of fiber type, firing pattern, and firing rate is probably the primary determinant of the quality of the neuropathic pain experienced by patients: steady burning, stabbing, cramping, or paroxysmal shock-like, for example. By the same token, sensory quality can provide diagnostic information on the nature of the ectopia. Such information, in principle at least, could inform the choice of drugs that might be effective.

Ectopic Mechanosensitivity

An intense stabbing or shock-like sensation is commonly evoked by gentle tapping over sites of nerve injury, nerve entrapments, and neuromas; this sensation is the Tinel sign. Other maneuvers that apply mechanical force to injured nerves or spinal roots evoke similar sensations. Examples are straight leg lifting in patients with radicular pain (Lasègue's sign) and the signs of Spurling and Lhermitte. Tapping along the length of a nerve with disseminated neuropathic changes, such as in painful diabetic neuropathy, may also evoke dysesthesias. These sensory signs correspond to the experimental observation, in animals and in microneurographic recordings from humans, that show that transient mechanical stimulation at ectopic pacemaker sites triggers ectopic discharge [25,63]. Interestingly, the discharge frequently outlasts the stimulus itself, fading away only after many seconds or a minute or two. This "afterdischarge" corresponds to the "aftersensation" that is felt. The fact that the ectopia and the sensation it drives outlast the stimulus indicates that the underlying mechanism is not just direct impulse activation, or signal amplification. Rather, it indicates that the stimulation has kindled an intrinsic repetitive firing process at the ectopic pacemaker site, a sort of transient spontaneous firing.

The presence of mechanosensitivity does not necessarily mean that there will also be spontaneous firing. In fact, mechanically evoked ectopia in the absence of spontaneous ectopia is a common observation. In contrast, it is unlikely that spontaneous ectopia ever occurs in the absence of mechanosensitivity. Experimental studies have shown that when an ectopic pacemaker site fires spontaneously, the spontaneously generated impulses and the ones evoked by mechanical stimulation originate at precisely the same location, and therefore they are probably due to the same pathophysiological impulse-generating process (discussed below). Because of the common impulse-generating process, in real-life situations it is easy to confuse pain due to mechanically evoked ectopia (and afterdischarge) with pain due to spontaneous firing. During movement (walking, running) and even simple weight bearing, mechanical forces are very likely to be applied to ectopic pacemaker sites [6]. Even while we are sitting quietly, mechanical forces may also apply as we adjust our posture.

Other Factors that Enhance Ectopic Firing

In addition to mechanical forces, ectopic pacemaker sites develop abnormal sensitivity to a variety of other factors. A potentially important factor is activation by circulating catecholamines and by norepinephrine released from postganglionic sympathetic axons [26]. Resulting sympathetic-sensory coupling is thought to contribute to sympathetically maintained neuropathic pain states. Abnormal sensitivity to local and circulating inflammatory mediators such as tumor necrosis factor α (TNF-α), interleukin 1- β (IL1-β), and bradykinin is a second example. The emergence of sensitivity to inflammatory mediators in injured afferents blurs the distinction between inflammatory pain and neuropathic pain. When these mediators sensitize or activate sensory endings, we speak of inflammatory pain. When the same molecules enhance activity at ectopic pacemaker sites, the resulting pain is neuropathic [25].

Temperature changes (notably cooling), ischemia, hypoxia, hypoglycemia, and other conditions capable of locally depolarizing afferent neurons at sites that have developed ectopic pacemaker capability can also enhance ectopic discharge and the resulting pain sensation [25]. Intact sensory endings may be sensitive to these chemical and physical stimuli, but mid-nerve axons (where neuromas form after trauma) generally are not. For example, press on the median nerve in your forearm. You feel your fingertip on the overlying skin. But if this pressure had activated axons in the median nerve, you would have felt a tingling sensation in the palm of your hand. If the nerve was healthy at the spot where you pressed, you presumably did not feel such tingling. This simple demonstration illustrates that intact sensory neurons are basically incapable of generating sustained impulse discharge at mid-nerve.

They are designed to fire exclusively in response to natural stimuli applied to the sensory endings in skin, muscle, etc., where the neuronal membrane is specialized for impulse initiation. The key change in neuropathy is not so much that physical and chemical activating factors are brought to bear at ectopic locations, but that nerve fibers develop an abnormal ability to respond to them.

Cellular Mechanisms of Ectopic Impulse Generation

The cascade of cellular events that lead to the development of ectopic pacemaker capability and ectopic neuronal discharge begins with the injured primary sensory neuron. Our current understanding of the process is as follows.

Altered Gene Expression

Axonal transection blocks the normal retrograde flow of neurotrophic signaling molecules—glial-derived neurotrophic factor (GDNF), nerve growth factor (NGF), and perhaps others—between the peripheral innervated tissue and the sensory cell body in the DRG. This process triggers a change in the quantity of various genes "expressed" (in effect, proteins synthesized) by the cell body. The proteins synthesized are exported by anterograde axoplasmic transport to the membrane of (1) the DRG cell soma, (2) patches of mid-nerve demyelination and (3) the ending(s) of the injured peripheral axon, notably neuroma endbulbs and sprouts [8,18,93]. These changes, in turn, alter the "phenotype" (functional behavior) of the injured afferent neuron, supporting the emergence of ectopic electrical impulse generation and abnormal sensitivity to its chemical and physical environment. Remarkably, the expression of several thousand genes are up- or downregulated (i.e., increased or decreased) in the DRG after nerve injury. Of these thousands of genes, it is not yet clear which are critical for neuronal hyperexcitability and pain, and which relate to other processes triggered by nerve injury such as nerve regeneration. Proteins synthesized in the cell body are also transported to the central (synaptic) endings of the afferent axon, where significant functional changes also, no doubt, occur. This aspect of nerve pathophysiology has not received as much experimental attention to date as the peripheral axon end.

Prominent among the numerous proteins whose synthesis is regulated by nerve injury are the ion channels that are known to form the basis of nerve impulse generation (the "molecules of excitability"). The most important of these are voltage-gated Na^+ channels, the channels that generate the upstroke of the nerve action potential. Voltage-sensitive K^+ channels tend to hold impulse generation in check. Interestingly, at least some of these channels are downregulated in afferent neurons after axotomy, "releasing the brakes" on repetitive firing. This downregulation is thought to contribute substantially to ectopia [49]. Other channel types and conductances thought to contribute are certain Ca^{2+} channels, hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (the Ih "pacemaker" channel), KCNQ potassium channels, the β_4-subunit-associated resurgent Na^+ current, and a persistent Na^+ conductance (gNaP). Their specific role, at this stage, remains uncertain.

Altered Protein Trafficking

In addition to changes in gene expression in injured sensory neurons, the delivery ("trafficking") of transported molecules to their intended address is disrupted. The most important change of this sort is the accumulation or depletion of molecules responsible for neuronal excitability at ectopic impulse generation sites. Most notable is the accumulation of Na^+ channels in axon sprouts, in neuroma endings, and in areas of demyelination at ectopic pacemaker sites [25]. This accumulation has been documented in painful neuromas dissected from neuropathic pain patients as well as in animal neuropathy models. Many other molecules involved in impulse initiation, both ion channels and membrane receptors of various sorts, are also known to accumulate in neuromas. Interestingly, even though the synthesis of most of the different types of Na^+ channels is downregulated within the cell soma, these channels still accumulate in excessive numbers at the axon ending. Altered synthesis and altered trafficking need to be considered as different processes.

Altered Behavior of Channel and Receptor Molecules

A third process that contributes to ectopic impulse generation is altered channel kinetics. The determinant of neuronal excitability is not ion channel proteins per se, but the ionic currents they carry. Increasing the mean time that a channel is open, for example, will increase current flow (and the activation of neurons) just as much as having an increase in the number of ion channels present. Proinflammatory

cytokines and other mediator molecules associated with neuropathy can affect the behavior of ion channels in this way. For example, cyclic adenosine monophosphate (cAMP)-dependent phosphorylation of Na^+ channel molecules reduces Na^+ current, whereas dephosphorylation returns it to normal (e.g., [41,52]. A variety of hormones, neurotrophic factors, cytokines, prostaglandins, and other inflammatory mediators can activate protein kinases A and C (PKA and PKC) and are hence positioned to affect ion channels and the basic excitability of afferents, not just to depolarize and excite sensory endings. Corresponding changes are known to occur also in the receptor molecules that accumulate in the axonal membrane in neuropathy, contributing to pathophysiological mechanosensitivity, chemosensitivity, and so on. Glial and immune cells have been identified as a significant source of molecules that alter neuronal excitability.

Drugs with Efficacy in Neuropathic Pain Affect Ectopic Impulse Generation Peripherally

One of the enduring embarrassments in the field of pain science and medicine is that most of the drugs with proven efficacy in the treatment of neuropathic pain were adopted post hoc from other disciplines, rather than having been developed with the aim of pain relief. Thus, the first-line analgesic agents used in the treatment of neuropathic pain are anticonvulsants, antidepressants, antiarrhythmics, and local anesthetics [38] (http://papas.cochrane.org/our-reviews). While on the face of it these agents represent highly diverse drug families, there is a common denominator. All of the agents that are effective analgesics in neuropathy are "membrane stabilizers"; they reduce membrane excitability and hence suppress ectopic neural discharge [24]. Importantly, only some members of each of these drug families can relieve neuropathic pain. For example, barbiturates and benzodiazepines are powerful anticonvulsants, but they act synaptically; they are not membrane stabilizers, do not suppress ectopia and are not analgesic. The ones that are analgesic, such as carbamazepine, phenytoin, and gabapentin, all suppress ectopia, mostly by acting on ion channels. The same holds true for antidepressants. Tricyclics, and to a lesser extent serotonin and norepinephrine-selective reuptake inhibitors (SNRIs), are effective pain relievers. Although they are best known as inhibitors of catecholamine reuptake, they are also powerful Na^+ channel blockers and hence membrane stabilizers. Selective serotonin reuptake inhibitors (SSRIs), in contrast, are ineffective at stabilizing membranes and correspondingly are ineffective at relieving pain [27].

Unfortunately, the clinical usefulness of these drugs is limited by the presence of difficult or intolerable side effects: sedation, vertigo, and nausea. The reason is that, just as they suppress the firing of afferent neurons, they also alter the firing of neurons in the brain. Since most of the desired analgesic action of these drugs is in the PNS (suppression of ectopia), whereas the unwanted side effects occur mostly in the CNS, in theory there are a number of potential avenues for improving their therapeutic profile. For example, one could better target drug delivery to PNS pain generators (e.g., DRGs), or reduce drug permeation through the blood-brain barrier (while preserving PNS activity).

Substrates of Central Sensitization

Beyond abnormal activity in primary afferents, misprocessing at the central level is a key element to exaggerated pain (hyperalgesia) but also aberrant pain sensation (allodynia). The phenomenon is often referred to as "central sensitization," where the gain of transmission from sensory fibers can be seen as being amplified at the different relay levels in the CNS (the dorsal horn of the spinal cord, brainstem/thalamic nuclei, or even at the cortical level) [71,99]. Yet, what is most likely more critical is the perception of pain in response to stimuli that do not activate nociceptors. In fact, it appears that for tactile allodynia in neuropathic pain, sensitization of nociceptors is not the explanation, and tactile pain results from activation of low-threshold afferents, including Aβ afferents [25]. How can innocuous input trigger signals that are processed centrally to be interpreted as painful? Several scenarios can be envisaged, and multiple etiologies are likely to be involved. Here, we will focus on spinal mechanisms because this is where most of the cellular signaling studies have been performed and knowledge is most advanced, but many of the principles illustrated can be transposed to higher relay stations in nociceptive pathways.

A large body of evidence indicates that injury either to the PNS or CNS causes a number of changes in the CNS, which lead to abnormal information processing and hyperexcitability. These changes include release of neuromodulatory compounds from sensory fibers, intrinsic spinal neurons, and glial

cells (microglia and astrocytes); changes in the intrinsic properties of neurons involved in the integration and relay of sensory information; plastic changes at synapses between sensory afferents and spinal neurons; and loss of cells or rewiring (for reviews see [71,96]). The end result can be summarized into two general phenomena: enhanced excitation and diminished inhibition. These changes will affect the gain of transmission at the central level, causing enhanced output to supraspinal areas in response to a given input, as well as unmasking normally silent interconnections, yielding responses to innocuous input within normally purely nociceptive pathways.

Enhanced Excitation

Enhancing the gain of transmission at the spinal level has been suggested as an important substrate of pain hypersensitivity [71,91]. It can take several forms, including increase in discharge of spinal output (summation) to repetitive input from C fibers (wind-up). The latter is a short-lived process that subsides immediately upon cessation of input. Interestingly, the established enhanced output in wind-up is very sensitive to NMDA-receptor blockade, which separates spinal pathways from other areas of the CNS in this respect and may have to do with the lower Mg^{2+} sensitivity of the NMDA-receptor subtypes prevailing in spinal lamina I excitatory neurons [84]. More relevant to central sensitization is likely the phenomenon of long-term potentiation (LTP) to C-fiber stimulation, which involves a longer-lasting increase in gain at afferent to dorsal horn synapses [71]. Whether LTP in nociceptive pathways can be maintained over very long periods (days to years) to explain chronic pathological pain remains to be elucidated. Evidence indicates that some form of sustained afferent input is necessary to maintain neuropathic pain [28,71,99].

A key feature of neuropathic pain is allodynia, which entails a central nociceptive response to innocuous stimulation. Evidence that normally nociceptive specific spinal neurons respond to innocuous input after nerve injury [46,51] indicates that normally silenced connections must be unmasked, or new connections formed, to explain the change in properties of these cells. For LTP to be a substrate of such phenomena would mean that enhancing the gain at some synapses causes normally subliminal interconnections between pathways to "break through" and enable input from low-threshold afferents to reach nociceptive output neurons. It is interesting in this context to note that polysynaptic interconnections exist between Aβ afferents and spinal lamina I neurons, although they do not normally receive direct monosynaptic input from these afferents. However, these functional polysynaptic connections appear to involve strong NMDA-receptor-mediated components [85]. Thus, analgesia by NMDA-receptor antagonists may reflect blockade of these polysynaptic pathways rather than LTP reduction.

Disinhibition

Disinhibition appears to be a common substrate with other pathophysiological conditions of the CNS, such as traumatic injuries and epilepsy [68,86]. It results in a general increase in the excitability of individual neurons and networks of neurons, but it also has the potential to allow inputs to be relayed through pathways that are normally kept silent by inhibition, thus destabilizing neuronal circuits.

Over 80% of spinal lamina I output neurons are nociceptive specific and do not receive direct input from low-threshold afferents [46,70] (although they can receive such input indirectly through feed-forward excitatory interneurons [17]). Yet, the majority spinal lamina I output neurons respond to innocuous touch after nerve injury [46]. Blocking γ-aminobutyric acid A ($GABA_A$) and glycine receptors replicates symptoms of neuropathic and inflammatory pain [77,78,80,82,94] and unmasks low-threshold responses to tactile stimulation in spinal and trigeminal lamina I neurons [46,60]. Similarly, normally nociceptive-specific thalamic neurons respond to innocuous input following blockade of spinal glycine receptors [78]. This response can be explained by the unmasking of polysynaptic links between Aβ fibers and lamina I neurons that are normally repressed by spinal inhibition [3,46,53,60,85], effectively allowing a crosstalk between low- and high-threshold pathways.

In consistency with this explanation, studies have shown that peripheral nerve injury causes loss of inhibition at the spinal level. Potential underlying mechanisms include degeneration of inhibitory interneurons [44,62,72], although hypersensitivity after nerve injury has been reported without any apparent loss of interneurons [66,67], and altered expression of GABA (or its synthesizing enzyme glutamate decarboxylase) and/or $GABA_A$ receptors, although findings appear to differ depending on chronic pain models and

animal strains [10–12,62,81]. An alternative mechanism that has emerged is the dysregulation of Cl⁻ transport associated with loss of activity of the K⁺-Cl⁻ co-transporter KCC2 in spinal dorsal horn neurons, causing intracellular Cl⁻ accumulation and an ensuing loss of hyperpolarizing inhibition [20,30,45,98].

Whether a similar switch from nociceptive-specific to polymodal occurs in other spinal nociceptive output pathways had remained elusive until recently. Indeed, in the deep spinothalamic tract (STT), multimodal (wide-dynamic-range [WDR]) neurons, able to respond to both innocuous and noxious mechanical input, are prominent [16], leaving open the question of whether allodynia results from a change in response dynamics within a common relay pathway (e.g., WDR neurons) or a switch in modality specificity within a normally dedicated nociceptive pathway (nociceptive-specific neurons). A recent quantitative neurometric study in rats showed that within the STT pathway, only neurons that are nociceptive-specific under normal conditions exhibited altered response properties after nerve injury; WDR STT neurons exhibited no change in threshold or input-output function, ruling out the participation of the latter cells to tactile allodynia [51] (in contrast to local, non-projection neurons, which explains why previous studies most likely missed this dichotomy). Blocking inhibition replicated the specific change in input-output properties of nociceptive specific neurons (with no effect on WDR STT cells), indicating that central disinhibition was sufficient to explain the change in threshold of the spinal neurons. In fact, combined blockade of $GABA_A$ and glycine receptors was necessary to fully replicate the effect of nerve injury. Consistent with the fact that both $GABA_A$ and glycine receptors are Cl⁻ channels, antagonizing KCC2 replicated the effect of combined $GABA_A$/glycine receptor blockade and of nerve injury, suggesting that disinhibition from impaired Cl⁻ transport underlies nerve-injury-induced allodynia [51].

Restoring central inhibition by rescuing KCC2 function [40] restored the modality specificity of normally nociceptive-specific STT neurons [51]. Hence, disinhibition was not only sufficient but also necessary to explain unmasking of low-threshold input to nociceptive-specific spinal output neurons. Crosstalk between low- and high-threshold pathways, which causes a switch in modality specificity within normally nociceptive-specific spinal relays, thus appears to be a general rule for allodynia [51]. Furthermore, the same

concept can be extended to supraspinal levels. While a functional switch in normally nociceptive-specific pathways can be explained by the establishment of crosstalk between parallel sensory lines at the spinal level, similar crosstalk can develop at supraspinal levels (Fig. 1). This possibility could explain the good, although short-term, efficacy of neurosurgical ablation of selected spinal ascending tracts in alleviating pain in patients [13,61].

A complex cascade of signaling events underlies Cl⁻ dysregulation in spinal neurons. It appears to involve upregulation and release of the chemokine monocyte chemoattracting protein 1 (MCP-1) from damaged afferents into the spinal cord to cause microglial activation and chemotaxis of circulating monocytes via activation of the CCR2 receptor [97]. Activated microglia proliferate and migrate to the site of projection of the damaged afferents into the dorsal spinal cord [4]. These microglia express de novo the $P2X_4$ receptors, and when stimulated by endogenously released ATP, they cause the release of brain-derived neurotrophic factor

Thalamus

Spinal cord

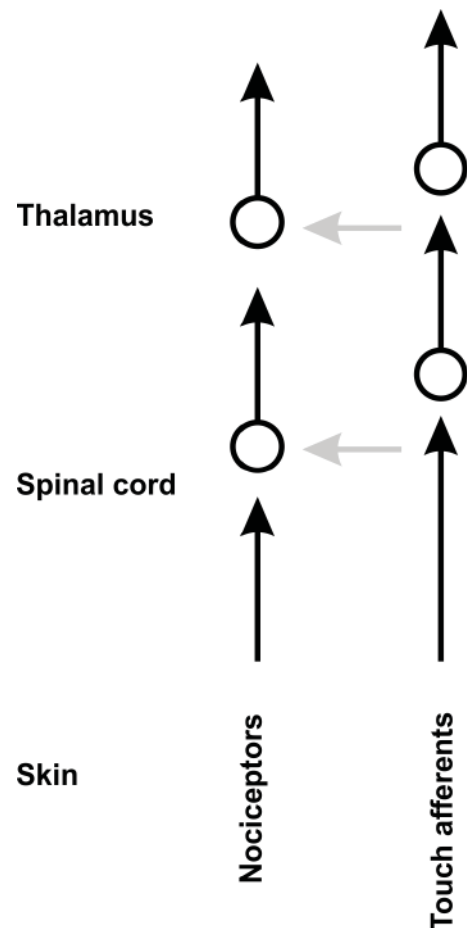**Skin**

Nociceptors

Touch afferents

Fig. 1. Crosstalk between nociceptive and non-nociceptive channels at any level in the ascending sensory pathways can explain tactile allodynia.

(BDNF) [19,35]. In turn, BDNF acts on neuronal tyrosine kinase B (TrkB) receptors to downregulate KCC2 in dorsal horn neurons [19,35]. Pain hypersensitivity after spinal cord injury, as well as paradoxical hyperalgesia following chronic opiate treatment, may follow a similar neuroinflammatory process as that seen with nerve injury [36,54]. However, in the case of peripheral inflammation, BDNF appears to be released by primary afferents [98]. In all cases, however, the final common pathway appears to be disinhibition of spinal sensory circuits as a result of KCC2 hypofunction [30]. Importantly, the spinal neuroimmune interaction triggered by peripheral nerve injury is maintained throughout the period of pain hypersensitivity [97], indicating that ongoing signaling, possibly originating from afferents, is necessary to maintain the pathology.

The identification of the cascade of signaling events leading to this form of disinhibition opens potential avenues for therapeutic interventions aimed at central sensitization. However, many of the culprits identified are involved in several neural processes, both central and peripheral, making them difficult targets for selective analgesia. Furthermore, the finding that disinhibition results from KCC2 hypofunction raises several constraints on therapeutic development. Indeed, it indicates that enhancing $GABA_A$-receptor function, albeit of some efficacy [48], is likely to have a limited therapeutic window because impaired Cl^- export will cause a collapse of the efficacy of $GABA_A$-mediated inhibition. Specifically, Cl^- influx through $GABA_A$ channels causes a collapse, and even a reversal, of the $GABA_A$ reversal potential, effectively impairing inhibitory efficacy and possibly even eventually causing inversion of $GABA_A$ action [2,31]. Hence, restoring KCC2 activity appears to represent a preferred therapeutic strategy [40], in particular because it would not affect neuronal excitability directly, but rather modulate the efficacy of endogenous inhibition [23].

Translational Aspects of Neuropathic Pain Pathophysiology

Considerable progress has been made toward bringing knowledge about the pathophysiology of pain, much of which is derived from animal models, closer to the clinic. This section will summarize recent advances in this field.

Pathological Activity in Nociceptors Underlies Neuropathic Pain

Genetic Findings in Voltage-Gated Sodium Channels

The finding of a point mutation in the *SCN9A* gene as the cause of primary erythromelalgia in 2004 [95] led the way to the discovery of a number other mutations. More than a dozen different mutations in *SCN9A* in patients with primary erythromelalgia have been described, and studies have clearly shown that these mutation lead to hyperexcitability of dorsal root ganglia neurons [22]. In 2006, a mutation in the *SCN9A* gene was detected in a family with paroxysmal extreme pain disorder, another rare hereditary pain disorder [37]. In the same year, a mutation was described, which leads to congenital analgesia, a rare disease in which the persons concerned are unable to perceive pain at all [21]. Also, an overlap syndrome with symptoms of both erythromelalgia and paroxysmal extreme pain disorder with underlying $Na_V1.7$ mutation has been described [32]. While gain-of-function mutations of $Na_V1.7$ ion channel underlie the symptoms in both erythromelalgia and paroxysmal extreme pain disorder and overlap syndromes, congenital analgesia is based on a loss-of-function mutation. All of the above diseases are very rare. However, in 2012, Faber et al. reported that almost 30% of a cohort of patients with idiopathic small-fiber neuropathy had mutations in the *SCN9A* gene [34]. Shortly thereafter, a study by the same group showed that at least 3% of patients with idiopathic small-fiber neuropathy had mutations in the *SCN10A* gene, which encodes the $Na_V1.8$ channel. Thus, the important role of voltage-gated sodium channel activity in nociceptors for pain perception has been convincingly shown in humans.

Furthermore, single-nucleotide polymorphisms may predispose to increased or decreased pain sensation. Estacion et al. and Reiman et al. were able to show a single-nucleotide polymorphism (rs6746030, amino acid substitution R1150W) in the *SCN9A* gene, which is associated with an increased perception of pain by an increased excitability of DRG neurons and is present in approximately 18% of Caucasians [33,69]. Further studies have demonstrated an association of this polymorphism with pain in Parkinson's disease, with multiple regional pain syndromes, and with pain in interstitial cystitis. Another single-nucleotide polymorphism in *SCN9A* was found to be increased in patients with severe fibromyalgia syndrome [90].

Microneurography Findings

Recording from single nociceptors is even more challenging in humans than in experimental animals. After pioneering work from Eric Torebjörk's and José Ochoa's groups [56,64], it took a while until researchers could reliably study patients using microneurography as refined by the methods of Hugh Bostock's laboratory [75]. Meanwhile, pathological activity in C fibers has been shown in patients with diabetic and other neuropathies [47,65], in small-fiber neuropathy [74], and in fibromyalgia syndrome [76]. Thus, without doubt, spontaneous activity and sensitization of C-fiber nociceptors underlie a large number of human neuropathic pain conditions.

Molecular Mediators of Neuropathic Pain in Humans

Various examples of "missed translation" have shown us that not all mediators that convincingly modulate pain or hyperalgesia in animal models have an equally important role in humans. Causes for these failures are various, from potentially inadequate animal models to differences in pain circuits between species or simply because experimental results were misinterpreted. For example, the neurokinin receptors seemed to be the perfect target for an analgesic according to animal data, but the concept did not translate to the clinic [42].

With increased standards in preclinical and clinical pain research, success stories have been published. One recent example is the glycation end-product methylglyoxal, which is increased in the plasma of patients with painful diabetic neuropathy, and which in preclinical studies depolarizes sensory neurons and induces post-translational modifications of the voltage-gated sodium channel $Na_V1.8$ [7]. Methylglyoxal had no effect in mice deficient in $Na_V1.8$, which supports this mechanism of action. Therapeutic interventions for painful diabetic neuropathy are planned using antagonists to methylglyoxal.

Proinflammatory cytokines and chemokines have unequivocally been shown to excite and sensitize nociceptors, in particular damaged nociceptors, and thus to underlie neuropathic pain [83]. While treatment directed at individual cytokines or chemokines has been only partially successful in controlled trials, disturbed cytokine profiles are prevalent in several chronic human pain diseases [89].

Among the neurotrophic factors, nerve growth factor (NGF) is known to play a key role in acute and chronic pain and hyperalgesia [58]. Human diseases illustrating the importance of NGF in pain perception are caused by mutations in NGF or its tyrosine kinase receptor TrkA. These rare autosomal recessive mutations lead to congenital insensitivity to pain or at least to decreased pain perception. These disorders have been named hereditary sensory and autonomic neuropathy (HSAN) type IV and type V. HSAN-IV, which was also called congenital insensitivity to pain with anhidrosis (CIPA), is caused by mutations in the gene coding for TrkA. It is a severe congenital disease where children do not respond to painful stimuli, which leads to mutilations, Charcot arthropathy, osteomyelitis, and autoamputation. Patients have hypo- or anhidrosis, recurrent episodes of fever, and a variable degree of mental retardation. They have a reduced life expectancy. Nerve biopsy samples show a complete absence of small-diameter nonmyelinated fibers and loss of sweat gland innervation. HSAN-V is caused by mutations in the NGF gene. The phenotype is similar to HSAN-IV, with higher variability among families and heterozygous cases with milder symptoms [9]. Given these facts, it is not surprising that drugs blocking NGF have turned out to be powerful analgesics [73].

The Central Nervous System in Neuropathic Pain: Evidence from Human Studies

In this field, translation lags far behind that of studies of the peripheral nerve and at the molecular level. First of all, CNS mechanisms in primary central neuropathic pain need to be distinguished from CNS changes caused by disorders associated with peripheral neuropathic pain. Furthermore, in contrast to findings from animal experiments, data on CNS mechanisms of neuropathic pain come from indirect measures. With the methods presently available, it is not possible to verify, for example, whether microglia play such an important role as might be assumed from animal experiments [35,88]. A trial with the microglia inhibitor minocycline failed to show any differences in comparison to placebo treatment [57]. Magnetic resonance spectroscopy may give some hints [14], but is not comparable with sophisticated morphological and functional studies in disease models. Optogenetics may be a tool of the future, if applications in humans are successful, but this possibility is far from reality [15]. Functional imaging methods such as functional magnetic resonance

Table I
Examples of successful translation between animal models and human neuropathic pain

Mechanism, Mediators, or Method	Animal Models	Human Conditions
Pathological C-fiber activity	Neuroma, diverse partial nerve injury models	Erythromelalgia, small-fiber neuropathy, fibromyalgia syndrome
Sensitization by nerve growth factor	Rat brachial plexus avulsion, chronic constriction injury of sciatic nerve	Hereditary sensory and autonomic neuropathy types IV and V; NGF blockade as analgesic therapy
Cytokines	Multiple nerve injury models, spinal cord injury	Altered cytokine profiles in several neuropathic pain conditions
Repetitive transcranial magnetic stimulation*	Naive animals, models of depression, CNS injury	Poststroke pain, spinal cord injury pain, migraine

Abbreviations: CNS, central nervous system; NGF, nerve growth factor.
* Reverse translation.

tomography (fMRI) may tell us where things happen, but cannot give information about molecular changes [61]. Positron emission tomography (PET) has been used to show changes in opioid receptor availability in neuropathic pain, for example [29], which may differ between peripheral and central neuropathic pain [55].

More traditional approaches to CNS function in neuropathic and other types of pain include neurophysiological techniques and selective peripheral blockades [100]. Some information has also been gathered from neurostimulatory methods such as repetitive transcranial magnetic stimulation (rTMS), which can reverse abnormal cortical excitability in patients with poststroke pain [43]. Animal models have recently been used to understand the molecular mechanisms of this treatment [50], an example of reverse translation (see Table I).

Acknowledgments

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