CHAPTER

# 54

# Pain

*Perrine Inquimbert, Joachim Scholz*

OUTLINE



## NOCICEPTIVE VERSUS CLINICAL PAIN

The ability to identify potentially harmful environmental stimuli and perceive internal signals of organ dysfunction or tissue damage is crucial for the protection of physical integrity and survival. Congenital insensitivity to pain, which occurs in rare hereditary disorders of the peripheral nervous system (Dib-Hajj et al., 2010; Indo et al., 1996), leads to recurrent injuries and mutilations, highlighting the importance of pain as a warning signal.

Subgroups of primary sensory neurons, so-called nociceptors, are equipped with distinct receptor molecules that recognize mechanical force of high intensity, cold and warm temperature and chemical compounds. Ascending pathways within the central nervous system (CNS) convey this information to the somatosensory cortex and areas of the brain associated with attention, emotion and autonomic functions. At each anatomical level along the path, nociceptive input is processed by local interneurons and fiber tracts that connect the spinal cord, brainstem nuclei and forebrain as discussed

later. Carefully controlled signal transmission ensures that the precise location, nature and intensity of a noxious insult can be determined, and potentially harmful mechanical, thermal or chemical stimuli are differentiated from innocuous input. The protective function of nociceptive pain is diminished in the presence of sustained inflammation or tissue injury, for example, joint damage caused by arthritis, and by a lesion or disease that affects the nervous system. Pain in these clinical conditions is characterized by an exaggerated response to noxious stimulation (hyperalgesia), painful sensations that are paradoxically evoked by innocuous stimulation (allodynia) and spontaneous pain in the absence of an identifiable stimulus. Complex cellular, molecular and biochemical changes contribute to the development of clinical pain. Once triggered, clinical pain may continue despite successful treatment of the underlying disorder that originally caused it. Such persistent pain must therefore be considered a disease in its own right.

## NOCICEPTORS ARE FIRST RESPONDERS

### Primary sensory neurons are located in the dorsal root ganglions (DRG) of spinal nerves and the semilunar ganglions of the trigeminal nerves

The axons of these pseudounipolar nerve cells produce a peripheral branch, which innervates skin, muscles, blood vessels and internal organs, and a central branch projecting to the spinal cord or the trigeminal sensory nuclei, respectively. The sensory nuclei of the trigeminal nerves extend from the midbrain to the cervical spinal cord; pain fibers from the head terminate in the spinal nucleus. Sensory neurons are classified by morphology and function. Large-diameter cells produce thickly myelinated Aα and Aβ fibers characterized by fast conduction. Aβ fibers convey innocuous mechanical stimulation; Aα fibers are specifically involved in proprioception. Nociceptors have a smaller cell body and unmyelinated fibers (C) or fibers surrounded by a thin myelin sheath (Aδ). The differences in axon myelination define the basic properties of nociceptors: Aδ fibers quickly mediate well-localized pain of a sharp character, whereas pain sensation conveyed by C fibers sets in slowly and has a dull character and a more diffuse localization. Biochemical characteristics divide C fiber nociceptors further, into peptidergic neurons that use substance P (SP) and calcitonin gene-related peptide (CGRP) as transmitters and express the high-affinity tyrosine kinase receptor (TRK) A for nerve growth factor (NGF) (Ch. 29). Nonpeptidergic neurons can be labeled immunohistochemically with isolectin B4, a product of the climbing shrub *Griffonia simplicifolia*, and express P2RX3, an ionotropic receptor for adenosine triphosphate (ATP) (Ch. 19).

### Receptor profiles define the response modalities of nociceptors

Ionotropic receptor molecules at the peripheral terminals of nociceptors convert noxious stimulation into depolarizing membrane currents (Fig. 54-1) (Woolf & Ma, 2007). Thermal stimuli activate transient receptor potential (TRP) channels; these are tetrameric receptors selectively permeable to cations. Approximately 30 different TRP channels are classified in six subfamilies (TRPC, TRPV, TRPM, TRPP, TRPML and TRPA). Six of the channels are involved in thermal sensation. Interestingly, and for evolutionary reasons that are unclear, the channels are also activated in the presence of chemical stimuli, including

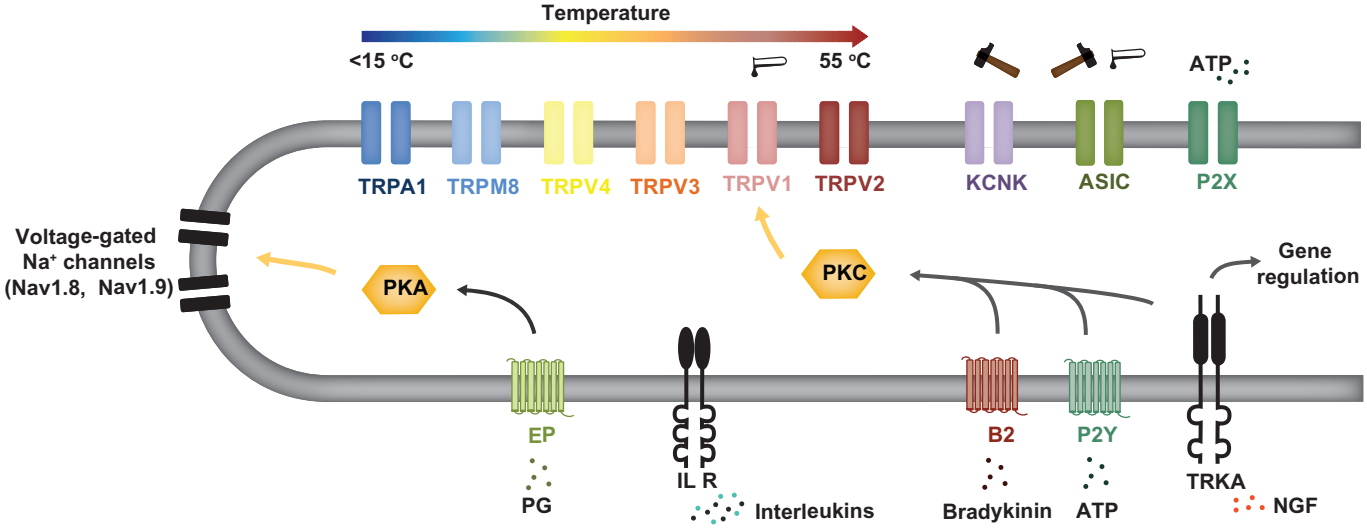


FIGURE 54-1 **Nociception and peripheral sensitization after inflammation**. Distinct ionotropic receptor molecules at the peripheral terminals of a nociceptor transduce noxious stimuli into depolarizing membrane currents. Upon depolarization, voltage-gated sodium channels (Nav) generate action potentials that propagate from the periphery to the cell body of the nociceptor in the dorsal root ganglion and its central terminal in the spinal cord. Inflammation leads to an enhancement of stimulus transduction and signal propagation (peripheral sensitization). Sensitization is mediated by protein kinases (PKA, PKC) that are activated in the presence of prostaglandins (PGs), bradykinin and adenosine triphosphate (ATP), which bind to G protein–coupled receptors, and interleukins and nerve growth factor (NGF), which activate tyrosine kinase (TRK) receptors. *ASIC*, acid sensing ion channel; *B2*, bradykinin receptor; *EP*, prostaglandin E receptor; *KCNK*, potassium channel, subfamily K; *P2X* and *P2Y*, purinergic receptors; *TRP*, transient receptor potential channel. Details on these molecules are in Chs. 4,19,20,21,26,29,34.

spicy food additives. TRPV1, which is expressed in both Aδ and C fibers, opens at temperatures ≥43°C, corresponding to the heat pain threshold in humans. In addition, TRPV1 is a receptor for capsaicin, the pungent ingredient in chili pepper, which explains the sensation of burning pain evoked by the consumption of chili. TRPV2 responds to temperatures between 52°C and 55°C, TRPV3 is activated by temperatures between 31°C and 39°C, and TRPV4 opens at temperatures <27°C. TRPV3 and TRPV4 are therefore likely to contribute to the sensation of (innocuous) warmth. These two channels are also found in keratinocytes and epithelial cells, suggesting that skin cells detect thermal stimuli. Cold temperatures between 8°C and 28°C lead to the opening of the TRPM8 ion channel, which also responds to icilin and menthol. TRPA1 is a receptor for allyl isothiocyanates in mustard and wasabi, cinnamaldehyde and pungent garlic ingredients. TRPA1 is activated below 15°C and may therefore be involved in the sensation of noxious cold (Dhaka et al., 2006; Karashima et al., 2009; Patapoutian et al., 2009) (see TRP also in Ch. 4).

Nociceptors not only detect chemical signals related to spicy flavors, but also sense changes that threaten biochemical homeostasis. Protons activate acid-sensing ion channels (ASICs) 1 (a, b), 2 (a, b), 3 and 4 expressed at peripheral terminals of primary sensory neurons (Wemmie et al., 2006). A pH drop below 6 will also open TRPV1.

The mechanisms of mechanical stimulus transduction are less clear. Mechanosensory abnormality (mec) genes 4 and 10, which encode pore-forming subunits of the degerin (deg) receptor family, are required for mechanotransduction in *caenorhabditis elegans* (Chatzigeorgiou et al., 2010). By homology, ASIC receptors, which belong to the epithelial sodium channel (ENaC)/degerin (deg) superfamily of ion channels have been proposed to be involved in noxious mechanoception. However, studies on mice deficient of ASIC channels do not support a prominent role in cutaneous mechanotransduction. Mice lacking ASIC2 or ASIC3 exhibit only subtle changes in sensitivity to light or painful mechanical stimulation. Osmotic stress and mechanical stimulation open TRPV1, TRPV2 and TRPV4 channels. Osmolarity and pressure, but also changes in temperature or pH modulate the activity of KCNK (potassium channel, subfamily K) 2 and 4, which generate outwardly rectifying potassium currents that contribute to the resting membrane potential of C fiber nociceptors (Honore, 2007). Intriguingly, epidermal cells express TRPV4 and TRPA1, which modulates action potential firing rates in mechanosensitive nociceptors and low-threshold mechanoreceptive fibers (Kwan et al., 2009).

The expression profile of receptor molecules defines the responsiveness of nociceptors. Whereas some sensory neurons specialize in the detection of either thermal, chemical or mechanical stimuli, others are polymodal, reacting to more than one class of stimuli. "Silent" nociceptors require sensitization after injury or inflammation before responding.

### Voltage-gated sodium channels determine the conduction of noxious information from the periphery to the spinal cord

Activation of ion channel receptors by external stimuli leads to membrane depolarization and, consequently, action potential generation at the peripheral terminal. Nerve fiber myelination determines the speed with which a signal is propagated to the CNS, whereas voltage-gated sodium channels (VGSCs) define critical parameters of axon membrane excitability (Dib-Hajj et al., 2010). VGSCs are composed of a large α subunit, which forms the ion channel, and one or more accessory β subunits, which modulate gating properties of the α subunit. Sensory neurons express the α subunits Nav1.1 Nav1.6, Nav1.7, Nav1.8 and Nav1.9. Two of these, Nav1.8 and Nav1.9, are almost exclusively present in C-fiber nociceptors; they are characterized by resistance to tetrodotoxin (TTX), a toxin found in the liver of the puffer fish. Nav1.8 has a high threshold for action potential generation (−30 to −40 mV), is slowly inactivated and quickly reprimes. In contrast, Nav1.9 is slowly activated at a voltage potential close to that of the resting membrane potential (−60 to −70 mV), inducing a tonic sodium current that facilitates depolarization of the cell. Local anesthetics such as lidocaine are nonselective sodium channel inhibitors that block conduction in both sensory and motor nerve fibers. They have to cross the axon membrane to reach their binding site at the α subunit. This characteristic can be exploited using positively charged lidocaine derivatives such as QX314. Unable to pass through the membrane and therefore ineffective when applied alone, QX314 can be introduced specifically into nociceptors by administering it together with capsaicin, which activates the TRPV1 channel, opening its large cation-permissible pore (Binshtok et al., 2007).

## PAIN TRANSMISSION IN THE SPINAL CORD

### Nociceptive information enters the dorsal horn of the spinal cord

Glutamate (Ch. 17) and neuropeptides (Ch. 20) are the major transmitters of nociceptors. Their release at the central terminal is regulated by voltage-gated N-type calcium channels ($Ca_V2.2$), which are activated upon depolarization. Glutamate acts on ionotropic receptors sensitive to α-amino-3-hydroxy-5-methyl-4-ioxazolepropionic acid (AMPA), kainate or N-methyl-D-aspartate (NMDA), and metabotropic glutamate receptors (mGluRs) 1 through 8 (Fig. 54-2). Activation of AMPA receptors causes a transient sodium flux into the cell that produces an excitatory postsynaptic current (EPSC) of less than tens of milliseconds duration. A magnesium ion in the channel pore of NMDA receptors prevents their activation, unless a substantial increase in afferent input leads to a voltage-dependent release of the magnesium ion. Recruitment of NMDA receptors strongly enhances the efficiency of synaptic transmission in the dorsal horn and is a major component of central sensitization (see below). Presynaptic NMDA and kainate receptors constitute an important feedback mechanism, regulating the release of glutamate in the spinal cord.

### Signals are modulated by spinal interneurons

The dorsal horn of the spinal cord contains both excitatory and inhibitory interneurons. Nociceptors directly synapse with inhibitory interneurons that are characterized by the production

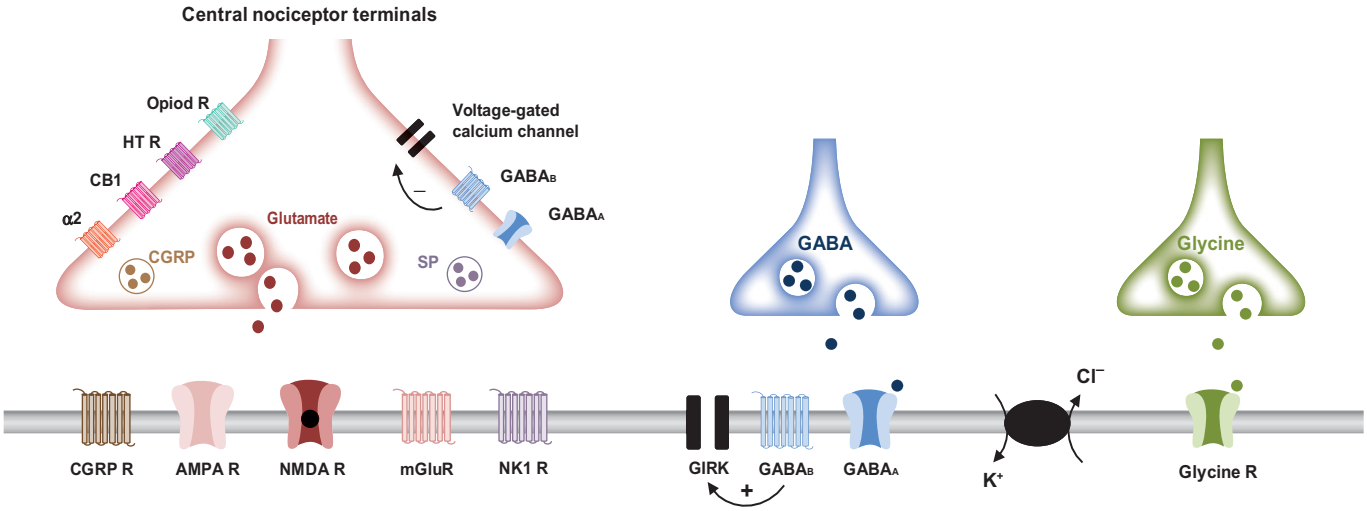


FIGURE 54-2 **Signal transmission in the dorsal horn**. Nociceptive input prompts the release of glutamate and neuropeptides. Glutamate acts on postsynaptic α-amino-3-hydroxy-5-methyl-4-ioxazolepropionic acid (AMPA) receptors and metabotropic glutamate receptors (mGluRs). Activation of the N-methyl-D-aspartate (NMDA)-type glutamate receptor, for example, after central sensitization requires removal of a magnesium ion from the channel pore. Signal transmission at synapses in the dorsal horn is subject to tight pre- and postsynaptic modulation through local interneurons and fiber tracts descending from brainstem nuclei, primarily located in the rostral ventromedial medulla (RVM). Spinal inhibitory interneurons are key regulators of nociceptive transmission. They release γ-aminobutyric acid (GABA) and glycine, which induce hyperpolarizing chloride currents. α2, α2-adrenergic receptor; *CB1*, cannabinoid receptor; *CGRP*, calcitonin gene related protein; *GIRK*, G-protein–gated inwardly rectifying potassium; *HTR*, hydroxytryptamine (serotonin) receptor; *KCC2*, potassium chloride cotransporter isoform 2; *NK1 R*, neurokinin 1 receptor; *opioid R*, opioids receptors; *SP*, substance P.

of γ-aminobutyric acid (GABA) (Ch.18) and glycine (Box 12). Activation of postsynaptic $GABA_A$ and glycine receptors on spinal neurons elicit inward chloride currents and hyperpolarization. This hyperpolarizing effect depends on potassium-chloride cotransporter 2 (KCC2) maintaining a low intracellular chloride concentration. $GABA_B$ receptors are metabotropic and mediate slow hyperpolarization through G protein-gated inwardly rectifying potassium (GIRK) channels (Fig. 54-2).

GABAergic interneurons also produce presynaptic inhibition, mediated through activation of both $GABA_A$ and $GABA_B$ receptors. Presynaptic inhibition decreases the release of glutamate, SP, CGRP and other signaling peptides from the central terminals of primary sensory neurons. Other presynaptic receptors include the G-protein–coupled cannabinoid receptor 1 (CB1), the noradrenaline receptor $\alpha_2$, and opioid receptors of the μ, δ and κ-type. (Fig. 54-2) They modulate transmitter release by reducing calcium flux into the terminal through voltage-gated calcium channels.

## BRAINSTEM, THALAMUS AND CORTEX

### Nuclei in the brainstem and thalamus, and distinct cortical areas are the major projection targets for nociceptive information

Neurons in laminae I, IV and V of the dorsal horn, laminae VII and VIII of the intermediate zone and the medial ventral horn of the spinal cord convey nociceptive input through the spinothalamic tract. Axons of lamina I neurons rise through the lateral portion of the spinothalamic tract and terminate in the posterior part of the ventral medial nucleus (VMpo), the ventral posterior inferior nucleus (VPI) and the ventral caudal part of the medial dorsal nucleus (MDvc), whereas axons from projection neurons in the deeper dorsal horn ascend through the anterior spinothalamic tract to the VPI, the ventral posterior lateral nucleus (VPL), and the ventral and central lateral nuclei (VL and CL). Most of the projections to the thalamus cross to the opposite side of the spinal cord before ascending (Fig. 54-3).

The involvement of the thalamus in pain perception was discovered at the beginning of the 20th century when Henry Head and Gordon Holmes examined brain lesions in patients with central pain. With the arrival of modern imaging techniques, noninvasive *in vivo* studies of forebrain connections involved in nociception became available. Magnetic resonance imaging (MRI) and positron emission tomography (PET) studies have demonstrated a functional association among the somatosensory cortex (SI and SII), anterior insula, anterior cingulate cortex, hypothalamus, amygdala, hippocampus and cerebellum (Fig. 54-3). This "pain matrix" serves the perception of pain and its contextual interpretation. Activation of the anterior cingulate cortex, for example, relates to the affective evaluation of painful events. Functional imaging further allows investigating complex pathophysiological processes that would be difficult to study in animal models. MRI, for example, has been used to demonstrate that placebo analgesia is not a "psychological trick"; instead it is associated with increased spinal inhibition and distinct changes in brain activity in regions that are involved in the processing of nociceptive input (Eippert

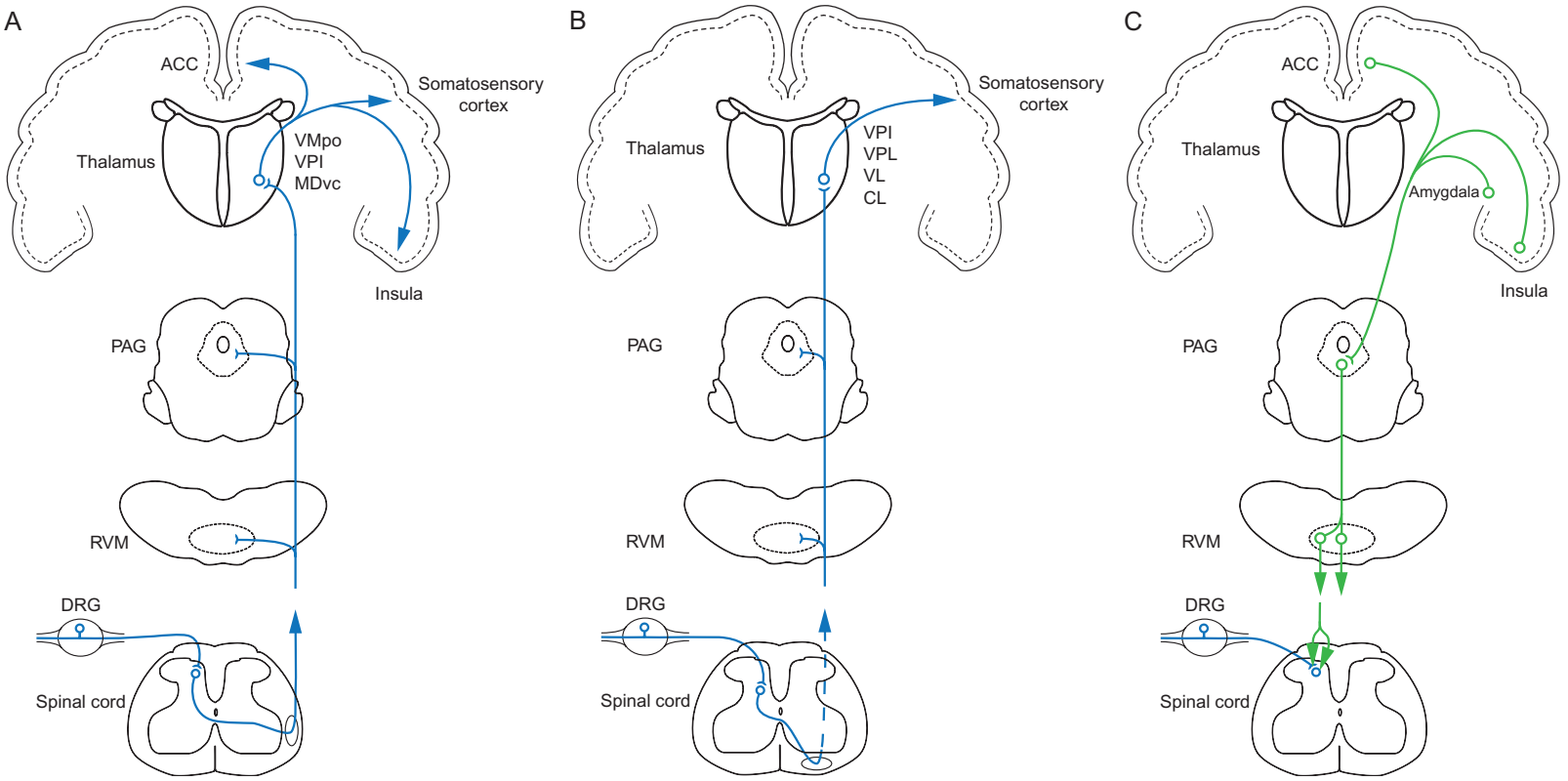


FIGURE 54-3 **Nociceptive pathways in the central nervous system. (A)** The dorsal horn of the spinal cord is the first relay station for nociceptive input. Projections from neurons in lamina I of the dorsal horn give rise to the main central pathway for pain, the lateral spinothalamic tract. It conveys information to the rostral ventromedial medulla (RVM), the periaqueductal gray (PAG) and thalamic nuclei. **(B)** Projections of dorsal horn neurons in deeper laminae (IV, V) ascend through the anterior spinothalamic tract. **(C)** Brainstem nuclei modulate the processing of afferent input through inhibition or facilitation. The PAG integrates information from the anterior insula, the anterior cingulate cortex, hypothalamus and amygdala. The RVM is the major origin of pathways descending to the spinal cord. *CL,* central lateral nucleus; *MDvc,* ventral caudal part of the medial dorsal nucleus; *VL,* ventral lateral nucleus; *VMpo,* ventral medial nucleus; *VPI,* ventral posterior inferior nucleus; *VPL,* ventral posterior lateral nucleus.

et al., 2009). Functional MRI studies have also highlighted the dynamic changes in cortical nociceptive fields that follow deafferentation, including cortical plasticity associated with the development of phantom pain after amputation (Flor et al., 2001). Techniques with high temporal resolution such as magnetoencephalography (MEG) are currently employed to determine the sequence in which components of pain pathways are activated.

### Brainstem nuclei play a major role in the modulation of pain

Brainstem nuclei are important mediators of nociceptive regulation. A spinobulbospinal loop originates at neurons in laminae I and III of the dorsal horn, which send their projections to the nucleus raphe magnus and the medullary dorsal reticular formation (Suzuki et al., 2002). Efferents from these brainstem regions converge back onto the dorsal horn of the spinal cord (Fig. 54-3). The serotoninergic nucleus raphe magnus gives rise to an inhibitory pathway, whereas signals from the medullary dorsal reticular formation facilitate pain transmission in the spinal cord. The nucleus raphe magnus is part of the rostral ventromedial medulla (RVM), a major relay station for descending pathways modulating nociception. The RVM receives serotoninergic and neurotensinergic input from the periaqueductal gray (PAG) and the nucleus cuneiformis in the midbrain, and noradrenergic input from the locus coeruleus in the dorsum of the rostrolateral pons. The PAG integrates information from forebrain regions involved in nociception, including the anterior insula, the anterior cingulate cortex, hypothalamus and amygdala. Activation of RVM neurons can have both excitatory and inhibitory effects on spinal neurotransmission. Serotoninergic RVM neurons stimulate transmitter release from primary sensory afferents through activation of presynaptic serotonin (5-hydroxytryptamine) receptors 2A and 3 (HTR2A, HTR3) (Ch. 15). Facilitation of pain is further mediated through activation of HTR3 expressed on dorsal horn neurons (Suzuki et al., 2002). Analgesic effects of RVM neurons may be produced by direct inhibition of spinal projection neurons or the activation of HTR3 on inhibitory interneurons.

Based on their electrical activity in response to pain, RVM neurons are divided into *on*, *neutral* and *off* cells. *Off* cells are thought to produce tonic inhibition, but precisely how the classification into *on*, *neutral* and *off* cells correlates with the biochemistry of RVM neurons is unclear. *On* and *neutral* cells are likely to use serotonin, but many descending projections from the RVM are known to operate through other transmitters (Braz & Basbaum, 2008). Opioid analgesia is in part mediated by direct inhibition of *on* cells and a release of *off* cells from GABAergic inhibitory control, resulting in their indirect activation (see also Addiction in Ch. 61).

The locus ceruleus and neurons in the dorsolateral pontine tegmentum are the origin of direct noradrenergic projections to the dorsal horn. These fiber tracts form a pathway parallel to the modulation of nociceptive input by the PAG and the RVM. Activation of $\alpha_2$ adrenoreceptors on the central terminals of nociceptors and on dorsal horn neurons, most of them excitatory interneurons (Olave & Maxwell, 2003), is a major mechanism through which tricyclic antidepressants and selective inhibitors of both noradrenalin and serotonin uptake, such as duloxetine, produce analgesia. Selective decrease of serotonin uptake alone is clinically less efficacious.

## OPIOID ANALGESIA

Naturally occurring opiates and synthetic derivatives (opioids) are among the most potent analgesics. Their efficacy varies depending on the nociceptive, inflammatory or neuropathic nature of the pain treated. Endogenous opiates derive from the precursor proteins proopiomelanocortin, preproenkephalin or preprodynorphin and are involved in stress-induced analgesia and the placebo response (Ch. 20). The opioid receptors $\mu$, $\kappa$ and $\delta$ and the opiate receptor-like protein 1 (ORL1) are highly homologous. However, ORL1 has low affinity for opioid ligands. All opioid receptors are coupled to $G_i/G_o$ proteins. Opioid binding leads to activation of inwardly rectifying potassium channels, inhibition of adenylyl cyclase and suppression of voltage-gated calcium currents. As a result, the membrane potential is hyperpolarized. Independent mechanisms of action include the activation of mitogen-activated protein (MAP) kinases and phospholipase C (see opioids also in Addiction, Ch. 61).

Opioid analgesia is mainly mediated by central effects. In the spinal cord, predominantly $\mu$ and $\delta$ receptors are found at the central terminals of A$\delta$ and C fibers, providing for a selective reduction of transmitter release from nociceptors. In addition, opioids inhibit spinal interneurons and projection neurons. Supraspinal sites involved in opioid analgesia include neurons in the PAG and RVM, thalamus, somatosensory cortex and amygdala. Animal studies using stereotaxic injections of morphine into these brainstem regions revealed that opioids reduce the activity of PAG neurons and *on* cells in the RVM. In addition, activation of $\mu$ receptors releases *off* cells from GABAergic inhibition. The net result is a reduction of nociceptive input transmission in the dorsal horn.

## CANNABINOIDS

The majority of primary sensory neurons carrying the cannabinoid receptor $CB_1$ are low-threshold, non-nociceptive afferents. But $CB_1$ is also highly expressed at important relay stations of nociceptive input, including laminae I, II and X of the dorsal horn, the PAG, RVM and the thalamus. In contrast, $CB_2$ is expressed by immune cells, mainly lymphocytes and monocytes. An endogenous cannabinoid, anandamide, is a partial agonist of both $CB_1$ and $CB_2$. Cannabinoid receptors are coupled to $G_i/G_o$ proteins. Their activation leads to adenylyl cyclase inhibition and increased signaling through MAP kinase pathways. Similar to opioid receptors, $CB_1$ activation reduces presynaptic voltage-gated calcium influx and increases inwardly rectifying potassium currents. The analgesic effect of CB1 activation is mediated through membrane hyperpolarization and diminished transmitter release (Agarwal et al., 2007). In addition, low-affinity binding of

cannabinoids to TRP channels, including TRPV1 and TRPA1, may desensitize nociceptors.

## INFLAMMATORY PAIN

### Tissue injury produces an "inflammatory soup" of signaling molecules

Infections, autoimmune reactions and wounds inflicted by mechanical trauma, burning or freezing lead to enhanced responsiveness of nociceptors (peripheral sensitization). Many inflammatory mediators set free by injured tissue or mast and immune cells recruited to the lesion site reduce the activation threshold of nociceptors. These mediators include protons, prostaglandins, kinins, cytokines and growth factors. Among immune cells, basophil and neutrophil granulocytes are the first to be recruited by chemokines. Resident macrophages follow, before circulating monocytes and lymphocytes begin to invade.

Protons, ATP and the oligopeptide bradykinin are the most potent messengers released from injured tissue cells to directly activate nociceptors. Protons bind to ASICs and TRPV1. ATP activates the cation-permeable ion channels P2RX2 and P2RX3, and the $G_{\alpha q}$-protein–coupled receptor P2RY2. Bradykinin acts on the B1 and B2 receptors, which are also G-protein coupled. Downstream of B2, protein kinase C (PKC), isoform $\varepsilon$, becomes activated (Fig. 54-1). Mast cells contribute serotonin to the inflammatory soup, which acts on the receptors HTR2 and HTR3. Activation of HTR2 depolarizes the cell membrane by reducing potassium currents, which are key regulators of the resting potential of neurons.

The most widely used analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), target cyclooxygenase (COX), the enzyme catalyzing the synthesis of prostaglandins from arachidonic acid (Ch.36). Intracellular calcium increase and activation of MAP kinases triggers phosphorylation of phospholipase $A_2$. Active phospholipase $A_2$ migrates to the cell membrane and hydrolyzes phospholipids, leading to arachidonic acid release. COX initiates the metabolization of arachidonic acid to prostaglandins. A constitutively expressed isoform, COX1, is involved in protective mucus secretion in the stomach and platelet aggregation. In contrast, COX2 is induced by cytokines and growth factors after tissue injury and is responsible for the generation of prostaglandin E2 (PGE2), an important mediator of inflammatory pain. PGE2 binds four different prostaglandin receptor subtypes expressed on sensory neurons: EP1, EP3a, EP3b and EP4. EP2 and EP4 receptors are coupled with $G_{\alpha s}$ protein and cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) (Dina et al., 2003). Their activation enhances transmitter release from afferent sensory fibers. PGE2 binding to EP2 expressed on inhibitory interneurons of the dorsal horn reduces glycinergic transmission via PKA-mediated phosphorylation of glycine receptor subunit $\alpha 3$. Most of the NSAIDs block COX indiscriminately. COX2 inhibitors were supposed to selectively reduce prostaglandin synthesis without gastric adverse effects or interference with platelet function. However, shortly after their introduction, all COX2 inhibitors except for celecoxib were withdrawn because they appeared to be associated with an increased risk of cardiovascular side effects such as heart attack and stroke.

### Molecular mechanisms involved in peripheral sensitization

Growth factors are potent signaling molecules in inflammation. NGF binds to the TRKA receptor, inducing TRPV1 sensitization. NGF is also retrogradely transported to the cell body of primary sensory neurons (see chapter 8), where it increases the expression of SP, CGRP and brain-derived neurotrophic factor (BDNF), ASICs, TRPV1 and Nav1.8. Enhanced synthesis of ASICs, TRPV1 and Nav1.8 and integration of these ion channels into peripheral terminals or the axon membrane, respectively, contribute critically to peripheral sensitization. SP and BDNF, which are normally exclusively expressed in nociceptors, are after peripheral inflammation also found in low-threshold $A\beta$ fibers, indicating a major phenotypic shift, which enables normally non-nociceptive neurons to target dorsal horn neurons involved in the transmission of pain.

Macrophages release tumor necrosis factor and the interleukins (ILs) $1\beta$ and 6. These cytokines induce an increase in the production of other inflammatory mediators such as bradykinin, PGE2 and NGF. PKA and PKC are major downstream targets of these signaling molecules. Activation of PKA and PKC leads to phosphorylation of TRPV1, decreasing the thermal threshold of this receptor ion channel and potentiating its response to activation. Phosphorylation of Nav1.8 shifts voltage-dependent gating to a more negative potential, facilitating the generation of action potentials and increasing the excitability of the nociceptor axons.

Inflammation leads to increased opioid receptor synthesis and membrane insertion. Immune cells release endogenous opioids, which through $G_i/G_o$ protein coupling decrease the excitability of sensory neurons. In inflamed tissue, endogenous opioids may therefore produce analgesia by attenuating nociceptor sensitization (Stein & Lang, 2009).

### Central sensitization

Exposure to pain-provoking stimuli during inflammation, reinforced by peripheral sensitization, drastically increases afferent input in the dorsal horn. This barrage of nociceptive input is insufficiently countered by inhibitory mechanisms. Instead it is further enhanced by central sensitization, perhaps because pain is so important for the protection of physical integrity.

Sustained nociceptor activity leads to a substantial rise in glutamate and release of SP and CGRP. This results in an activation of G-protein–coupled receptors: group 1 mGluR (mGluR1 and mGluR5), NK1 and CGRP receptor. As a consequence, phospholipase C is activated and calcium is mobilized from the endoplasmic reticulum. Rising intracellular calcium produces slow postsynaptic depolarization, lasting tens of seconds. This provides for a temporal summation of EPSCs and cumulative depolarization of the postsynaptic membrane potential. The magnesium block is removed from

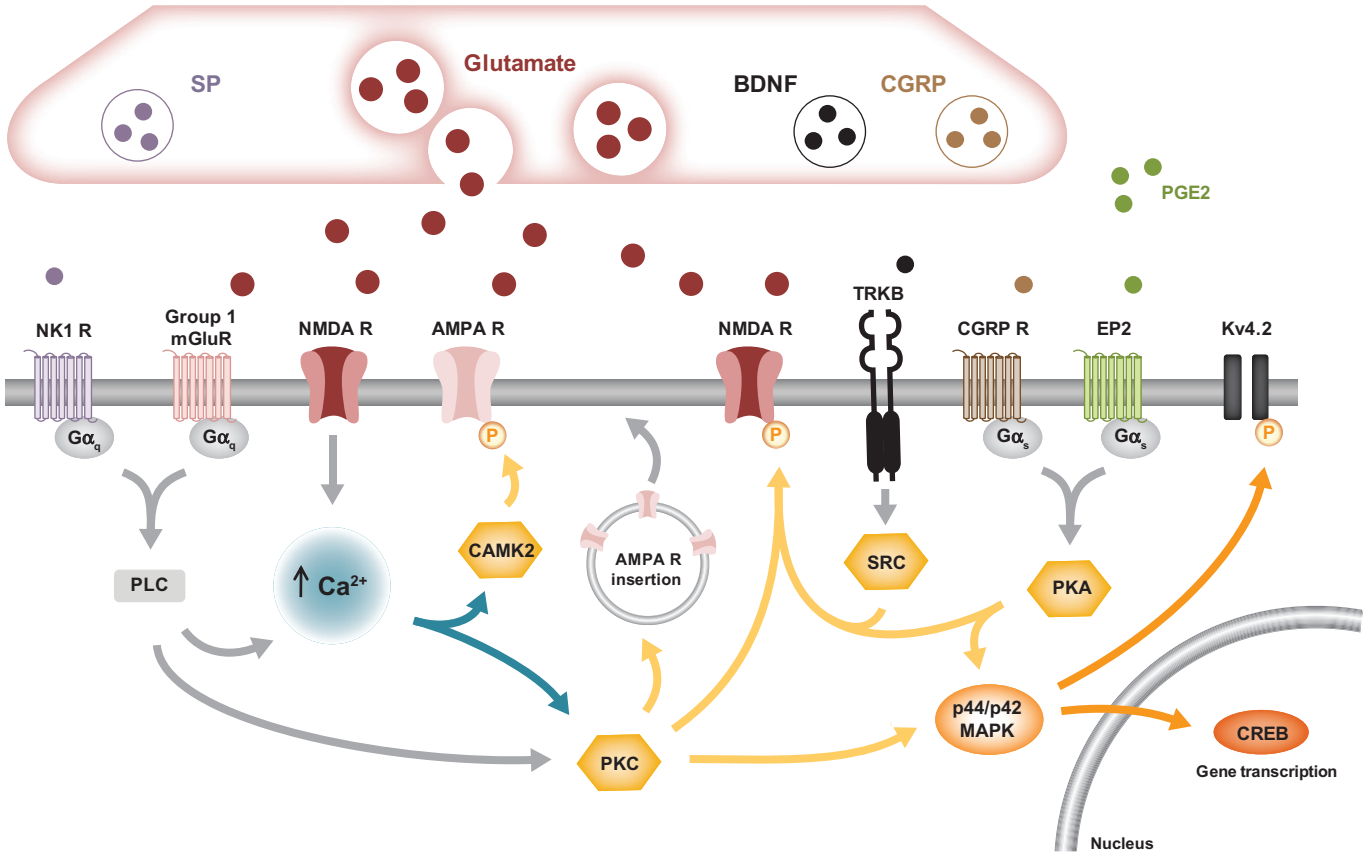


FIGURE 54-4 **Central sensitization**. Signal transmission in the dorsal horn is amplified in the presence of enhanced nociceptor activity. Increased release of glutamate, substance P (SP), calcitonin gene related protein (CGRP) and brain-derived neurotrophic factor (BDNF) leads to rising intracellular calcium in dorsal horn neurons and activation of multiple protein kinases (PK): PKA, PKC, SRC, calcium-calmodulin–dependent protein kinase 2 (CAMK2) and mitogen-activated protein (MAP) kinases. Phosphorylation of receptor subunits and ion channels and the insertion of additional α-amino-3-hydroxy-5-methyl-4-ioxazolepropionic acid (AMPA) receptors increase the excitability of the postsynaptic membrane substantially. Removal of the magnesium ion block from the pore of N-methyl-D-aspartate (NMDA) receptors augments glutamatergic transmission. Activation of p44 and p42 MAP kinases (MAP kinases 1 and 3, respectively) triggers phosphorylation of cyclic adenosine monophosphate (cAMP)-responsive element binding protein (CREB). Subsequent changes in gene transcription maintain dorsal horn neurons in a sensitized state. *EP2*, prostaglandin E receptor 2; *$G\alpha_q$*, G-protein subunit. *Kv4.2*, voltage-gated potassium channel; *mGluR*, metabotropic glutamate receptor; *NK1R*, neurokinin 1 receptor; *PGE2*, prostaglandin E2; *PLC*, phospholipase C; *TRKB*, tyrosine kinase receptor B.

NMDA receptor ion channels and activation of NMDA receptors participates in the enhancement of intracellular calcium increase (Fig. 54-4). Temporal summation can be demonstrated in patch clamp recordings as "wind-up," a progressive increase in the action potentials of postsynaptic neurons upon repetitive stimulation of C fibers at low frequency.

Central sensitization of dorsal horn neurons results from use-dependent plasticity. The term encompasses multiple molecular mechanisms that enhance membrane excitability and increase synaptic efficiency (Latremoliere et al., 2009). Elevated intracellular calcium triggers distinct phosphorylation cascades involving PKA, PKC, members of the Src kinase family, calcium-calmodulin-dependent protein kinase 2 (CAMK2) and p44/42 MAP kinases (Fig. 54-4). Among the targets of these kinases are glutamate receptors. Phosphorylation of NMDA receptor subunits 2A and 2B facilitates the release of magnesium from the ion channel, raising the probability and prolonging the time of the ion channel remaining open. AMPA receptor phosphorylation promotes its trafficking and insertion into the postsynaptic density. MAP kinase 1 phosphorylates the Kv4.2 potassium channel, which is involved in the generation of rapidly inactivated (A-type) currents. Such synaptic plasticity occurs within minutes and may last for hours after afferent input ceases. Phosphatases and receptor endocytosis return synaptic activity to baseline. However, in the presence of sustained input changes in gene transcription may generate more profound alterations. MAP kinases entering the nucleus and phosphorylating cAMP responsive element binding protein (CREB) induce the transcription of immediate early genes such as c-fos, TRKB and COX2 (Fig. 54-4).

## Prolonged homosynaptic facilitation

Activity-dependent long-lasting homosynaptic facilitation of the glutamatergic synapse occurs in response to trains of both low- (2 Hz) and high- (100 Hz) frequency stimulations

of afferent fibers. This process is comparable to long-term potentiation in the hippocampus (Ch.56). It involves simultaneous activation of the SP receptor, the NMDA receptor and voltage-gated calcium channels, and leads to a massive mobilization of calcium from intracellular stores (Ikeda et al., 2006). Different from wind-up, homosynaptic facilitation induced by high-intensity stimulation increases the amplitude of EPSCs for hours. Homosynaptic facilitation is likely to contribute to hyperalgesia. In contrast, central sensitization, which enhances the responsiveness of the dorsal horn neuron in its entirety, offers an explanation not only for hyperalgesia but also for allodynia, because normally innocuous input may now be amplified and enter nociceptive pathways.

Pro-inflammatory cytokines (TNFa, IL1B and Il6) released during peripheral inflammation contribute to the enhanced excitability of spinal cord neurons by increasing synaptic input and reducing inhibition (Kawasaki et al., 2008). IL1B is directly involved in the development of central sensitization, as it leads to COX2 induction and PGE2 synthesis (Samad et al., 2001).

## NEUROPATHIC PAIN

### Paradoxically, nervous system injury may produce not only sensory loss but also chronic pain

A lesion or disease involving the somatosensory nervous system may induce the development of lasting pain (neuropathic pain). Persistent pain may result from very different types of injuries: trauma; compression of a peripheral nerve or spinal nerve root; metabolic disorders such as diabetes mellitus; exposure to neurotoxic chemicals or drugs; infections, for example, by human immunodeficiency virus (HIV); or tumor infiltration. Neural damage initiates complex cellular and molecular changes that lead to increased activity of primary sensory neurons and enhanced transmission of sensory input in the spinal cord. The clinical presentation of neuropathic pain varies. Pain may be present continuously, occur in spontaneous episodes or be evoked by painful as well as normally nonpainful stimuli. These phenotypical differences imply different underlying mechanisms.

### Spontaneous discharges and enhanced excitability of sensory neurons

Spontaneous pain arises as a result of stimulus-independent (ectopic) action potential generation within nociceptive pathways. Following peripheral nerve injury, spontaneous activity is recorded at the proximal stump and along axons of lesioned nerve fibers and in the dorsal root ganglion (Amir et al., 2005). Injured sensory neurons first generate a very intense but transient burst of action potentials, which subsides within minutes. In the following one or two days, a second, sustained wave of activity emerges. Tingling and other nonpainful sensations (paresthesia) may occur when such spontaneous activity develops in low-threshold, non-nociceptive sensory nerve fibers. Some sensations have a disturbing character (dysesthesia) without being painful.

Changes in the expression of membrane ion channels are likely to play a major role for nociceptor activity in the absence of external stimulation. Hyperpolarization-activated cyclic nucleotide-modulated channels (HCN) accumulate at the site of a nerve lesion and produce a pacemaker current, $I_h$, which facilitates the development of spontaneous discharges (Jiang et al., 2008). The voltage-gated sodium channel subunit Nav1.3 is markedly upregulated after nerve injury, potentially increasing the membrane excitability of nociceptors. However, Nav1.3 knockout in mice does not alter spontaneous activity in the injured nerve, indicating that increased Nav1.3 expression may contribute to, but is not required for, the generation of ectopic discharges. Spontaneous activity in injured sensory neurons is further facilitated by a decrease in the activity of voltage-gated potassium channels, which regulate membrane excitability. It is conceivable that spontaneous pain also occurs as a consequence of changes in the sensitivity of injured sensory neurons to endogenous stimuli. For example, a decrease in the thermal threshold of TRPV1 may prompt activation of this receptor at body temperature and produce sensations of burning pain in the absence of an external trigger.

T-type low voltage-gated calcium channels (Cav3.2) modulate neuronal excitability, whereas high voltage-gated N-type calcium channels (Cav2.2) regulate transmitter release from the central terminals of primary sensory neurons. Both types of calcium channels are upregulated after nerve injury (Yaksh, 2006). Gabapentin and pregabalin, two first-line treatments of neuropathic pain, target $\alpha_2\delta_1$ and $\alpha_2\delta_2$ auxiliary subunits of calcium channels and inhibit the release of glutamate and peptide transmitters. Potentially more important for its analgesic effect, gabapentin also disrupts the insertion of calcium channels into the cell membrane (Hendrich et al., 2008). In addition, gabapentin reduces excitatory synapse formation in the CNS; it is unclear, however, whether this mechanism of action reduces neuropathic pain. Components of snail venoms, ω-conotoxins, block N-type voltage-gated calcium channels with high affinity. Intrathecal delivery of a synthetic ω-conotoxin, ziconotide, is used for the treatment of severe pain that resists other forms of therapy.

### Allodynia signals a crossover of sensory modalities

Painful sensations evoked by normally subthreshold tactile or thermal stimuli are a characteristic feature of neuropathic pain. Pain may be elicited by gentle touch of the skin or a moving tactile stimulus—for example, when putting on a shirt. Patients with neuropathic pain also often complain about cold allodynia; a puff of cool air across the skin in the territory of an injured nerve may trigger intense pain. Increased excitability of temperature-sensitive nociceptors may explain thermal allodynia, but tactile allodynia indicates a crossover of sensory modalities that are normally anatomically and functionally separated. Light touch is detected by low-threshold Aβ fibers that send central projections to deeper laminae (III, IV, and the adjacent margins of laminae II and V) of the dorsal horn. Projection neurons in these laminae convey information to the brain through the posterior funiculus of the spinal cord. After nerve injury, however, Aβ fibers sprout into

lamina II (Kohama et al., 2000; Woolf et al., 1992) and form new synapses with projection neurons that normally receive input from Aδ and C fibers. As a consequence, low-threshold input is entering nociceptive pathways and falsely interpreted as pain. Whereas peripheral axon growth is an important repair mechanism after nerve injury, the sprouting of central axons of Aβ fibers into the superficial dorsal horn demonstrates the maladaptive nature of neuropathic pain (Costigan et al., 2009).

## Central sensitization and descending facilitation

Increased afferent input caused by ectopic activity and enhanced transmitter release from the terminals of injured nerve fibers sensitizes projection neurons in the spinal cord, analogous to the central sensitization that occurs after inflammation. Nerve injury-induced changes include alterations in the membrane insertion, subunit composition and phosphorylation of NMDA receptors (Tao et al., 2003). Similar synaptic changes take place in structures that are involved in supraspinal pain processing, including the amygdala, anterior cingulate gyrus and prefrontal cortex. In addition, nerve injury prompts a shift of descending pain-modulating pathways toward facilitation that contributes to the persistence of neuropathic pain. Experiments involving a destruction of RVM neurons or lesions of the dorsolateral funiculus have shown that the enhanced release of excitatory transmitters from injured primary sensory neurons is subject to direct supraspinal regulation (Gardell et al., 2003). Activation of presynaptic HTR3 appears to mediate the facilitation of afferent input.

## Disinhibition

Central sensitization and facilitation after nerve injury are aggravated by reduced spinal inhibition. Tonic inhibitory control

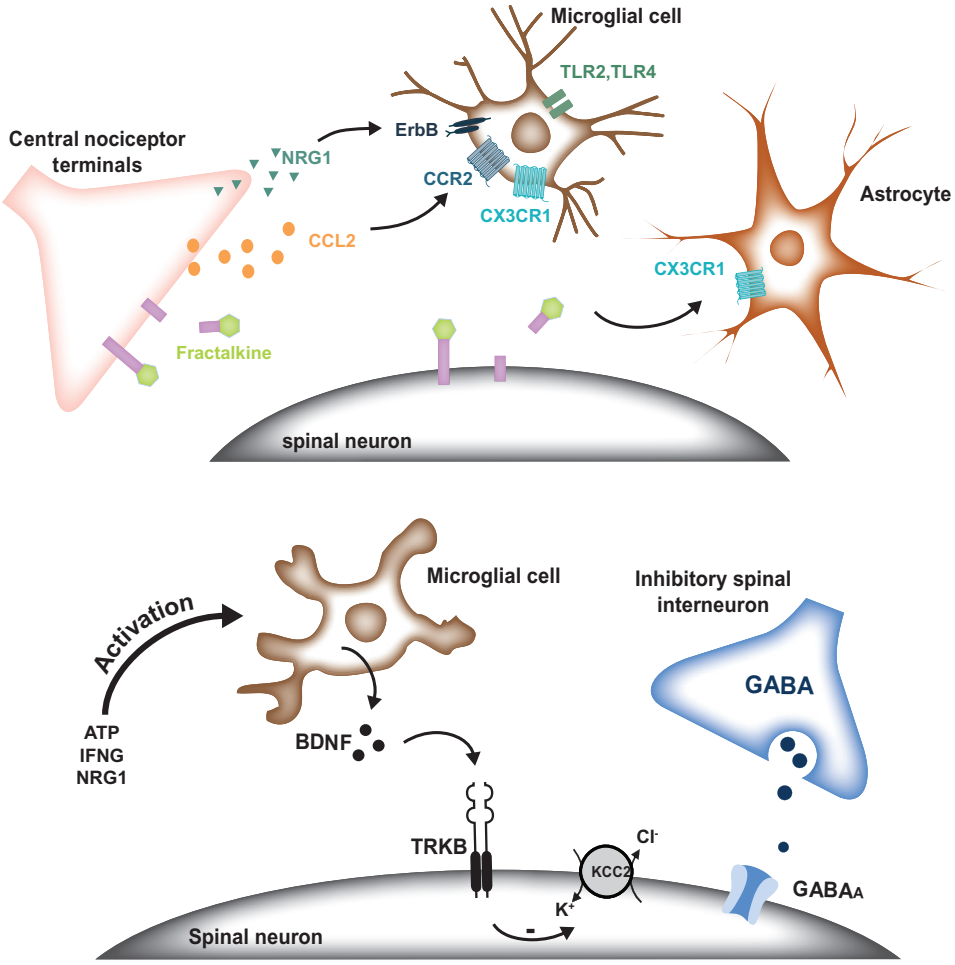


FIGURE 54-5 **Neuroimmune signaling after peripheral nerve injury**. **(A)** Microglial cells are recruited to the central projections of injured sensory neurons. Signals involved in the chemotaxis of spinal microglia include fractalkine, CCL2, neuregulin 1 (NRG1) and (as yet unknown) ligands of the Toll-like receptors (TLR) 2 and 4. Fractalkine has a chemokine domain that is cleaved from its membrane-bound portion by cathepsin S. Because astrocytes also express the fractalkine receptor CX3CR1, this chemokine may attract microglia as well as astrocytes. **(B)** Adenosine triphosphate (ATP) binding to the purinergic receptor P2RX4, interferon γ (IFNG) and NRG1 trigger microglial activation. Active microglia release brain-derived neurotrophic factor (BDNF), which induces in a subpopulation of dorsal horn lamina I neurons downregulation of potassium chloride cotransporter isoform 2 (KCC2) and inversion of inhibitory GABAergic currents. In addition, microglial cytokines may act directly on the central terminals of nociceptors. *TRK B*, tyrosine kinase receptor B.

through descending noradrenergic pathways decreases, and both the central terminals of sensory neurons and dorsal horn neurons become less sensitive to inhibition mediated by μ opioid receptors. In lamina I neurons the transmembrane gradient for chloride ions changes so that activation of $GABA_A$ receptors may produce depolarization instead of hyperpolarization, potentially provoking paradoxical excitation and spontaneous activity. Independently, segmental inhibition in the superficial dorsal horn of the spinal cord is compromised by a loss of GABAergic interneurons as sustained ectopic activity and glutamate-mediated excitotoxicity trigger apoptosis of dorsal horn neurons (Fig. 54-5). Preventing apoptotic neuron death restores GABAergic inhibition and attenuates in rats the behavioral equivalents of mechanical allodynia, hyperalgesia and cold allodynia (Scholz et al., 2005). Enhanced nociceptive input furthermore provokes an increase in endocannabinoids such as 2-arachidonoyl glycerol and anandamide, which after activation of CB1 on inhibitory interneurons may suppress the synaptic release of GABA and glycine (Pernia-Andrade et al., 2009). See Box below.

## Immune response to nerve injury

Nerve injury prompts an immune response that involves activation of macrophages, T lymphocytes and Schwann cells at the site of the nerve lesion and in the dorsal root ganglion. Microglia, macrophages and lymphocytes are recruited to regions in the dorsal horn, corresponding to the distribution of central projections of injured nerve fibers (Fig. 54-5). Chemotactic signals involved in this immune cell recruitment include fractalkine (CX3CL1), CCL2, neuregulin 1 (NRG1), complement factor C5a and activation of Toll-like receptors (Scholz et al., 2007). Fractalkine is released from neuronal membranes after cleavage by cathepsin S (Clark et al., 2007) and acts on a single receptor expressed by resident microglia and astrocytes.

Adenosine triphosphate (ATP) has a chemotactic effect and activates the purinergic receptors P2RX4 and P2RX7, expressed on microglial cells and monocytes, respectively (Ch. 19). Activation of P2RX4 causes the release of BDNF, which binds to TRKB receptors of dorsal horn neurons. This induces

## PAIN: THE CLINICAL CHALLENGE

*Perrine Inquimbert, Joachim Scholz*

Despite tremendous improvement in the understanding of neurobiological pain mechanisms, pain management, particularly the treatment of chronic pain, remains a largely unmet need. Millions of patients suffer from persistent pain that is incompletely relieved by analgesics.

Adverse effects of analgesics and comorbidity often limit the options for pain treatment. Conditions associated with chronic pain are common among elderly people, who have an increased risk of potentially harmful side effects, such as cardiotoxicity caused by tricyclic antidepressants. Experimental pain research is required to identify drug targets with reduced toxicity and reveal mechanisms of action that explain the side effects of analgesics.

Two recent studies illustrate the need for a translational approach to pain research. Insufficient inhibition of nociceptive input is a key factor in inflammatory and neuropathic pain. The major inhibitory transmitter in the spinal cord is γ-aminobutyric acid (GABA). Treatment with GABA receptor agonists should therefore be a viable strategy for alleviating pain. However, use of GABA receptor agonists is hampered by a loss of muscle tone, sedation and the provocation of epileptic seizures. Knabl et al. (2008) generated transgenic mice with point mutations in distinct $GABA_A$ receptor subunits to determine which subunits mediate the analgesic effect of ligands at the benzodiazepine site of the receptor, and which subunits are responsible for common side effects of benzodiazepines such as motor impairment, sedation and the development of tolerance. They identified $\alpha_2$ and $\alpha_3$ subunits as specific targets for analgesia and demonstrated that L-838,417, a selective agonist at $\alpha_2$ and $\alpha_3$ receptor subunits and antagonist at the $\alpha_1$ subunit, reduces inflammatory and neuropathic pain. Mice treated with L-838,417 did not exhibit signs of sedation or tolerance and performed well in a rotarod test. Selective targeting of the $GABA_A$ receptor subunits $\alpha_2$ and $\alpha_3$ has the potential of improving the analgesic specificity of benzodiazepine-site ligands.

Neurotrophic factors are essential for neuronal survival, differentiation and axon guidance during development. In the mature nervous system, they serve as signaling molecules that regulate synaptic function and stimulus response properties of neurons, and are involved in the communication between neurons and non-neuronal cells. Nerve growth factor (NGF) is specifically required for the development of nociceptors. In the adult organism, activation of tyrosine kinase receptor A (TRKA) by NGF triggers signaling pathways that contribute to nociceptor sensitization, including an increase in the responsiveness of transient receptor potential channel V1 (TRPV1). NGF also induces an enhanced expression of ion channels, neuropeptides and brain-derived neurotrophic factor (BDNF) (Pezet & McMahon, 2006). Tanezumab is a humanized monoclonal antibody that neutralizes NGF. In a recent phase 2 research trial, its analgesic efficacy for inflammatory pain was tested in 450 patients with osteoarthritis of the knee (Lane et al., 2010). Subjects receiving tanezumab had less pain while walking, experienced less joint stiffness and showed improved physical function in tasks that required movement of the knee. However, in a different phase 3 study, several subjects receiving tanezumab developed bone necrosis that required total joint replacement. This prompted the Food and Drug Administration (FDA) to suspend further clinical testing of the drug. Was the inhibition of NGF signaling responsible for the necrosis of bone tissue? One possible explanation would be that tanezumab not only blocked excess pain caused by osteoarthritis but also protective (nociceptive) pain, so that subjects treated with tanezumab put too much load on their damaged knees. Joint degeneration is observed in patients with peripheral neuropathy who have a

## PAIN: THE CLINICAL CHALLENGE (*cont'd*)

deficit in nociceptive and proprioceptive input. And congenital mutations in the NGF receptor TRKA prevent the development of nociceptors, leading to complete pain insensitivity and, as a consequence, repeated injuries of joints and soft tissue.

An ideal analgesic would remove clinical pain while preserving the protection provided by nociceptive pain. It would be directed at molecular targets with sufficient specificity to prevent intolerable or harmful side effects. A thorough understanding of the complex biological changes that are responsible for clinical pain is crucial for the development of better analgesics. Experimental pain research should inspire new treatment strategies. Likewise, important lessons are to be learned from clinical experience before we will be able to tackle chronic pain.

### References

Knabl, J., Witschi, R., Hösl, K., Reinold, H., Zeilhofer, U. B., Ahmadi, S., et al. (2008). Reversal of pathological pain through specific spinal GABAA receptor subtypes. *Nature*, *451*(7176), 330–334.

Lane, N. E., Schnitzer, T. J., Birbara, C. A., Mokhtarani, M., Shelton, D. L., Smith, M. D., et al. (2010). Tanezumab for the treatment of pain from osteoarthritis of the knee. *The New England Journal of Medicine*, *363*(16), 1521–1531.

Pezet, S., & McMahon, S. B. (2006). Neurotrophins: Mediators and modulators of pain. *Annual Review of Neuroscience*, *29*, 507–538.

downregulation of potassium chloride cotransporter isoform 2 (KCC2). KCC2 is responsible for the capacity of a cell to move chloride ions across the membrane. Reduced KCC2-mediated transport leads to chloride accumulation and a collapse in the transmembrane gradient for chloride. As a consequence, GABA no longer has a hyperpolarizing effect, diminishing an important component of spinal inhibitory control (Coull et al., 2005).

Active microglia release a number of cytokines. IL1, IL6 and tumor necrosis factor enhance excitatory transmission and suppress inhibitory currents in the dorsal horn (Fig. 54-5). Cleavage of IL1 involves matrix metalloproteinases (MMP) 2 and 9. MMP9 is released from sensory neurons, MMP2 from astrocytes. Whereas MMP9 appears to be involved in the development of neuropathic pain, MMP2 more likely contributes to its persistence, because astrocytes are activated late (within weeks) after nerve injury. MMP9 further induces activation of p38 MAP kinase (MAP kinase 14) in microglial cells, whereas MMP2 increases the activity of extracellular signal-regulated kinase (MAP kinase 1) in astrocytes (Kawasaki et al., 2008).

## GENETIC FACTORS

### Nociceptive responses and the susceptibility to clinical pain depend on genetic factors

Studies examining cellular or molecular changes involved in the development of inflammatory or neuropathic pain rely on animal models. Inbred strains of rats and mice are usually employed in these models, eliminating genetics as a potential source of variability in study outcomes. However, in humans both nociceptive pain sensitivity and clinical pain phenotypes differ substantially among individuals. Depending on the painful condition, genetic factors may be responsible for >50% of the variability (Lacroix-Fralish & Mogil, 2009). The complexity of pain, be it the physiological response to noxious stimulation or the result of a clinical disorder, strongly suggests that a combination of multiple genes and environmental factors determines the phenotype of pain. Several genetic associations have been identified, for example, between catechol-O-methyl transferase (COMT) and temporomandibular joint disorder; fibromyalgia and opioid analgesia (Diatchenko et al., 2005); guanosine triphosphate cyclohydrolase 1 and neuropathic pain (Tegeder et al., 2006); and melanocortin 1 receptor and opioid analgesia (Mogil et al., 2003). Insight into the genetics of pain not only improves the understanding of pain mechanisms and their variability, but also has implications for therapeutic decisions and the choice of analgesics in the individual patient.

## CONCLUSION

Nociceptive pain protects from injury; it is the physiological response to potentially harmful stimuli in the environment. However, sustained exposure to noxious stimulation in the presence of inflammation or tissue damage causes changes in signal transduction and processing that result in exaggerated pain sensitivity. Inflammatory pain is still protective, but the precision with which somatosensory stimuli are normally identified is lost. In contrast, neuropathic pain often lacks a protective function. Pain may persist long after the nerve injury that initially caused it, and neuropathic pain may be evoked by innocuous stimuli or occur spontaneously, without an identifiable trigger. The complex and profound changes that underlie clinical disorders associated with pain involve neurons at every level of the somatosensory pathway as well as the immune system and glia. Functional imaging in humans and genetic research on pain are critical for the validation of animal studies and will support the translation of experimental research into the development of new analgesic therapies.

### Acknowledgments

We thank Emily A. Jacobs and Denise J. Xu for assistance, and the National Institute of Neurological Disorders and Stroke (NINDS) for supporting this work through grant NS050408.

## References


Agarwal, N., Pacher, P., Tegeder, I., Amaya, F., Constantin, C. E., Brenner, G. J., et al. (2007). Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nature Neuroscience*, *10*(7), 870–879.

Amir, R., Kocsis, J. D., & Devor, M. (2005). Multiple interacting sites of ectopic spike electrogenesis in primary sensory neurons. *Journal of Neuroscience*, *25*(10), 2576–2585.

Binshtok, A. M., Bean, B. P., & Woolf, C. J. (2007). Inhibition of nociceptors by TRPV1-mediated entry of impermeant sodium channel blockers. *Nature*, *449*(7162), 607–610.

Braz, J. M., & Basbaum, A. I. (2008). Genetically expressed transneuronal tracer reveals direct and indirect serotonergic descending control circuits. *Journal of Comparative Neurology*, *507*(6), 1990–2003.

Chatzigeorgiou, M., Yoo, S., Watson, J. D., Lee, W. H., Spencer, W. C., Kindt, K. S., et al. (2010). Specific roles for DEG/ENaC and TRP channels in touch and thermosensation in C. elegans nociceptors. *Nature Neuroscience*, *13*(7), 861–868.

Clark, A. K., Yip, P. K., Grist, J., Gentry, C., Staniland, A. A., Marchand, F., et al. (2007). Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(25), 10655–10660.

Costigan, M., Scholz, J., & Woolf, C. J. (2009). Neuropathic pain: A maladaptive response of the nervous system to damage. *Annual Review of Neuroscience*, *32*, 1–32.

Coull, J. A., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., et al. (2005). BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature*, *438*(7070), 1017–1021.

Dhaka, A., Viswanath, V., & Patapoutian, A. (2006). Trp ion channels and temperature sensation. *Annual Review of Neuroscience*, *29*, 135–161.

Diatchenko, L., Slade, G. D., Nackley, A. G., Bhalang, K., Sigurdsson, A., Belfer, I., et al. (2005). Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Human Molecular Genetics*, *14*(1), 135–143.

Dib-Hajj, S. D., Cummins, T. R., Black, J. A., & Waxman, S. G. (2010). Sodium channels in normal and pathological pain. *Annual Review of Neuroscience*, *33*, 325–347.

Dina, O. A., McCarter, G. C., de Coupade, C., & Levine, J. D. (2003). Role of the sensory neuron cytoskeleton in second messenger signaling for inflammatory pain. *Neuron*, *39*(4), 613–624.

Eippert, F., Finsterbusch, J., Bingel, U., & Buchel, C. (2009). Direct evidence for spinal cord involvement in placebo analgesia. *Science*, *326*(5951), 404.

Flor, H., Denke, C., Schaefer, M., & Grusser, S. (2001). Effect of sensory discrimination training on cortical reorganisation and phantom limb pain. *Lancet*, *357*(9270), 1763–1764.

Gardell, L. R., Vanderah, T. W., Gardell, S. E., Wang, R., Ossipov, M. H., Lai, J., et al. (2003). Enhanced evoked excitatory transmitter release in experimental neuropathy requires descending facilitation. *Journal of Neuroscience*, *23*(23), 8370–8379.

Hendrich, J., Van Minh, A. T., Heblich, F., Nieto-Rostro, M., Watschinger, K., Striessnig, J., et al. (2008). Pharmacological disruption of calcium channel trafficking by the alpha2delta ligand gabapentin. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(9), 3628–3633.

Honore, E. (2007). The neuronal background K2P channels: Focus on TREK1. *Nature Reviews Neuroscience*, *8*(4), 251–261.

Ikeda, H., Stark, J., Fischer, H., Wagner, M., Drdla, R., Jager, T., et al. (2006). Synaptic amplifier of inflammatory pain in the spinal dorsal horn. *Science*, *312*(5780), 1659–1662.

Indo, Y., Tsuruta, M., Hayashida, Y., Karim, M. A., Ohta, K., Kawano, T., et al. (1996). Mutations in the TRKA/NGF receptor gene in patients with congenital insensitivity to pain with anhidrosis. *Nature Genetics*, *13*(4), 485–488.

Jiang, Y. Q., Xing, G. G., Wang, S. L., Tu, H. Y., Chi, Y. N., Li, J., et al. (2008). Axonal accumulation of hyperpolarization-activated cyclic nucleotide-gated cation channels contributes to mechanical allodynia after peripheral nerve injury in rat. *Pain*, *137*(3), 495–506.

Karashima, Y., Talavera, K., Everaerts, W., Janssens, A., Kwan, K. Y., Vennekens, R., et al. (2009). TRPA1 acts as a cold sensor *in vitro* and *in vivo*. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(4), 1273–1278.

Kawasaki, Y., Xu, Z. Z., Wang, X., Park, J. Y., Zhuang, Z. Y., Tan, P. H., et al. (2008). Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. *Nature Medicine*, *14*(3), 331–336.

Kawasaki, Y., Zhang, L., Cheng, J. K., & Ji, R. R. (2008). Cytokine mechanisms of central sensitization: Distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. *Journal of Neuroscience*, *28*(20), 5189–5194.

Kohama, I., Ishikawa, K., & Kocsis, J. D. (2000). Synaptic reorganization in the substantia gelatinosa after peripheral nerve neuroma formation: Aberrant innervation of lamina II neurons by Abeta afferents. *Journal of Neuroscience*, *20*(4), 1538–1549.

Kwan, K. Y., Glazer, J. M., Corey, D. P., Rice, F. L., & Stucky, C. L. (2009). TRPA1 modulates mechanotransduction in cutaneous sensory neurons. *Journal of Neuroscience*, *29*(15), 4808–4819.

Lacroix-Fralish, M. L., & Mogil, J. S. (2009). Progress in genetic studies of pain and analgesia. *Annual Review of Pharmacology and Toxicology*, *49*, 97–121.

Latremoliere, A., & Woolf, C. J. (2009). Central sensitization: A generator of pain hypersensitivity by central neural plasticity. *Journal of Pain*, *10*(9), 895–926.

Mogil, J. S., Wilson, S. G., Chesler, E. J., Rankin, A. L., Nemmani, K. V., Lariviere, W. R., et al. (2003). The melanocortin-1 receptor gene mediates female-specific mechanisms of analgesia in mice and humans. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(8), 4867–4872.

Olave, M. J., & Maxwell, D. J. (2003). Neurokinin-1 projection cells in the rat dorsal horn receive synaptic contacts from axons that possess alpha2C-adrenergic receptors. *Journal of Neuroscience*, *23*(17), 6837–6846.

Patapoutian, A., Tate, S., & Woolf, C. J. (2009). Transient receptor potential channels: Targeting pain at the source. *Nature Reviews Drug Discovery*, *8*(1), 55–68.

Pernia-Andrade, A. J., Kato, A., Witschi, R., Nyilas, R., Katona, I., Freund, T. F., et al. (2009). Spinal endocannabinoids and CB1 receptors mediate C-fiber-induced heterosynaptic pain sensitization. *Science*, *325*(5941), 760–764.

Samad, T. A., Moore, K. A., Sapirstein, A., Billet, S., Allchorne, A., Poole, S., et al. (2001). Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. *Nature*, *410*(6827), 471–475.

Scholz, J., Broom, D. C., Youn, D. H., Mills, C. D., Kohno, T., Suter, M. R., et al. (2005). Blocking caspase activity prevents transsynaptic neuronal apoptosis and the loss of inhibition in lamina II of the dorsal horn after peripheral nerve injury. *Journal of Neuroscience*, *25*(32), 7317–7323.

Scholz, J., & Woolf, C. J. (2007). The neuropathic pain triad: Neurons, immune cells and glia. *Nature Neuroscience*, *10*(11), 1361–1368.

Stein, C., & Lang, L. J. (2009). Peripheral mechanisms of opioid analgesia. *Current Opinion in Pharmacology*, *9*(1), 3–8.

Suzuki, R., Morcuende, S., Webber, M., Hunt, S. P., & Dickenson, A. H. (2002). Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. *Nature Neuroscience*, *5*(12), 1319–1326.

Tao, Y. X., Rumbaugh, G., Wang, G. D., Petralia, R. S., Zhao, C., Kauer, F. W., et al. (2003). Impaired NMDA receptor-mediated postsynaptic function and blunted NMDA receptor-dependent persistent pain in mice lacking postsynaptic density-93 protein. *Journal of Neuroscience*, *23*(17), 6703–6712.

Tegeder, I., Costigan, M., Griffin, R. S., Abele, A., Belfer, I., Schmidt, H., et al. (2006). GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence. *Nature Medicine*, *12*(11), 1269–1277.

Wemmie, J. A., Price, M. P., & Welsh, M. J. (2006). Acid-sensing ion channels: Advances, questions and therapeutic opportunities. *Trends in Neurosciences*, *29*(10), 578–586.

Woolf, C. J., & Ma, Q. (2007). Nociceptors—noxious stimulus detectors. *Neuron*, *55*(3), 353–364.

Woolf, C. J., Shortland, P., & Coggeshall, R. E. (1992). Peripheral nerve injury triggers central sprouting of myelinated afferents. *Nature*, *355*(6355), 75–78.

Yaksh, T. L. (2006). Calcium channels as therapeutic targets in neuropathic pain. *Journal of Pain*, *7*(1 Suppl. 1), S13–30.