nature medicine

Central mechanisms of pathological pain

Rohini Kuner

Chronic pain is a major challenge to clinical practice and basic science. The peripheral and central neural networks that mediate nociception show extensive plasticity in pathological disease states. Disease-induced plasticity can occur at both structural and functional levels and is manifest as changes in individual molecules, synapses, cellular function and network activity. Recent work has yielded a better understanding of communication within the neural matrix of physiological pain and has also brought important advances in concepts of injury-induced hyperalgesia and tactile allodynia and how these might contribute to the complex, multidimensional state of chronic pain. This review focuses on the molecular determinants of network plasticity in the central nervous system (CNS) and discusses their relevance to the development of new therapeutic approaches.

The origin of pain and its dual role as a key physiological function and a debilitating disease has fascinated scientists and philosophers for centuries. Charles Darwin described pain as a 'homeostatic emotion,' which is essential for the survival of species[1]. The seventeenth-century philosopher René Descartes described pain as an outcome of the activation of a defined channel running from the skin to the brain, a concept that was a forerunner of two nineteenth-century theories that explained pain on the basis of either intense stimulation of any kind of nerve fibers (intensity hypothesis) or specific nociceptors (specificity hypothesis)[2]. The idea that pain is strictly hard-wired can explain why acute pain is caused upon injury to a specific part of the body, but would predict that pain is restricted to the injury site and should be abolished after healing. However, chronic pain can persist long after the initial injury is healed and can arise even in the absence of any obvious pathological trigger. Furthermore, a rigid, hard-wired pain pathway cannot account for the clinical observation that pathological pain neither obeys somatotopic borders (for example, phantom limb pain) nor complies with segregation of sensory modalities (for example, brushing or stroking of skin evokes pain after injury). This is perhaps best exemplified by the paradox of neuropathic pain—nerve loss induced by injury should lead to numbness alone, but can evoke chronic hypersensitivity to painful and innocuous stimuli instead. Therefore, the concept emerged that the neural substrates that mediate pain are plastic—that is, modifiable in a manner that depends on use or modulatory influences[3]. Since then, tremendous efforts have been made to understand the cellular and molecular basis of chronic pain, and the field has been marked by important conceptual advances, mechanistic triumphs and frustrating discrepancies.

Pharmacology Institute, University of Heidelberg, Im Neuenheimer Feld, 366 Heidelberg, Germany. Correspondence should be addressed to R.K. (rohini.kuner@pharma.uni-heidelberg.de).

Published online 14 October 2010; doi:10.1038/nm.2231

Physiological pain and its conversion to chronic pain

Noxious stimuli of various modalities are sensed by a specialized set of nerve fibers: unmyelinated C fibers and thinly myelinated Aδ fibers, which are distinct from myelinated tactile sensors (Aβ fibers) and proprioceptors (**Fig. 1a**). The physicochemical properties of noxious stimuli, such as heat, extreme cold, pressure and chemicals, are converted to electrical activity by transient receptor potential–generating channels (TRP channels) and purinergic channels, and this electrical activity is amplified by sodium channels to elicit action potentials. Nociceptive afferents carrying these peripheral inputs form glutamatergic synapses onto second-order neurons mostly in the superficial laminae (I and II) in the spinal dorsal horn, whereas inputs from non-nociceptive fibers form synapses in deeper laminae (**Fig. 1a**). Some integration and processing of sensory inputs occurs in the spinal dorsal horn, and the net output from spinal networks is carried by several pathways to distinct projection sites in the brain (**Fig. 1a**). For example, the lateral spinothalamic tract projects multimodal sensory inputs from spinal wide-dynamic range neurons to the lateral thalamus and has been implicated in processing sensory and discriminative aspects of pain. By contrast, the medial aspect of the spinothalamic tract and the spinoparabrachial tract project to the medial thalamus and limbic structures and are believed to mediate the emotional and aversive components of pain. The experience of pain is perceived in the cortex, and information is accordingly sent to the spinal cord to enable withdrawal from the noxious stimulus.

Although this experience of physiological pain serves an important protective function, pain can take on a disease character in pathological states such as inflammation, neuropathy, cancer, viral infections, chemotherapy and diabetes. This state is manifest as hyperalgesia (increased sensitivity to painful stimuli; **Fig. 1b**). Furthermore, individuals with chronic pain often show disease-induced, therapy-resistant deviations from normal tactile sensation, such as paraesthesias and dysesthesias. The counterpart of these changes in experimental animals is tactile allodynia (**Fig. 1b**), which represents withdrawal behavior in response to innocuous stimuli[4]. Finally, the most common complaint from individuals with chronic pain is spontaneous,

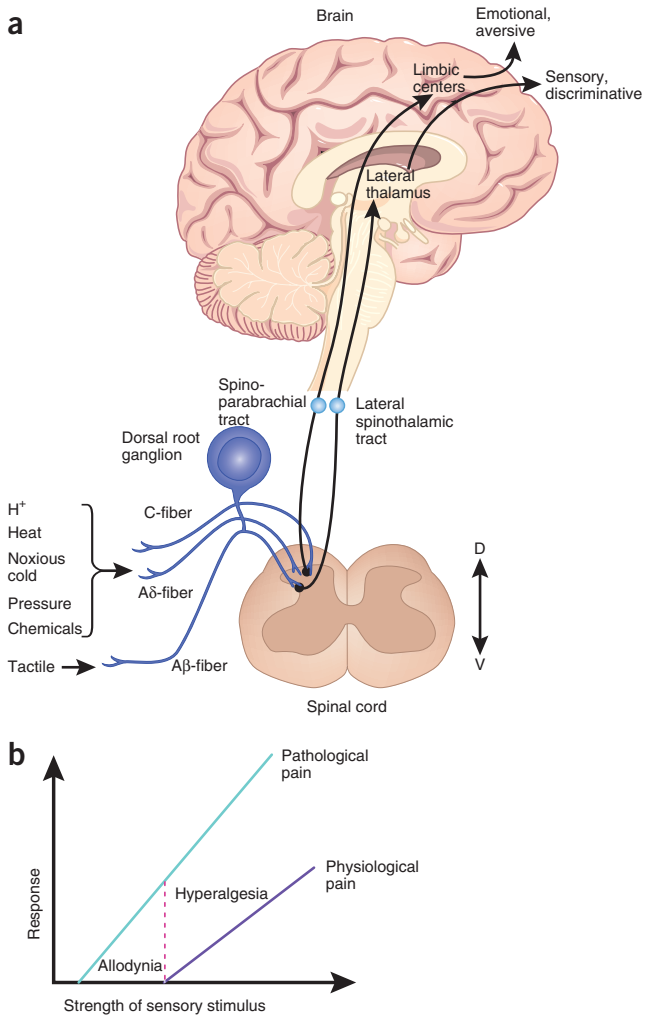

Figure 1 Pain circuits. (**a**,**b**) A schematic overview of the main circuits mediating physiological pain (**a**) and some manifestations of chronic pain (**b**).

ongoing pain, which might come about through mechanisms distinct from those that underlie evoked pain. Whereas evoked pain has been well studied, spontaneous pain is only beginning to be addressed in experimental animals[5,6]. Neural plasticity is a key element of chronic pain and can account to a large extent for the clinical manifestations of chronic pain[3,7].

The different forms and levels of plasticity

Dynamic changes in the neural matrix of pain can occur over several temporal scales (acute to chronic) and on the molecular, synaptic, cellular and network levels (**Fig. 2**).

Molecules may change in an activity-dependent manner (for example, by phosphorylation) and thereby alter their function (for example, a drop in the activation threshold of an ion channel) or localization (for example, endocytosis or trafficking; **Fig. 2a**). At the synaptic level, the strength of synaptic contacts can vary from a failure to produce any postsynaptic response at one extreme (silent synapse) to a state in which low levels of transmitter release can evoke action potentials in the target neurons (potentiated state; **Fig. 2a**). Classical postsynaptic mechanisms of synaptic potentiation result in amplified excitatory postsynaptic potentials in spite of unchanged neurotransmitter availability and typically involve the insertion of glutamatergic AMPA receptors (AMPARs) into postsynaptic membranes, driven by activation of glutamatergic NMDA receptors (NMDARs; **Fig. 2a**). Conversely, alterations in the strength of synaptic inputs mediated, for example, by changing the probability of neurotransmitter release or quantal content, could be a key mechanism for eliciting changes in the net excitation of neurons, particularly at synapses with a low release probability under physiological conditions (**Fig. 2a**). Long-term potentiation of nociceptive transmission has been reported in the spinal dorsal horn[8] and anterior cingulate cortex (ACC)[9].

Plasticity at the level of neurons in nociceptive pathways is seen as an increase in the magnitude of responses to a defined sensory stimulus, an increase in the level of spontaneous activity, or after-discharges, which represent continued activity after the termination of a nociceptive stimulus (**Fig. 2a**), leading to central amplification of pain (central sensitization)[7,10]. Furthermore, the peripheral receptive fields of neurons can expand, allowing hyperalgesia to spread to uninjured regions (**Fig. 2a**).

There is tremendous potential for plasticity at network-level processing of nociceptive inputs. Depending upon how the networks that underlie the sensory and affective dimensions of pain are wired and how diverse inputs are coordinated, filtered and integrated, entirely different outputs may emerge from the same given peripheral input[11] (**Fig. 2a**). The net output of the spinal dorsal horn represents a balance between diverse excitatory processes and spinal inhibitory interneurons (**Fig. 3a**), which can be disrupted in pathological states (**Fig. 3b**). For example, in naive rodents unilateral nociceptive stimulation evokes unilateral spinal calcium transients, and these are potentiated and spread contralaterally in states of peripheral inflammation[12] or neuropathy[13] (**Fig. 2a**); these changes can be mimicked by blocking spinal GABAergic and glycinergic inhibition[13].

Finally, tremendous complexity and dynamic range is added by the fact that plasticity not only occurs at a functional level, but can also take place at a structural level (**Fig. 2b**). Examples of this include an increase or a decrease in the density of synaptic spines, degeneration or regeneration of axons leading to aberrant connectivity, degeneration of neurons and proliferation of astrocytes and microglia, which influence nociceptive processing by releasing modulatory substances (**Figs. 2b** and **3b**). Importantly, structural plasticity can account for the long-term persistence of changes that arise in pathological pain states.

Diverse molecules modulate spinal pain processing by activating cell surface receptors in discrete spatial and temporal patterns[7,14,15]. The main receptors that mediate these influences include ligand-gated ion channels, which regulate neuronal excitability at a scale of microseconds to seconds (**Fig. 4**). Most prominent amongst ion channel receptors are NMDA and AMPA-type glutamate receptors and ATP-gated P2X3-type ion channels[7]. Second, G protein–coupled receptors (GPCRs) are activated by diverse neurotransmitters and neuromodulators, such as glutamate, adenosine, ATP, cannabinoids, opioids and prostaglandins, and modulate pain processing over seconds to minutes (**Fig. 4**). Finally, receptor tyrosine kinases (RTKs) are activated in nociceptive pathways by several growth factors[16,17] (for example, trkA by nerve growth factor[16]) and act over temporal scales of minutes to hours (**Fig. 4**). In addition, signaling transducers activated by each of these three kinds of receptor can indirectly or directly modulate gene transcription, which allows long-term modulation of pain. Some of these mechanisms are described in more detail below.

AMPARs as determinants of spinal hyperalgesia

Glutamate is the main nociceptive neurotransmitter at the synapse between the primary afferent and the second-order neuron (**Fig. 3a**).

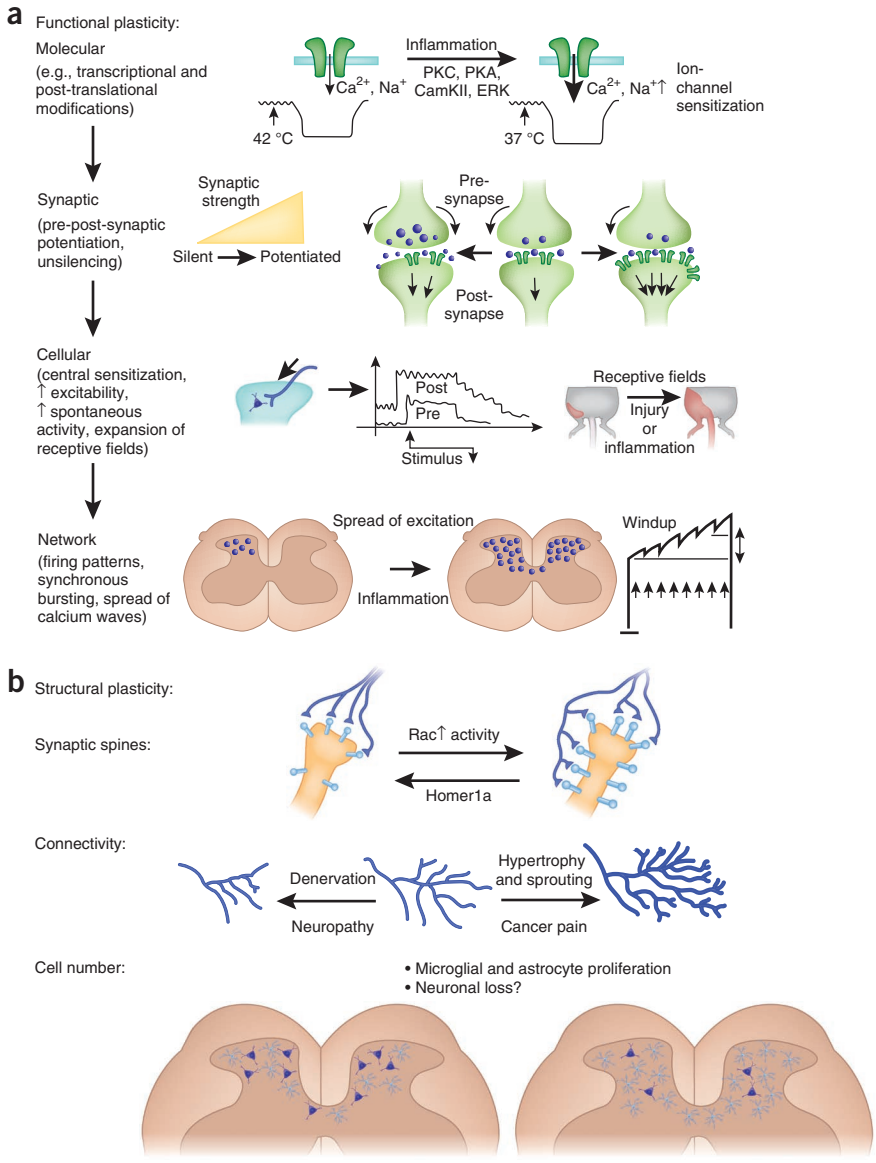

Figure 2 Disease-induced functional and structural plasticity in neural substrates of pain. (**a**) Different levels of activity-dependent functional plasticity. Molecules may become functionally sensitized (top), synaptic transmission may become potentiated by presynaptic mechanisms (second row, arrow to the left) or by postsynaptic plasticity (arrow to the right), cells may respond to noxious stimuli with increased activity and expanded receptive fields after injury (third row) and network function may change so that more cell ensembles respond to noxious stimuli, collectively leading to a higher net spinal output after injury or inflammation (bottom). (**b**) Examples of nociceptive activity-induced structural plasticity. From the top, synaptic spines may increase in size and density; axons may sprout or degenerate; and cells may atrophy (for example, loss of inhibitory interneurons) or proliferate (for example, microglia and astrocytes).

Owing to their high Ca^{2+} permeability and Mg^{2+} block under physiological conditions, NMDARs are key mediators of pathological pain[7]. Whereas NMDARs always permit Ca^{2+} entry upon activation, AMPARs encode a regulatable switch that controls glutamate-evoked entry of Ca^{2+} into neurons[18]. This is brought about by regulated expression and inclusion of the subunit GluA2 (GluR-B or GluR2), which imparts low Ca^{2+} permeability to AMPAR channels because it carries an arginine residue (inserted by Q/R site RNA editing) in its pore-forming M2 segment[18]. The dorsal horn of the spinal horn contains an unusually high density of calcium-permeable AMPARs[19], and their activation can strengthen the AMPAR-mediated component of synaptic transmission in the spinal cord[20].

Mice lacking the GluA1 subunit (GluR-A or GluR1 subunit), which is highly expressed in the spinal dorsal horn, show a loss of nociceptive plasticity and a marked reduction in acute inflammatory hyperalgesia[21]. By contrast, deletion of the GluA2 subunit, which enhances the permeability of AMPAR to calcium channels and modifies current rectification and channel conductance, leads to facilitation of nociceptive plasticity and inflammatory hyperalgesia[21]. This indicates that spinal AMPARs are crucially involved in activity-dependent changes in synaptic processing of nociceptive inputs. Importantly, potentiation and spatial spread of spinal calcium transients induced by peripheral inflammation are lost in mice that do not possess calcium-permeable AMPARs, showing that GluR-A-containing AMPARs are a key source of calcium in spinal nociceptive laminae in pain states[12].

Protein-protein interactions in spinal circuits

Various excitatory and inhibitory receptors interact with transmembrane proteins or cytosolic modulators, which can alter the cell surface expression and function of transmembrane receptors. For example, the long-form Homer proteins, Homer1b and Homer1c, link metabotropic glutamate receptors ($mGluR_1$ and $mGluR_5$) to sources of calcium release (inositol 1,4,5-triphosphate receptors) at synapses, and to transient receptor potential C channels, calcium channels and components of the NMDA receptor complex at the cell surface[22,23]. These interactions are antagonized by the short activity-dependent splice variant of the Homer1 gene, Homer1a, which is upregulated in the spinal dorsal horn in models of peripheral inflammation and neuropathy in an NMDA receptor–dependent manner[23]. Homer1a strongly attenuates calcium mobilization induced by glutamate receptors and the subsequent activation of MAP kinases, thereby reducing inflammatory hyperalgesia[23]. Thus, activity-dependent upregulation of the Homer1a protein represents a negative feedback loop that uncouples glutamatergic receptors from intracellular nociceptive mediators and counteracts central sensitization.

Interactions between the AMPAR subunits GluA2 and GluA3 and proteins containing PDZ (postsynaptic density 65–discs large–zonula occludens 1) domains such as glutamate receptor-interacting protein (GRIP) are important for the activity-induced unsilencing of silent synapses in the spinal dorsal horn[24]. Similarly, interactions between GluA2 subunits and intracellular adaptor proteins such as PICK2 and NSF have also been implicated in neuropathic sensitization of nociceptive inputs[25]. The properties and configuration of AMPARs

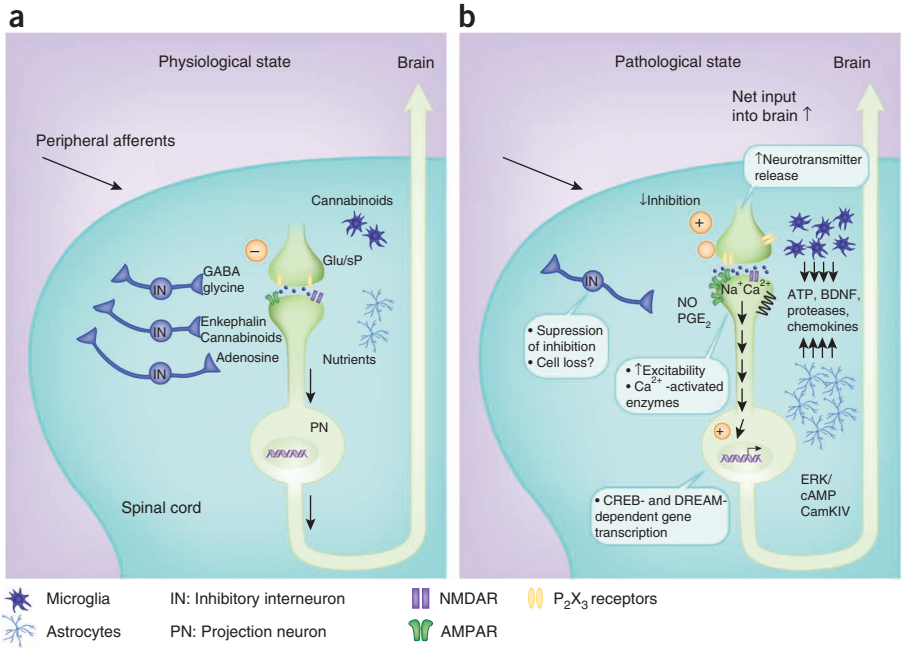

Figure 3 Spinal mechanisms of physiological pain and disease-induced pain hypersensitivity. (**a**,**b**) The diagrams show a few prominent of many possible mediators and cell-cell interactions in the spinal cord dorsal horn in physiological states (**a**) and disease states (**b**). Putative changes in pathological states include mechanisms involving suppression of inhibition, potentiation of presynaptic release and postsynaptic excitability, increases in synapse-to-nucleus communication and gene transcription, release of neuromodulators from activated microglia and astrocytes and a net increase in nociceptive input onto higher brain structures. Glu, glutamate; sP, substance P.

are dynamically regulated in pain states. Peripheral inflammation induces the internalization of the GluA2 subunit in dorsal horn neurons[26], which is mediated by binding of GluA2 to GRIP and leads to an increase in the calcium permeability of AMPARs. Therefore, preventing the internalization of GluA2 subunits impairs nociceptive hypersensitivity in inflammatory states[26]. Activity can also induce the insertion of new AMPARs at spinal synapses (**Fig. 3b**), which suggests that rapid activity-dependent modification of AMPAR properties is a key mechanism for rapid short-term plasticity in the spinal dorsal horn[27].

The Shank family of postsynaptic density (PSD) proteins, which work with Homer proteins to form a molecular bridge that links NMDARs and $mGluR_1$ and $mGluR_5$ in the postsynaptic space, are also upregulated in spinal dorsal horn neurons after peripheral nerve injury[28]. Similarly, the multivariant adaptor protein PSD95 is required for the coupling of NMDARs, nitric oxide (NO) synthase and calcium/calmodulin–dependent protein kinase IIα (CamKIIα) at the postsynaptic density (**Fig. 3b**). Mice that express a truncated form of PSD95 show a loss of NMDAR-mediated hyperalgesia and allodynia and a reduction in CamKIIα activation upon peripheral injury[29]. Nociceptive activity also switches on the expression of proteins in the spinal dorsal horn such as the NO inhibitory protein (NOSIP)[30], which blocks NMDAR signaling through NO. This suggests that expression of NOSIP is an activity-induced defense mechanism to block spinal amplification of pain.

GPCR-mediated mechanisms in the spinal facilitation of pain

G proteins of the $G_{q/11}$ family couple numerous GPCRs to the activation of phospholipase Cβ (PLC-β), protein kinase C (PKC) and intracellular calcium release[31] and mediate the facilitatory effects of glutamate, substance P and other neuromodulators on spinal neuronal function. The G_s family proteins link GPCR activation to the production of cAMP and subsequent activation of protein kinase A (PKA)[31]. Numerous ion channels, GPCRs and intracellular effectors carry recognition sites for PKC and PKA, and both kinases have major roles in modulating pain processing[32,33] (**Fig. 4**).

GPCRs also contribute to the actions of proteolytic enzymes, which are an exciting and newly recognized potential target for modulating nociceptive processing in the spinal cord. These serine proteases include members of the coagulation cascade (for example, thrombin factor, plasminogen and tissue plasminogen activator), proteases from inflammatory cells (for example, cathepsin G) and proteases from epithelial tissues and neurons (for example, trypsin)[34–39] (**Fig. 3b**). Most of the functions of serine proteases are carried out by cleaving and activating protease-activated receptors (PARs), which are a family of GPCRs[36,37]. In most tissues, PARs act by coupling to $Gα_{q/11}$, $Gα_{12/13}$ and inhibitory $Gα_i$ proteins (**Fig. 4**). Proteases contribute to peripheral neurogenic inflammation by sensitizing TRP channels[40] and also act at the spinal level to enhance neuronal excitability in inflammatory states[34–39]. Similarly, other proteases such as cathepsin[35,41] and the matrix metalloproteases[39] have been implicated in potentiating spinal output by mediating interactions between neurons, glia and astrocytes[42]. However, there is some evidence that spinal proteases have antinociceptive functions[34]. Recently, a transmembrane isoform of prostatic acid phosphatase, which avidly and selectively binds subsets of nociceptive neurons, was found to block acute and chronic pain by functioning as a ecto-5′-nucleotidase and dephosphorylating extracellular AMP to adenosine, which then activates spinal $Gα_i$-coupled A1-adenosine receptors[43]. As enzymes make good drug targets, and proteases and related enzymes have marked functional roles in spinal hyperalgesia, these molecules provide new hope for the development of therapeutic strategies.

In addition to $Gα_{q/11}$ and $Gα_s$, $Gα_{12/13}$ might also contribute to chronic pain. For example, a single injection of lysophosphatidic acid (LPA) into the intrathecal space elicits profound mechanical allodynia for several days, accompanied by a rapid loss of myelin in peripheral nerves that is induced by activation of the LPA_1 receptor-$Gα_{12/13}$-Rho pathway[44]. However, whether the two phenotypes are linked, and whether and how demyelination could induce allodynia, needs to be clarified.

Common signatures for pain-sensitizing molecules

A recurring theme with most mediators of pain is their effect on intracellular calcium, which leads to the activation of several calcium-dependent kinases, such as CamKIIα, cyclooxygenase-2 (COX-2) and the NO synthase (NOS) family (**Figs. 3b** and **4**). Prostaglandin E_2 (PGE_2) and NO, the products of COX-2 and neuronal NOS, respectively, have been proposed to function as retrograde messengers and to facilitate neurotransmitter release from primary afferent terminals in the spinal dorsal horn (**Fig. 3b**).

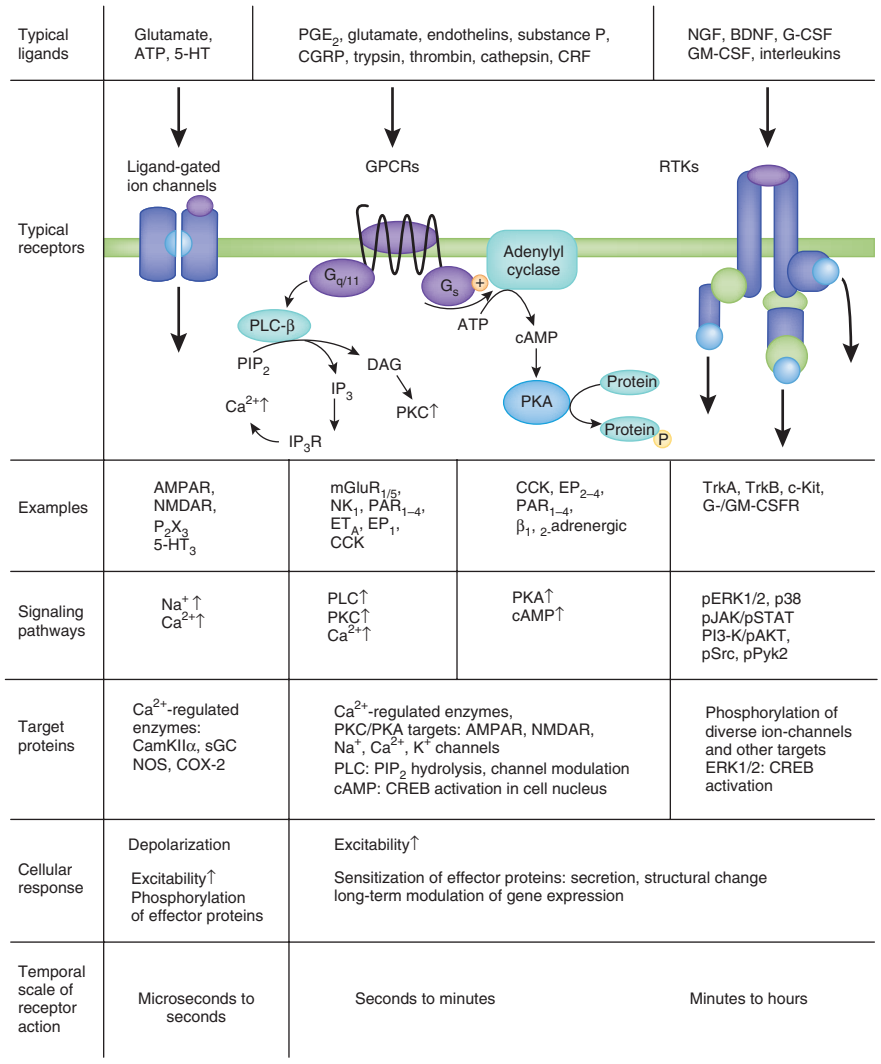

Figure 4 Overview of typical signaling pathways used by pronociceptive molecules that mediate disease-induced pain hypersensitivity. Shown are typical ligands and the types of receptors and signaling mediators that they use to induce changes in the expression or function of target proteins, thereby leading to characteristic functional changes over diverse timescales. 5HT, serotonin; CGRP: calcitonin gene-related peptide; CRF, corticotrophin releasing factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IP3R, inositol 1,4,5-triphosphate receptor; DAG, diacylglycerol; P_2X_3, ATP-gated ion channel; $5\text{-}HT_3$, serotonin-gated ion channel; NK_1: neurokinin receptor-1; $PAR_{1\text{-}4}$, protease-activated receptors 1-4; ET_A, endothelin receptor A; EP_1, prostaglandin receptor-1; CCK, cholecystokinin; TrkA, neurotrophin receptor A; TrkB, neurotrophin receptor B; G-/GM-CSFR, G-CSF receptor and GM-CSF receptor pJAK, phosphorylated Janus-activated kinase; pSTAT, phosphorylated signal transducer and activator of transcription; PI3-K, phosphoinositol 3-kinase; pAKT, phosphoprotein kinase B; sGC, soluble guanylyl cyclase; PIP_2, phosphoinositol diphosphate.

The mitogen-activated protein kinases extracellular signal–regulated kinase-1 (ERK1) and ERK2 are also activated downstream of diverse ion channels, GPCRs and RTKs and have gained a prominent place in the field of nociceptive sensitization[45]. The $K_v4.2$ potassium channel, which regulates the excitability of neurons, has been identified as a prominent target of ERK1 and ERK2 (ref. 46).

Synapse-to-nucleus communication

The ability of ERK1 and ERK2 to phosphorylate ion channels probably mediates only the acute component of ERK-induced hyperalgesia. However, ERK1 and ERK2 can also induce long-lasting changes in pain sensitivity by migrating to the cell nucleus and acting on gene transcription. Moreover, cAMP and CamKIV are also synapse-to-nucleus communicators and thereby recruit a chronic 'memory' component for pathological pain[25] (**Fig. 3b**). In the cell nucleus, cAMP and ERK trigger the activation of the cAMP response element (CREB), which drives the expression of a variety of pain-related proteins, such as COX-2, transient receptor potential vanilloid-1 (TRPV1) and calcium channels (**Fig. 3b**). Another transcriptional repressor, DREAM, acts constitutively to suppress prodynorphin expression in spinal cord neurons and thereby to elicit hyperalgesia[47].

Inhibitory networks and pain processing

An important way in which the net output of the spinal dorsal horn can be modulated is by controlling the degree of tonic and phasic inhibition, which is determined by GABAergic and glycinergic neurotransmission and by endogenously released opioids, cannabinoids and adenosine (**Fig. 3a**). A balance between the activation of metabotropic glutamate and GABA receptors can control the intrinsic firing properties of deep dorsal horn neurons and switch them between diverse activation modes, such as tonic, plateau or bursting patterns, by modulating inwardly rectifying potassium channels[48]. It has been proposed that a selective loss of GABAergic interneurons in the spinal dorsal horn after nerve injury causes an imbalance between excitation and inhibition[49,50].

PGE_2 causes protein kinase A–dependent phosphorylation and inhibition of glycine receptors that contain the α3 subunit and thereby relieves dorsal horn neurons from glycinergic inhibition[51]. Another intriguing mechanism for disinhibition of spinal neurons is related to a nerve injury–induced collapse of the chloride gradient, which is coupled with enhanced excitability in postsynaptic neurons[52]. Loss of the postsynaptic potassium chloride exporter KCC2 mediates this phenomenon and leads to a reduction in GABA-mediated inhibitory postsynaptic currents[52], a process that is aided by bone-derived neurotrophic factor (BDNF) released from microglia[53] (**Fig. 3b**). Depletion of KCC2 has also been implicated in the pathogenesis of pain associated with spinal cord injury[54], diabetic neuropathy[55] and other forms of chronic pain. Modulating the activation of $GABA_A$ receptors with subunit-specific agonists may constitute a promising approach for inhibiting pathological pain without eliciting the numerous typical side effects of $GABA_A$-modifying drugs[56].

Mechanisms of tactile allodynia

Because the peripheral Aβ fibers that conduct mechanical touch are distinct from nociceptors (C and Aδ fibers), mechanical allodynia is

generally considered to be determined by central changes that are triggered by increased activity of nociceptors. In some animal models of chronic pain, such as knee arthritis[57], sensitization of C fibers might even contribute to the maintenance of mechanical allodynia and persistent changes in C fiber activity are seen in many clinical settings of chronic pain. In contrast, it has also been suggested that nociceptors are not required for the induction of mechanical hypersensitivity: toxin-based ablation of $Na_v1.8$-expressing nociceptors, which affects nearly all nociceptive neurons of the dorsal root ganglia, was reported to elicit deficits in responses to noxious mechanical pressure and cold stimuli, but not in nerve injury–induced mechanical allodynia[58]. This is mostly consistent with previous observations in animals in which capsaicin has been used to produce neonatal ablation of TRPV1-expressing C fibers[59]. Although these results are compelling, they do not fully rule out the possibility that peripheral nociceptors contribute to neuropathic allodynia, because these approaches may not target all nociceptors and because inducing widespread death of nociceptors during development could elicit adaptive plasticity and trigger compensatory mechanisms. Indeed, recent results suggest that a distinct class of C-type sensory neuron (unmyelinated, low-threshold C mechanoreceptors, which express the vesicular glutamate transporter VGLUT3) are involved in mediating mechanical hypersensitivity caused by injury[60].

Despite these advances, it is unclear why low-threshold inputs are read or interpreted as painful or unpleasant after nerve injury. Collateral sprouting of low-threshold Aβ fibers on to 'nociceptive' second-order neurons in the spinal dorsal horn has been observed after peripheral injury[61], but this might not occur consistently or in a sufficiently large magnitude[62]. Furthermore, the fact that disinhibition of spinal networks by acute blockade of spinal GABAergic or glycinergic transmission can induce immense tactile allodynia within minutes[63] suggests that there must be a hard-wired pathway already in place that is normally under strong inhibitory control. Electrophysiological studies show that whereas only high-threshold monosynaptic inputs are found on pain- and temperature-sensitive spinal projection neurons under normal circumstances, blockade of local inhibition uncovers substantial Aβ, low-threshold fiber inputs, which are polysynaptic and require NMDA receptor activation to be functional[64,65].

Nociceptive processing and hyperalgesia at supraspinal sites

Thalamic relay nuclei have a key role in gating, filtering and processing sensory information *en route* to the cerebral cortex and are subject to similar activity-induced plasticity processes as the spinal cord[46,66]. However, the role of corticothalamic and thalamocortical loops in regulating sensory gating in the thalamus has not been widely studied in the context of pain. Two modes of firing of thalamocortical neurons, tonic and burst firing, are believed to reflect the divergent states of sensory signal transmission from the thalamus to the cortex. The $mGluR_1$/$mGluR_5$-PLCβ pathway increases burst firing and decreases tonic firing in thalamocortical neurons by concurrently regulating T-type and L-type calcium currents[67], and this process is associated with reduced visceral pain responses, suggesting that switching between the firing modes of thalamocortical neurons is a key mechanism for gating of incoming sensory nociceptive inputs.

The ACC mediates key emotional-aversive aspects of pain[68] and may also have a mnemonic role in which it allows transient storage of information during pain processing. Peripheral nerve injury triggers long-term changes in excitatory synaptic transmission in layer 2/3 neurons in the ACC, recruiting both pre- and postsynaptic mechanisms of potentiation[9,69], which involve GluR-A–containing AMPARs, activation of ERK1 and ERK2 and the calcium-stimulated adenylyl cyclase-1 (refs. 68,69).

Another key limbic system structure that has been implicated in the affective component of pain is the central nucleus of the amygdala (CeA; nociceptive amygdala). Interestingly, rats with arthritic pain show enhanced transmission at synapses in the CeA with afferents that bring nociceptive inputs from the parabrachial nucleus as well as those that bring polymodal sensory inputs from the basolateral amygdala[65]. This enhanced transmission is mediated by G protein signaling through $mGluR_1$ and $mGluR_5$ and corticotrophin-releasing factor receptors[70,71]. Furthermore, at glutamatergic synapses between CeA neurons and nociceptive inputs from the pontine parabrachial nuclues, endogenously released noradrenaline acting at presynaptic α_2 receptors decreases the number of active release sites for glutamate with no change in release probability, suggesting that the CeA might be an important target region for the antinociceptive actions of noradrenaline[72].

Descending modulation of spinal gating of pain

Descending inhibitory systems block spinal transmission, leading to hyposensibility or a lack of pain, in spite of inputs coming in from the periphery. Such inhibitory mechanisms have evolutionary value because they can enable the organism to ignore pain in critical situations, such as flight or fight, and serve as a mechanistic basis for placebo-induced analgesia[73]. Furthermore, descending modulatory systems may contribute to analgesia produced by a variety of non-pharmacological pain control approaches, such as transcutaneous electrical nerve stimulation, acupuncture and hypnosis.

Converging lines of evidence from anatomical, electrophysiological and pharmacological studies show that the axis of the periaqueductal gray (PAG) and rostroventral medulla (RVM) can inhibit or facilitate sensory processing in the spinal dorsal horn[74]. Descending control can also arise from the lateral and caudal dorsal reticular nucleus and the ventrolateral medulla. Owing to their therapeutic role and their contribution to opioidergic control of pain and placebo analgesia, much attention was initially focused on descending adrenergic and serotonergic pathways, originating from neurons in the locus coeruleus and nucleus raphe magnus, respectively, which finally lead to the activation of local encephalenergic neurons in the spinal dorsal horn. The differential contributions of the noradrenergic and serotonergic components to opioid-induced analgesia have been a topic of much debate. Pharmacological manipulations that increase synaptic levels of serotonin and noradrenaline, such as the use of tricyclic antidepressants and other classes of antidepressant, have gained prominence in the clinical management of chronic pain, particularly in therapy-resistant states such as neuropathic pain and fibromyalgia[74,75]. The *in vivo* analgesic efficacy of selective serotonin reuptake inhibitors in clinical trials is lower than that of drugs that affect both serotonin and noradrenaline (for example, tricyclic antidepressants). It has been suggested that the analgesic effects of antidepressant drugs occur mainly through the modulation of noradrenaline in the spinal dorsal horn. However, mice lacking a LIM homeobox transcription factor called Lmx1b, which lack serotonergic neurons in the adult CNS, show markedly reduced analgesia in response to opioids and antidepressants, suggesting that central serotonergic neurons constitute an important part of the descending pain modulatory circuitry that mediates analgesia induced by opioids and antidepressants[76,77].

Recent years have brought substantial advances in the understanding of descending facilitation of pain by the PAG-RVM axis[74]. Site-specific microinjections of local anaesthetics and lesion studies have helped to

work out the circuitry that underlies this process and its functional role in mediating the facilitatory influences of supraspinal sites[74,75]. On a mechanistic level, it has been reported that both NMDA receptor–NO signaling and cholecystokinin are involved in the control of RVM excitability. Neurons in the RVM that express both cholecystokinin receptor 2 and the μ-opioid receptor, which are directly activated by cholecystokinin input to the RVM, are important for descending facilitation and their ablation markedly reduces the duration of neuropathic pain[78]. Persistent afferent inputs that arise from peripheral injury or inflammation produce neuroplastic changes in the RVM, such as activation and proliferation of microglia and astrocytes, phosphorylation of the p38 MAP kinase, release of BDNF and upregulation of NMDAR subunits[79,80]. Interestingly, the ablation of lamina 1 projection neurons, which express neurokinin 1 receptors, leads to a decrease in activity-induced activation of serotonergic neurons in the brain stem and to loss of descending facilitation[81]. These and other observations have led to the notion that upon activation by primary afferent input, spinal projection neurons recruit PAG-RVM modulatory loops to trigger descending facilitation of nociceptive transmission in the spinal dorsal horn and that this could be further amplified by feedback from the amygdala and the ACC in states of chronic pain.

Anatomical and pharmacological considerations suggest that although facilitatory and inhibitory pathways arising from the RVM are distinct, they are likely to be activated simultaneously in conditions of acute nociception. However, in pathological pain states, neuroplastic changes such as those described above may yield sustained facilitation, tipping the balance in favor of amplifying pain. A key question is how this bidirectional control of spinal transmission can be achieved by the circuitry in the RVM. A widely accepted theory proposes that two distinct populations of neurons in the brainstem, 'on cells' and 'off cells', are differentially recruited by higher brain structures in conditions of chronic pain, stress or fear to facilitate or inhibit pain at the spinal level[82]. It is intriguing to note that in conditions of tissue injury or persistent activation of nociceptors, a phenotypic switch is seen in RVM neurons such that the incidence of on and off cells in the population increases, coupled with a corresponding decrease in neutral cells[74,75]. For example, application of inflammatory mediators to the dura leads to an activation of on cells and a transient inhibition of off cells in the RVM, which is associated with facial allodynia in headache-related pain[83].

The precise mechanism by which spinal synaptic transmission is facilitated by descending influences is a matter of much interest. Spinal depletion of serotonin reduces mechanically evoked responses of deep dorsal horn neurons in electrophysiological studies and pharmacological studies indicate that spinal 5-HT3 receptors make a key contribution to facilitation. Importantly, serotonin applied spinally can transform silent glutamatergic synapses into functional ones by insertion of AMPARs[84]. Mice that genetically lack serotonergic neurons[76] show enhanced inflammatory pain, which is attenuated by spinal delivery of serotonin, but also show decreased sensitivity to mechanical painful stimuli in basal (naive) conditions. One interpretation of these findings is that descending serotonergic pathways facilitate mechanical sensitivity in circumstances of acute pain, but that in inflammatory conditions the inhibitory influences of descending serotonergic neurons prevail[76]. However, this notion is not fully supported by a recent study, which reports that selective depletion of serotonin in RVM neurons by local RNA interference of tryptophan hydroxylase-2, the rate-limiting enzyme in the synthesis of neuronal serotonin, attenuates tissue or nerve injury-induced allodynia and hyperalgesia[85]. These contrasting observations may stem from differences in the depletion of serotonin in terms of spatial (global versus RVM specific) and temporal (onset at prenatal versus adult stages) profiles. In summary, descending serotonergic influences not only serve as a key mechanism to regulate the gain of pain transmission in a complex and context-dependent manner, but also lay the basis for pharmacological development of drugs.

Structural plasticity in neural networks underlying pain

A tremendous level of modulation can be achieved by structural modifications of nociceptive pathways. Structural plasticity can occur at various anatomical and temporal scales (**Fig. 1b**). At the macroscopic anatomical level, long-term neuropathic pain in humans has widespread effects on brain anatomy related to the duration and magnitude of pain[86]. Local morphological alterations in the brain, mostly representing a decrease in the brain gray matter, have been reported in people with phantom pain, chronic back pain, irritable bowel syndrome, fibromyalgia and headaches, among others[86,87]. The important question is whether these changes are the cause or the consequence of pain. Furthermore, specificity is an issue, as other distinct neural disorders, such as depression, are also accompanied by changes in brain volume. One interesting observation that supports a role for brain volume changes in chronic pain is that different pain syndromes appear to be associated with distinct patterns of alterations spanning the ACC, orbitofrontal cortex, insular cortex and dorsal pons. Furthermore, the decrease in gray matter associated with chronic pain is at least partially reversible when pain is successfully treated, suggesting that these structural changes are a reversible consequence of frequent nociceptive inputs[88]. Interestingly, these changes have been modeled successfully in rats, making it possible to carry out mechanistic studies into the functional relevance of the observations made in chronic pain patients[89].

Another level of structural plasticity is that of activity-dependent changes in connectivity. Striking changes in the structure of nerves such as denervation, renervation, sprouting and hypertrophy have been reported in peripheral tissues, such as the skin, bone or visceral organs in pathological pain states in humans and experimental animals[90–93] (**Fig. 1b**). The functional role of such morphological changes is not clear, especially as most of the morphological studies have been done on fixed tissue in biopsies, which precludes an unequivocal causal association with changes in pain perception[90–92]. For example, in animal models of cancer-induced pain, recurring cycles of denervation and renervation occur, which may lead to confounding results across studies depending upon which time point was examined[93]. It will be imperative in future studies to apply noninvasive *in vivo* imaging techniques to models of chronic pain to establish causal associations between structural plasticity of nerves and pain levels over the temporal progression of the disease.

Perhaps the most exciting form of structural plasticity refers to activity-dependent changes in dendritic spines, which define the strength of excitatory synaptic transmission[94] (**Fig. 1b**). Sensory inputs can profoundly alter both the stability and function of synaptic contacts by inducing activity-dependent changes in spines over a time scale ranging from seconds to hours or even days[95]. It has been proposed that in adult animals, most spines that are newly formed by sensory inputs are transient and changes in spine morphology and shape rather than spine number may reflect the dynamic state of the associated synapse. However, new evidence shows that a small fraction of new spines generated by a novel sensory experience are preserved and are associated with life-long memories[96]. Interestingly, several key mediators of spine stabilization and turnover, which have been studied

in brain circuits[95], overlap with molecules that mediate spinal pain hypersensitivity, such as AMPARs, NMDARs, CamKIIα and ephrins as well as other RTKs (**Fig. 2b**). Neuropathic pain resulting from spinal cord injury is associated with both increased *de novo* formation and elaboration of dendritic spines in spinal laminae IV and V[97].

From studies on the brain, it is known that changes in the shape and size of dendritic spines are determined by rapid remodelling of the underlying actin cytoskeleton[98]. Furthermore, most signaling pathways that link synaptic activity to spine morphology influence local actin dynamics[97,98]. For example, the Rho/Rac families of GTPases transduce signals coming from extracellular stimuli (such as ephrins or glutamate) to the actin cytoskeleton and contribute to plasticity in dendritic spines[99]. In this context, it is interesting to note that modulation of spine morphology and density in the spinal dorsal horn induced by spinal cord injury is reversed by inhibiting Rac1, which also leads to amelioration of injury-induced hyperalgesia[97] (**Fig. 2b**). Furthermore, Homer1a also reduces the density of spines in lamina IV and V neurons in the spinal dorsal horn[23] (**Fig. 2b**). Finally, recent evidence connects gabapentin, a key pharmacological drug used in the therapy of epilepsy and neuropathic pain, to remodelling of synaptic contacts. Gabapentin is an inhibitor of α2δ-1, which was proposed to be a component of voltage-sensitive calcium channels; however, it was recently found that α2δ-1 constitutes a thrombospondin receptor and gabapentin inhibits excitatory synaptogenesis in the brain by antagonizing thrombospondin binding to α2δ-1, a mechanism that has been linked to the antiepileptic actions of gabapentin[100]. This raises the exciting possibility that a similar mechanism in spinal networks might produce an antihyperalgesic effect of gapapentin in neuropathic pain states.

Beyond molecular mechanisms—a need for improved translation

Although there are many targets that could be selected to take forward to therapeutic and clinical development, several hindrances have to be overcome to enable bench-to-bedside translation. For one, redundancy between nociceptive mediators and mechanisms limits the clinical utility of single approaches. Second, most of the key intracellular mediators of nociceptive plasticity, such as MAP kinases, PKC, PLC and CREB, are not specific for the pain modulatory system and have global functions in physiology, thereby necessitating the development of site-specific delivery tools. One possibility to side-step central side effects is to target peripheral mechanisms early on and to use peripheral analgesia, as has been shown for opioids and cannabinoids. Finally, a focus on understanding and targeting the mechanisms that underlie spontaneous pain in pathological states is urgently required. Despite these challenges, the sheer breadth and depth of pain research and the rapid pace at which new insights have been attained over recent years provides a strong basis for the belief that excellent therapeutics are within reach.

ACKNOWLEDGMENTS

I thank R. Lefaucheur for secretarial assistance and members of the laboratory for discussions.

COMPETING FINANCIAL INTERESTS

The author declares no competing financial interests.

Published online at http://www.nature.com/naturemedicine/.
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/.

1. Darwin, C. *The Expression of the Emotions in Man and Animals* (Murray, J., London, 1872).
2. Todd, E.M. & Kucharski, A. Pain: historical perspectives. in *Principles and Practice of Pain Medicine* (eds. Warfield, C.A. & Bajwa, Z.H.) 1–10 (McGraw-Hill, 2004).
3. Melzack, R. & Wall, P.D. Pain mechanisms: a new theory. *Science* **150**, 971–979 (1965).
4. Baron, R. Neuropathic pain: a clinical perspective. *Handb. Exp. Pharmacol.* **194**, 3–30 (2009).
5. King, T. *et al.* Unmasking the tonic-aversive state in neuropathic pain. *Nat. Neurosci.* **12**, 1364–1366 (2009).
6. Kurejova, M. *et al.* An improved behavioural assay demonstrates that ultrasound vocalizations constitute a reliable indicator of chronic cancer pain and neuropathic pain. *Mol. Pain* **6**, 18 (2010).
7. Woolf, C.J. & Salter, M.W. Neuronal plasticity: increasing the gain in pain. *Science* **288**, 1765–1769 (2000).
8. Ikeda, H., Heinke, B., Ruscheweyh, R. & Sandkuhler, J. Synaptic plasticity in spinal lamina I projection neurons that mediate hyperalgesia. *Science* **299**, 1237–1240 (2003).
9. Toyoda, H. *et al.* Roles of the AMPA receptor subunit GluA1 but not GluA2 in synaptic potentiation and activation of ERK in the anterior cingulate cortex. *Mol. Pain* **5**, 46 (2009).
10. Woolf, C.J. Evidence for a central component of post-injury pain hypersensitivity. *Nature* **306**, 686–688 (1983).
11. Price, D.D. Psychological and neural mechanisms of the affective dimension of pain. *Science* **288**, 1769–1772 (2000).
12. Luo, C., Seeburg, P.H., Sprengel, R. & Kuner, R. Activity-dependent potentiation of calcium signals in spinal sensory networks in inflammatory pain states. *Pain* **140**, 358–367 (2008).
13. Schoffnegger, D., Ruscheweyh, R. & Sandkuhler, J. Spread of excitation across modality borders in spinal dorsal horn of neuropathic rats. *Pain* **135**, 300–310 (2008).
14. Ji, R.R., Kohno, T., Moore, K.A. & Woolf, C.J. Central sensitization and LTP: do pain and memory share similar mechanisms? *Trends Neurosci.* **26**, 696–705 (2003).
15. Sandkühler, J. Models and mechanisms of hyperalgesia and allodynia. *Physiol. Rev.* **89**, 707–758 (2009).
16. Pezet, S. & McMahon, S.B. Neurotrophins: mediators and modulators of pain. *Annu. Rev. Neurosci.* **29**, 507–538 (2006).
17. Milenkovic, N. *et al.* Nociceptive tuning by stem cell factor/c-Kit signaling. *Neuron* **56**, 893–906 (2007).
18. Burnashev, N., Monyer, H., Seeburg, P.H. & Sakmann, B. Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. *Neuron* **8**, 189–198 (1992).
19. Tong, C.K. & MacDermott, A.B. Both Ca^{2+}-permeable and -impermeable AMPA receptors contribute to primary synaptic drive onto rat dorsal horn neurons. *J. Physiol. (Lond.)* **575**, 133–144 (2006).
20. Gu, J.G., Albuquerque, C., Lee, C.J. & MacDermott, A.B. Synaptic strengthening through activation of Ca^{2+}-permeable AMPA receptors. *Nature* **381**, 793–796 (1996).
21. Hartmann, B. *et al.* The AMPA receptor subunits GluR-A and GluR-B reciprocally modulate spinal synaptic plasticity and inflammatory pain. *Neuron* **44**, 637–650 (2004).
22. Xiao, B., Tu, J.C. & Worley, P.F. Homer: a link between neural activity and glutamate receptor function. *Curr. Opin. Neurobiol.* **10**, 370–374 (2000).
23. Tappe, A. *et al.* Synaptic scaffolding protein Homer1a protects against chronic inflammatory pain. *Nat. Med.* **12**, 677–681 (2006).
24. Li, P. *et al.* AMPA receptor-PDZ interactions in facilitation of spinal sensory synapses. *Nat. Neurosci.* **2**, 972–977 (1999).
25. Garry, E.M. *et al.* Specific involvement in neuropathic pain of AMPA receptors and adapter proteins for the GluR2 subunit. *Mol. Cell. Neurosci.* **24**, 10–22 (2003).
26. Park, J.S. *et al.* Persistent inflammation induces GluR2 internalization via NMDA receptor–triggered PKC activation in dorsal horn neurons. *J. Neurosci.* **29**, 3206–3219 (2009).
27. Galan, A., Laird, J.M. & Cervero, F. *In vivo* recruitment by painful stimuli of AMPA receptor subunits to the plasma membrane of spinal cord neurons. *Pain* **112**, 315–323 (2004).
28. Miletic, G., Dumitrascu, C.I., Honstad, C.E., Micic, D. & Miletic, V. Loose ligation of the rat sciatic nerve elicits early accumulation of Shank1 protein in the post-synaptic density of spinal dorsal horn neurons. *Pain* **149**, 152–159 (2010).
29. Garry, E.M. *et al.* Neuropathic sensitization of behavioral reflexes and spinal NMDA receptor/CaM kinase II interactions are disrupted in PSD-95 mutant mice. *Curr. Biol.* **13**, 321–328 (2003).
30. Dreyer, J. *et al.* Nitric oxide synthase (NOS)-interacting protein interacts with neuronal NOS and regulates its distribution and activity. *J. Neurosci.* **24**, 10454–10465 (2004).
31. Wettschureck, N. & Offermanns, S. Mammalian G proteins and their cell type specific functions. *Physiol. Rev.* **85**, 1159–1204 (2005).
32. Willis, W.D. Role of neurotransmitters in sensitization of pain responses. *Ann. NY Acad. Sci.* **933**, 142–156 (2001).
33. Gold, M.S. & Gebhart, G.F. Nociceptor sensitization in pain pathogenesis. *Nat. Med.* advance online publication, doi:10.1038/nm.2235 (14 October 2010).
34. Fang, M., Kovacs, K.J., Fisher, L.L. & Larson, A.A. Thrombin inhibits NMDA-mediated nociceptive activity in the mouse: possible mediation by endothelin. *J. Physiol. (Lond.)* **549**, 903–917 (2003).
35. Clark, A.K. *et al.* Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain. *Proc. Natl. Acad. Sci. USA* **104**, 10655–10660 (2007).

36. Vergnolle, N. *et al.* Proteinase-activated receptor-2 and hyperalgesia: a novel pain pathway. *Nat. Med.* **7**, 821–826 (2001).
37. Narita, M. *et al.* Protease-activated receptor-1 and platelet-derived growth factor in spinal cord neurons are implicated in neuropathic pain after nerve injury. *J. Neurosci.* **25**, 10000–10009 (2005).
38. Yamanaka, H. *et al.* Tissue plasminogen activator in primary afferents induces dorsal horn excitability and pain response after peripheral nerve injury. *Eur. J. Neurosci.* **19**, 93–102 (2004).
39. Kawasaki, Y. *et al.* Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. *Nat. Med.* **14**, 331–336 (2008).
40. Dai, Y. *et al.* Proteinase-activated receptor 2–mediated potentiation of transient receptor potential vanilloid subfamily 1 activity reveals a mechanism for proteinase-induced inflammatory pain. *J. Neurosci.* **24**, 4293–4299 (2004).
41. Clark, A.K., Yip, P.K. & Malcangio, M. The liberation of fractalkine in the dorsal horn requires microglial cathepsin S. *J. Neurosci.* **29**, 6945–6954 (2009).
42. McMahon, S.B. & Malcangio, M. Current challenges in glia-pain biology. *Neuron* **64**, 46–54 (2009).
43. Zylka, M.J. *et al.* Prostatic acid phosphatase is an ectonucleotidase and suppresses pain by generating adenosine. *Neuron* **60**, 111–122 (2008).
44. Inoue, M. *et al.* Initiation of neuropathic pain requires lysophosphatidic acid receptor signaling. *Nat. Med.* **10**, 712–718 (2004).
45. Ji, R.R., Baba, H., Brenner, G.J. & Woolf, C.J. Nociceptive-specific activation of ERK in spinal neurons contributes to pain hypersensitivity. *Nat. Neurosci.* **2**, 1114–1119 (1999).
46. Hu, H.J. *et al.* The kv4.2 potassium channel subunit is required for pain plasticity. *Neuron* **50**, 89–100 (2006).
47. Cheng, H.Y. *et al.* DREAM is a critical transcriptional repressor for pain modulation. *Cell* **108**, 31–43 (2002).
48. Derjean, D. *et al.* Dynamic balance of metabotropic inputs causes dorsal horn neurons to switch functional states. *Nat. Neurosci.* **6**, 274–281 (2003).
49. Moore, K.A. *et al.* Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord. *J. Neurosci.* **22**, 6724–6731 (2002).
50. Scholz, J. *et al.* Blocking caspase activity prevents transsynaptic neuronal apoptosis and the loss of inhibition in lamina II of the dorsal horn after peripheral nerve injury. *J. Neurosci.* **25**, 7317–7323 (2005).
51. Harvey, R.J. *et al.* GlyR α3: an essential target for spinal PGE2-mediated inflammatory pain sensitization. *Science* **304**, 884–887 (2004).
52. Coull, J.A. *et al.* BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* **438**, 1017–1021 (2005).
53. Coull, J.A. *et al.* Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature* **424**, 938–942 (2003).
54. Lu, Y., Zheng, J., Xiong, L., Zimmermann, M. & Yang, J. Spinal cord injury-induced attenuation of GABAergic inhibition in spinal dorsal horn circuits is associated with down-regulation of the chloride transporter KCC2 in rat. *J. Physiol. (Lond.)* **586**, 5701–5715 (2008).
55. Jolivalt, C.G., Lee, C.A., Ramos, K.M. & Calcutt, N.A. Allodynia and hyperalgesia in diabetic rats are mediated by GABA and depletion of spinal potassium-chloride co-transporters. *Pain* **140**, 48–57 (2008).
56. Knabl, J. *et al.* Reversal of pathological pain through specific spinal GABAA receptor subtypes. *Nature* **451**, 330–334 (2008).
57. Schaible, H.G., Ebersberger, A. & Von Banchet, G.S. Mechanisms of pain in arthritis. *Ann. NY Acad. Sci.* **966**, 343–354 (2002).
58. Abrahamsen, B. *et al.* The cell and molecular basis of mechanical, cold, and inflammatory pain. *Science* **321**, 702–705 (2008).
59. Shir, Y. & Seltzer, Z. A-fibers mediate mechanical hyperesthesia and allodynia and C-fibers mediate thermal hyperalgesia in a new model of causalgiform pain disorders in rats. *Neurosci. Lett.* **115**, 62–67 (1990).
60. Seal, R.P. *et al.* Injury-induced mechanical hypersensitivity requires C-low threshold mechanoreceptors. *Nature* **462**, 651–655 (2009).
61. Neumann, S., Doubell, T.P., Leslie, T. & Woolf, C.J. Inflammatory pain hypersensitivity mediated by phenotypic switch in myelinated primary sensory neurons. *Nature* **384**, 360–364 (1996).
62. Bao, L. *et al.* Peripheral axotomy induces only very limited sprouting of coarse myelinated afferents into inner lamina II of rat spinal cord. *Eur. J. Neurosci.* **16**, 175–185 (2002).
63. Zhang, Z., Hefferan, M.P. & Loomis, C.W. Topical bicuculline to the rat spinal cord induces highly localized allodynia that is mediated by spinal prostaglandins. *Pain* **92**, 351–361 (2001).
64. Baba, H. *et al.* Removal of GABAergic inhibition facilitates polysynaptic A fiber-mediated excitatory transmission to the superficial spinal dorsal horn. *Mol. Cell. Neurosci.* **24**, 818–830 (2003).
65. Torsney, C., Anderson, R.L., Ryce-Paul, K.A. & MacDermott, A.B. Characterization of sensory neuron subpopulations selectively expressing green fluorescent protein in phosphodiesterase 1C BAC transgenic mice. *Mol. Pain* **2**, 17 (2006).
66. Kolhekar, R., Murphy, S. & Gebhart, G.F. Thalamic NMDA receptors modulate inflammation-produced hyperalgesia in the rat. *Pain* **71**, 31–40 (1997).
67. Cheong, E. *et al.* Tuning thalamic firing modes via simultaneous modulation of T- and L-type Ca^{2+} channels controls pain sensory gating in the thalamus. *J. Neurosci.* **28**, 13331–13340 (2008).
68. Jeon, D. *et al.* Observational fear learning involves affective pain system and Cav1.2 Ca2+ channels in ACC. *Nat. Neurosci.* **13**, 482–488 (2010).
69. Cao, H. *et al.* Activation of extracellular signal-regulated kinase in the anterior cingulate cortex contributes to the induction and expression of affective pain. *J. Neurosci.* **29**, 3307–3321 (2009).
70. Fu, Y. & Neugebauer, V. Differential mechanisms of CRF1 and CRF2 receptor functions in the amygdala in pain-related synaptic facilitation and behavior. *J. Neurosci.* **28**, 3861–3876 (2008).
71. Neugebauer, V., Li, W., Bird, G.C., Bhave, G. & Gereau, R.W. Synaptic plasticity in the amygdala in a model of arthritic pain: differential roles of metabotropic glutamate receptors 1 and 5. *J. Neurosci.* **23**, 52–63 (2003).
72. Delaney, A.J., Crane, J.W. & Sah, P. Noradrenaline modulates transmission at a central synapse by a presynaptic mechanism. *Neuron* **56**, 880–892 (2007).
73. Eippert, F. *et al.* Activation of the opioidergic descending pain control system underlies placebo analgesia. *Neuron* **63**, 533–543 (2009).
74. Porreca, F., Ossipov, M.H. & Gebhart, G.F. Chronic pain and medullary descending facilitation. *Trends Neurosci.* **25**, 319–325 (2002).
75. Heinricher, M.M., Tavares, I., Leith, J.L. & Lumb, B.M. Descending control of nociception: specificity, recruitment and plasticity. *Brain Res. Rev.* **60**, 214–225 (2009).
77. Zhao, Z.Q. *et al.* Mice lacking central serotonergic neurons show enhanced inflammatory pain and an impaired analgesic response to antidepressant drugs. *J. Neurosci.* **27**, 6045–6053 (2007).
76. Zhao, Z.Q. *et al.* Central serotonergic neurons are differentially required for opioid analgesia but not for morphine tolerance or morphine reward. *Proc. Natl. Acad. Sci. USA* **104**, 14519–14524 (2007).
78. Zhang, W. *et al.* Neuropathic pain is maintained by brainstem neurons co-expressing opioid and cholecystokinin receptors. *Brain* **132**, 778–787 (2009).
79. Wei, F., Guo, W., Zou, S., Ren, K. & Dubner, R. Supraspinal glial-neuronal interactions contribute to descending pain facilitation. *J. Neurosci.* **28**, 10482–10495 (2008).
80. Guo, W. *et al.* Supraspinal brain-derived neurotrophic factor signaling: a novel mechanism for descending pain facilitation. *J. Neurosci.* **26**, 126–137 (2006).
81. Suzuki, R., Morcuende, S., Webber, M., Hunt, S.P. & Dickenson, A.H. Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. *Nat. Neurosci.* **5**, 1319–1326 (2002).
82. Fields, H.L. Pain modulation: expectation, opioid analgesia and virtual pain. *Prog. Brain Res.* **122**, 245–253 (2000).
83. Edelmayer, R.M. *et al.* Medullary pain facilitating neurons mediate allodynia in headache-related pain. *Ann. Neurol.* **65**, 184–193 (2009).
84. Li, P. & Zhuo, M. Silent glutamatergic synapses and nociception in mammalian spinal cord. *Nature* **393**, 695–698 (1998).
85. Wei, F. *et al.* Molecular depletion of descending serotonin unmasks its novel facilitatory role in the development of persistent pain. *J. Neurosci.* **30**, 8624–8636 (2010).
86. Seminowicz, D.A. *et al.* Regional gray matter density changes in brains of patients with irritable bowel syndrome. *Gastroenterology* **139**, 48–57 (2010).
87. May, A. Morphing voxels: the hype around structural imaging of headache patients. *Brain* **132**, 1419–1425 (2009).
88. Rodriguez-Raecke, R., Niemeier, A., Ihle, K., Ruether, W. & May, A. Brain gray matter decrease in chronic pain is the consequence and not the cause of pain. *J. Neurosci.* **29**, 13746–13750 (2009).
89. Seminowicz, D.A. *et al.* MRI structural brain changes associated with sensory and emotional function in a rat model of long-term neuropathic pain. *Neuroimage* **47**, 1007–1014 (2009).
90. Schweizerhof, M. *et al.* Hematopoietic colony-stimulating factors mediate tumor-nerve interactions and bone cancer pain. *Nat. Med.* **15**, 802–807 (2009).
91. Ceyhan, G.O. *et al.* Pancreatic neuropathy and neuropathic pain—a comprehensive pathomorphological study of 546 cases. *Gastroenterology* **136**, 177–186 (2009).
92. Siau, C., Xiao, W. & Bennett, G.J. Paclitaxel- and vincristine-evoked painful peripheral neuropathies: loss of epidermal innervation and activation of Langerhans cells. *Exp. Neurol.* **201**, 507–514 (2006).
93. Cain, D.M. *et al.* Functional interactions between tumor and peripheral nerve: changes in excitability and morphology of primary afferent fibers in a murine model of cancer pain. *J. Neurosci.* **21**, 9367–9376 (2001).
94. Hofer, S.B. & Bonhoeffer, T. Dendritic spines: the stuff that memories are made of? *Curr. Biol.* **20**, R157–R159 (2010).
95. Saneyoshi, T., Fortin, D.A. & Soderling, T.R. Regulation of spine and synapse formation by activity-dependent intracellular signaling pathways. *Curr. Opin. Neurobiol.* **20**, 108–115 (2010).
96. Yang, G., Pan, F. & Gan, W.B. Stably maintained dendritic spines are associated with lifelong memories. *Nature* **462**, 920–924 (2009).
97. Tan, A.M. *et al.* Neuropathic pain memory is maintained by Rac1-regulated dendritic spine remodeling after spinal cord injury. *J. Neurosci.* **28**, 13173–13183 (2008).
98. Hotulainen, P. & Hoogenraad, C.C. Actin in dendritic spines: connecting dynamics to function. *J. Cell Biol.* **189**, 619–629 (2010).
99. Luo, L. Rho GTPases in neuronal morphogenesis. *Nat. Rev. Neurosci.* **1**, 173–180 (2000).
100. Eroglu, C. *et al.* Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell* **139**, 380–392 (2009).