Can J Anesth/J Can Anesth (2014) 61:112–122
DOI 10.1007/s12630-013-0087-4

REVIEW ARTICLE/BRIEF REVIEW

The transition from acute to chronic pain: understanding how different biological systems interact

La transition d'une douleur aiguë vers une douleur chronique – comprendre l'interaction entre différents systèmes biologiques

Katherine A. Mifflin, BSc · Bradley J. Kerr, PhD

Received: 14 June 2013 / Accepted: 18 November 2013 / Published online: 26 November 2013

Abstract

Purpose *Although pain is an adaptive sensory experience necessary to prevent further bodily harm, the transition from acute to chronic pain is not adaptive and results in the development of a chronic clinical condition. How this transition occurs has been the focus of intense study for some time. The focus of the current review is on changes in neuronal plasticity as well as the role of immune cells and glia in the development of chronic pain from acute tissue injury and pain.*

Principal findings *Our understanding of the complex pathways that mediate the transition from acute to chronic pain continues to increase. Work in this area has already revealed the complex interactions between the nervous and immune system that result in both peripheral and central sensitization, essential components to the development of chronic pain. Taken together, a thorough characterization of the cellular mechanisms that generate chronic pain states is essential for the development of new therapies and treatments. Basic research leading to the development of new therapeutic targets is promising with the development of chloride extrusion enhancers. It is hoped that one day they will provide relief to patients with chronic pain.*

Conclusions *A better understanding of how chronic pain develops at a mechanistic level can aid clinicians in treating their patients by showing how the underlying biology of chronic pain contributes to the clinical manifestations of pain. A thorough understanding of how chronic pain develops may also help identify new targets for future analgesic drugs.*

Résumé

Objectif *Bien que la douleur soit une expérience sensorielle adaptative nécessaire à la prévention d'atteintes corporelles supplémentaires, la transition d'une douleur aiguë à une douleur chronique n'est pas un phénomène adaptatif et entraîne l'apparition d'une condition clinique chronique. Depuis un certain temps, la façon dont cette transition survient fait l'objet d'études poussées. Cet article se concentre sur les changements au niveau de la plasticité neuronale ainsi que sur le rôle des cellules immunitaires et de la glie dans l'apparition de la douleur chronique à partir d'une lésion tissulaire et d'une douleur aiguës.*

Constatations principales *Notre compréhension des voies complexes qui jouent un rôle dans la transition d'une douleur aiguë vers une douleur chronique continue de s'étendre. Les travaux dans ce domaine ont déjà révélé les interactions complexes entre le système nerveux et le système immunitaire, lesquelles entraînent une sensibilisation périphérique et centrale, des composantes essentielles à l'apparition de douleur chronique. Dans son ensemble, la caractérisation exhaustive des mécanismes cellulaires qui génèrent des états de douleur chronique est essentielle à la mise au point de nouvelles thérapies et de nouveaux traitements. La recherche fondamentale menant à la mise au point de nouvelles cibles thérapeutiques est prometteuse, grâce à la mise au point de molécules*

K. A. Mifflin, BSc · B. J. Kerr, PhD
Centre for Neuroscience, University of Alberta, Edmonton, AB, Canada

B. J. Kerr, PhD (✉)
Department of Anesthesiology and Pain Medicine, University of Alberta, 8-120, Clinical Sciences Building, Edmonton, AB T6G 2G3, Canada
e-mail: bradley.kerr@ualberta.ca

B. J. Kerr, PhD
Department of Pharmacology, University of Alberta, Edmonton, AB, Canada

favorisant la sortie des ions chlorure des cellules nerveuses, lesquelles permettront peut-être un jour de soulager les patients atteints de douleur chronique.

Conclusion *Une meilleure compréhension de la façon dont la douleur chronique se manifeste à un niveau mécaniste peut aider les cliniciens à traiter leurs patients en démontrant comment la biologie sous-jacente à la douleur chronique contribue aux manifestations cliniques de la douleur. Une compréhension plus complète de la façon dont la douleur chronique se développe pourrait également nous permettre d'identifier de nouvelles cibles pour les médicaments analgésiques futurs.*

Pain is a universal sensation necessary for survival. From a purely sensory perspective, "nociception", the neural process of encoding and processing noxious stimuli, has been observed in the simplest to the most complex organism.[1,2] Although acute pain is an important warning signal for bodily harm, pain that persists long after tissue damage may become a clinical problem.

Chronic pain can be defined as any pain lasting more than three to six months.[2] The World Health Organization has declared chronic pain to be one of the most common world health problems, with more than a quarter of the population suffering from chronic pain.[3] In Canada, estimates indicate that approximately 29% of the population suffers from chronic pain, with 80% of those individuals suffering from moderate to severe pain.[4] Despite the large prevalence of this condition, chronic pain is still poorly understood and notoriously difficult to treat. Acute pain is easily modelled in experimental settings as physiological or behavioural responses to noxious stimuli or tissue damage, while chronic pain can arise from different types of tissue damage. Chronic pain may spontaneously arise without tissue damage or it can paradoxically exist in areas where damaged tissue is no longer present (i.e., phantom limb pain).[1,3,5] Chronic pain also violates the boundaries of the thresholds for what normally causes pain, resulting in "*allodynia*" (pain in response to a stimuli that does not normally provoke pain), "*hyperalgesia*" (increased sensitivity to stimulation), and "*sensitization*" (an increased responsiveness of nociceptive neurons to their normal input and/or recruitment of a response to normally subthreshold inputs).[1] Overall, chronic pain is a paradoxical phenomenon with no adaptive purpose when compared with acute pain.

Understanding the difficulty in treating chronic pain compared with treating acute pain has been the focus of an intense research effort. The main focus of research in this area has been on trying to understand the transition from injury and acute pain to the development of chronic pain.

Although this transition is still not fully understood, many advances have been made at the cellular and molecular levels using model systems that have advanced our understanding of the basic mechanisms underlying this process. The plastic nature of the central nervous system has been well established, and both functional and structural changes in pain pathways play a role in the development of chronic pain. The plasticity of the nervous system can be seen in processes such as peripheral and central sensitization, which increase sensitivity and lower the threshold to stimuli that cause pain through various mechanisms.[6-8] In addition to changes in neurons, non-neuronal components of the nervous system, glia, and immune cells also have a role in the development of chronic pain.[9-15] In this review, we consider the current body of research examining the mechanisms that mediate the transition from acute to chronic pain with particular focus on spinal pathways, the plastic changes involved, and the role of glial cells in this process. Although there are many other reviews on the development of chronic pain from acute pain, in this review, we examine the various roles played by neuron, glial, and immune cells and how they work together and interact at the molecular level to cause cellular changes that result in a chronic pain state. In addition, we explore how research in the transition from acute to chronic pain has led to the identification of new therapeutic targets and has revealed new roles for sex differences and bacteria in the development of persistent pain.

Peripheral sensitization: how neuron, immune, and glial cells interact in chronic pain

Neurons in peripheral sensitization

Peripheral sensitization is responsible for the initial sensitization of nociceptors and the development of primary hyperalgesia. It is thought that peripheral sensitization has a role in altered thermal sensitivity.[16,17] There are essentially three main steps in the development of peripheral sensitization. First, there is an initial release of inflammatory mediators due to tissue damage. These mediators then act on G-protein-coupled receptors (GPCRs) or tyrosine kinase receptors (TKRs) at nociceptor terminals. This leads to an activation of different intracellular signalling pathways that culminates in the phosphorylation of different receptors and ion-channels. The net result of this cascade is a change in the threshold and kinetics of the nociceptor.[18-20] For example, inflammation causes the release of prostaglandin E2, bradykinin, and nerve growth factor (NGF).[18-20] Prostaglandin E2 and bradykinin then bind to receptors

expressed on nociceptor nerve terminals, which leads to the activation of cyclic adenosine monophosphate (cAMP) dependent protein kinase A (PKA) and the Ca^{2+}/phospholipid-dependent protein kinase C (PKC).[18,19] Subsequent phosphorylation of the transient receptor potential vanilloid receptor subtype 1 (TRPV1), an endogenous receptor for "heat" by PKA/PKC, results in a lower activation threshold for the channel (< 40°C).[18,19,21,22]

Heat hyperalgesia (e.g., after a sunburn) can also be caused by changes in posttranscriptional regulation. This is seen when tissue injury leads to the release of the inflammatory mediator, NGF, causing an induced activation of p38 mitogen-activated protein kinase (MAPK) in primary sensory neurons.[20] Increased activity of p38 MAPK can lead to an increased expression and transport of the TRPV1 channel which results in increased heat hyperalgesia.[20]

Immune responses in the periphery

While the role of neurons in the development of chronic pain is essential, it is also important to acknowledge the role of glial and immune cells in this process. Immune cells in the peripheral nervous system and dorsal root ganglion are critical for mediating the initial responses to injury and the development of pain. This initial response is followed by activation of glial cells in the central nervous system (see below), specifically microglial and astrocytes.

In the early immune response to injury, neutrophil granulocytes (the most common type of white blood cells that make up the innate immune response) are attracted to the site of injury by the release of chemokine ligand 1 and leukotriene-B4.[23-27] Though the initial neutrophil response is brief, neutrophils may release chemoattractants and cytokines to potentiate the recruitment of macrophages. The recruitment and activation of resident macrophages at the injury site can be mediated by chemokine (C-C motif) ligand 2 (CCL2, also known as MCP-1) and chemokine (C-C motif) ligand 3 (CCL3, also known as MIP-1α).[28,29] Macrophages can release prostaglandins and cytokines, such as interleukin 1β (IL-1β), interleukin 6 (IL-6), tumour necrosis factor (TNF)α, and leukemia inhibitory factor (LIF). Tumour necrosis factor α has autocrine effects that increase cytokine synthesis and release, promoting further macrophage infiltration.[30]

Other growth factors, such as neuregulin bind to v-erb-b2- erythroblastic leukemia viral oncogene homolog 2 and 3 receptors (ERBB2 and ERBB3) on Schwann cells.[31] Early ERBB2 activation results in demyelination, while late activation of ERBB2 causes Schwann cell proliferation. Proliferating Schwann cells release NGF as well as the neurotrophin glial-derived neurotrophic factor, prostaglandins, and cytokines, such as IL-1β and IL-6. All of these factors can sensitize nociceptors and alter gene transcription and expression in sensory neurons.[31]

Activated macrophages along with Schwann cells also release matrix metalloproteins that attack and break down the blood nerve barrier.[12] Vasoactive mediators, such as nitric oxide (NO), are also released, causing hyperemia and swelling, and they have also been implicated in the development of neuropathic pain.[32] In addition, breakdown of the blood nerve barrier and the release of these vasoactive mediators also potentiate the infiltration of macrophages into the dorsal root ganglia.

The ability of macrophages to infiltrate and evoke changes in the dorsal root ganglia is essential to the development of central sensitization, a main component of chronic pain states (see following section). The invasion of macrophages into the dorsal root ganglia is mediated by the release of chemokine (C-X3-C motif) ligand 1 (CX3CL1, also known as fractalkine) and CCL2 from dorsal root ganglion neurons.[28,33,34] Once they have infiltrated into the ganglia, macrophages can enhance excitatory tetrodotoxin-resistant voltage-gated sodium channel currents in sensory neurons via a TNFα-mediated pathway.[12,35] Tumour necrosis factor α binds to the TNFα receptor 1 (TNFR1) and activates the p38 MAPK signalling pathway.[36,37] This is important, as multiple studies have shown that increased activation of p38 contributes to the development of pain hypersensitivity.[13,38-41] Additional activation of the purinergic receptors, P2X2, P2X3, and P2X7, on immune cells can lead to increased release of IL-1β, IL-6, and LIF.[37,42] The release of IL-6 can trigger sprouting of sympathetic nerve fibres into the dorsal root ganglia.[43] The sprouting of sympathetic fibres around dorsal root ganglia neurons is thought to enhance communication between the two types of neurons as well as contribute to the long-term maintenance of neuropathic pain, showing how the nervous and immune systems can interact in the periphery to lead to chronic pain development.[43]

Central sensitization

Although peripheral sensitization is an important mechanism in the development of short-term pain sensitivity, sensitization of peripheral nociceptors is normally short-lived, reversible, and confined to the site of injury (Table). It is the development of central sensitization after injury that leads to the spread of pain, allodynia, and secondary hyperalgesia. Central sensitization can be defined as a "facilitated excitatory synaptic response and depressed inhibition, causing

TABLE Comparison of the components in peripheral and central sensitization that contribute to the development of chronic pain

Peripheral Sensitization	Central Sensitization
-primary hyperalgesia	-secondary hyperalgesia
-transient	-long-term
-confined to site of injury	-spreads to outside site of injury
-altered thermal sensitivity	-allodynia and hyperalgesia
Neuronal Changes:	***Neuronal Changes:***
-changes occur in peripheral nociceptors	-changes occur in dorsal spinal cord neurons
-release of inflammatory mediators leads to signalling pathway activation and phosphorylation of receptors: results in altered nociceptor thresholds and kinetics	-recruitment and phosphorylation of AMPA and NMDA receptors lead to classic central sensitization development
	-Late-onset central sensitization requires transcriptional changes mediated by increases in pERK and activation of the MAPK pathway. This can result in phenotypic switches, such as in Aβ neurons
Immune Involvement:	***Glial Involvement:***
-neutrophil granulocytes are initially attracted to the injury site	-microglial and astrocyte recruitment and activation in the spinal cord via TLRs
-recruitment and activation of macrophages via chemokines	-decrease in KCC2 in dorsal lamina I neurons after BDNF release from activated microglia. Leads to a depolarizing shift and loss of inhibition
-Schwann cell proliferation	
-infiltration of macrophages into the DRG	

AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF = Brain-derived neurotrophic factor; DRG = dorsal root ganglia; KCC2 = Potassium-chloride exchanger 2; MAPK = Mitogen-activated protein kinase; NMDA = N-methyl-D-aspartate; pERK = phosphorylated extracellular signal-regulated kinase; TLRs = Toll-like receptors

amplified responses to noxious and innocuous inputs."[44] This heightened synaptic transmission causes a reduced pain threshold, an amplification of pain responses, and the spread of pain to non-injured areas.[45] These changes typically occur in somatosensory neurons of the spinal cord dorsal horn after exposure to intense peripheral noxious stimuli, tissue injury, or nerve damage. Normally, excitatory input is subthreshold, but the increased sensitivity of spinal neurons caused by central sensitization results in the recruitment of inputs that can now cause the neurons to generate action potentials in response to these normally ineffective inputs.[45,46] This results in pain being produced by low-threshold afferent inputs and hypersensitivity in undamaged areas of tissue around the primary injury site.

There are several main triggers of central sensitization that are neuronal, immune, and glial related. The principal neuronal changes in central sensitization involved the recruitment of N-methyl-D-aspartate receptors (NMDARs) and their activation in the dorsal horn.[47-49] After a repeated train of stimuli that activates peripheral nociceptors, there is a release of glutamate, substance P (SP), calcitonin gene-related peptide (CGRP), and brain-derived neurotrophic factor (BDNF) at central synapses.[50-54] Each of these neuromodulators binds to different receptors and all play a part in the development of central sensitization.

Glutamate, the major excitatory neurotransmitter in the central nervous system, binds both to ionotropic receptors (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors [AMPARs] and NMDARs) and to metabotropic glutamate receptors (mGluRs). When glutamate binds to its respective ionotropic receptors, levels of Ca^{2+}, Na^{+}, and calcium/calmodulin-dependent protein kinase II (CaMKII) increase.[55,56] This leads to increased depolarization and excitability. The binding of glutamate to its metabotropic receptor, mGluR, and the binding of other neuromodulators to other GPCRs, such as the binding of SP to neurokinin-1 receptors (NK1R) and CGRP to the CGRP receptors 1 (CGRP1), ultimately lead to increases in PKA and PKC as well as increases in intracellular Ca^{2+}.[55,56] Protein kinase A and PKC, along with CaMKII, then act to boost synaptic efficiency by phosphorylating different subunits on NMDARs and AMPARs.[47,57] Protein kinase A phosphorylation of AMPAR increases its calcium permeability, and this has been shown to increase synaptic strength.[57] Phosphorylation of different AMPAR subunits by PKC and CaMKII also contributes to increasing nociceptor excitability.[57] Protein kinase A/PKC phosphorylation of NMDAR subunits facilitates hypersensitivity by increasing the response of the receptor to glutamate.[47] Protein kinase C also acts to reduce the Mg^{2+} blockade of NMDA, increasing the channel's open probability, again prolonging its active state.[48] Also, it is important to highlight that the activation of mGluRs and NK1Rs and the subsequent increase in levels of intracellular Ca^{2+} and PKC can also lead to the activation of the sarcoma (Src) family kinases (a family of non-receptor kinases).[58,59] Sarcoma family kinases act to phosphorylate tyrosine restudies on the NR2B subunit of the NMDA receptor to increase NMDA activity and hyperalgesia.[56,58]

Sarcoma family kinases can also act through receptor tyrosine kinases (TrKs) to increase NMDA activity and produce hyperalgesia.[59,60] This occurs when tissue damage detected by primary somatosensory neurons causes a redistribution and accumulation of ephrinB in dorsal horn neurons.[60] This redistribution leads to a postsynaptic cascade, which is initiated by the binding of ephrinB to its receptor EphB to cause activation of the Src family kinase signalling and tyrosine phosphorylation of the NMDAR.[60] Phosphorylation results in increased NMDA activation and the development of hyperalgesia.[59,60] Other

signalling pathways involved in the development of pain also act through receptor tyrosine kinases mediated by modulators such as BDNF. Once released, BDNF binds to the tyrosine receptor kinase B (TrkB) and acts to increase levels of phosphorylated extracellular signal-regulated kinase (pERK).[61,62] Phosphorylated extracellular signal-regulated kinase acts to decrease potassium currents by phosphorylating K_v 4.2 channels to increase membrane excitability.[61,62] In addition, pERK is involved in the sustainment of long-term pain, a process discussed below. Overall, multiple signalling pathways are involved in the development of central sensitization and pain hypersensitivity.

Late-onset central sensitization

The long-term maintenance of chronic pain involves late-onset central sensitization, so named because the changes involved in its development can take hours to occur. These processes require the activation of transcription. One signal that may trigger changes in transcription is the release of BNDF.[38] Brain-derived neurotrophic factor release plays a key role in the long-term maintenance of central sensitization as this pathway can modulate transcriptional processes in the cell. Binding of BNDF to TrkB leads to phosphorylation of ERK through the mitogen kinase activated pathway (MAPK pathway: Ras-Raf-MEK-MAPK/ERK). Phosphorylated extracellular signal-regulated kinase can enter the nucleus of the cell, resulting in transcriptional changes by increasing levels of RSK2 (an AGC group kinase of the ribosomal s6 kinase [RSK] family), which allows for the phosphorylation of cAMP response element-binding protein at the serine 133 residue.[63,64] Transcriptional changes then occur in the dorsal root ganglion of the spinal cord that contribute to pain.[63,65] Although there are hundreds of gene modifications that contribute to pain, their specific roles and pathways are still poorly understood.[66] It has been shown that increased transcription of the genes for c-Fos, cyclooxygenase-2 (known as early gene encoding), neurokinin (NK), TrkB, and prodynorphin (known as late-response gene encoding) contribute to long-term central sensitization and pain.[10,38,67-69]

It has also been shown that a phenotypic switch in dorsal root ganglia neurons in the periphery can occur, possibly due to changes in transcription.[70] It has been observed that large-diameter Aβ neurons may begin to express SP under pathological conditions associated with pain hypersensitivity.[70] Since these Aβ fibres now express high levels of SP, they now function like C-fibres and can increase central excitability.[70] In addition, as Aβ fibres terminate in the lamina III of the dorsal horn, an area where there are NK1R-expressing cells, this phenotypic switch in the afferent cell body may be involved in postsynaptic alterations in excitability.[70] It has been suggested that phenotypic switches of this nature serve as a key mechanism whereby previously non-nociceptive afferents now have the ability to induce central sensitization and tactile pain hypersensitivity.[11,71]

Glial recruitment and central sensitization

Phenotypic switches and the maintenance of chronic pain can also be influenced by the actions of glial cells in the spinal cord dorsal horn. Although injury initially triggers changes in the periphery, it can also trigger major changes to the reactivity and functioning of spinal microglia and astrocytes in the dorsal horn. Microglia and astrocytes are normally activated by pathogens through their recognition of pathogenic protein patterns using different pattern-recognition receptors, such as toll-like receptors (TLRs). Toll-like receptors 2 expressed on astrocytes and microglia and TLR4 expressed on microglia mediate neuropathic pain processing.[14,72] Toll-like receptors 2 and TLR4 activation leads to an immune-like response that results in the release of pro-inflammatory cytokines (such as IL-1β, TNFα, and IL-6) and an increase in phagocytosis.[14,15,72] Release of these cytokines can result in an excitatory positive feedback loop that modulates glial-neuronal communications where TLRs are also directly activated by IL-1β, TNFα, IL-6, and NO.[14,15,72] Tumour necrosis factor α and Il-1β released from astrocytes also act to increase neuronal excitability by increasing synaptic strength as a result of increased AMPAR/NMDAR number and connectivity.[73,74] Interleukin-1β does this by increasing the inward Ca^{2+} conductance of NMDARs.[73] The increased Ca^{2+} conductance induces phosphorylation of NMDARs for a further increase in Ca^{2+} influx.[75] Increases in NMDARs can create a feedback loop where increased production of NO increases neuronal excitability.[76,77]

Toll-like receptors 4 is also specifically involved in pain processing via activation by its endogenous ligand, fibronectin. Fibronectin is produced in response to tissue injury and is also involved in the up-regulation of the purinergic receptor, $P2X_4$, in microglia.[78] Purinergic receptors on microglia also promote neuropathic pain signalling when activated by adenosine triphosphate (ATP) released in the dorsal horn. It has been shown that direct intrathecal injections of ATP or the intrathecal administration of ATP-stimulated microglia in rats can cause mechanical hypersensitivity.[79,80]

Adenosine triphosphate action on spinal microglial $P2X_4$ receptors has also been shown to cause a biphasic BDNF release.[81] The release of BDNF is thought to occur

both through an extracellular Ca^{2+} dependent SNARE-(soluble N-ethylmaleimide-sensitive factor attachment protein receptors) mediated pathway and through activation of the p38-MAPK pathway.[81] Brain-derived neurotrophic factor released from microglia has been shown to play a major role in chronic pain development.[82-84] The release of BDNF from spinal microglia leads to a depolarizing shift in dorsal horn lamina I neurons (Figure A & B).[81,82] This is caused by an inversion of normally inhibitory gamma-aminobutyric acid (GABA) signals to excitatory ones, which facilitates pain.[81,83] This inversion in GABA inhibitory signalling is thought to be due to a decrease in the levels of the potassium-chloride exchanger KCC2, which results in an increase in intracellular chloride and a depolarizing shift.[83] The decrease in KCC2 is mediated by the binding of BDNF to TrkB.[83] Overall, the decrease in KCC2 and reversal of inhibitory signalling results in hypersensitivity.[83]

Discovery of this microglia-mediated pathway has recently led to the development of a new class of therapeutic targets, i.e., chloride extrusion enhancers.[84] High-throughput screening led to the discovery of KCC2 activators that help reduce the high levels of intracellular chloride seen with chronic pain.[84] A KCC2-specific activator (CLP257) has been shown to restore chloride transport, lower intracellular chloride, restore plasma membrane KCC2 levels, and normalize sensitized spinal nociceptive pathways (Figure C).[84] Most importantly, the target compound successfully reversed hypersensitivity in a rat model of neuropathic pain, demonstrating that chloride

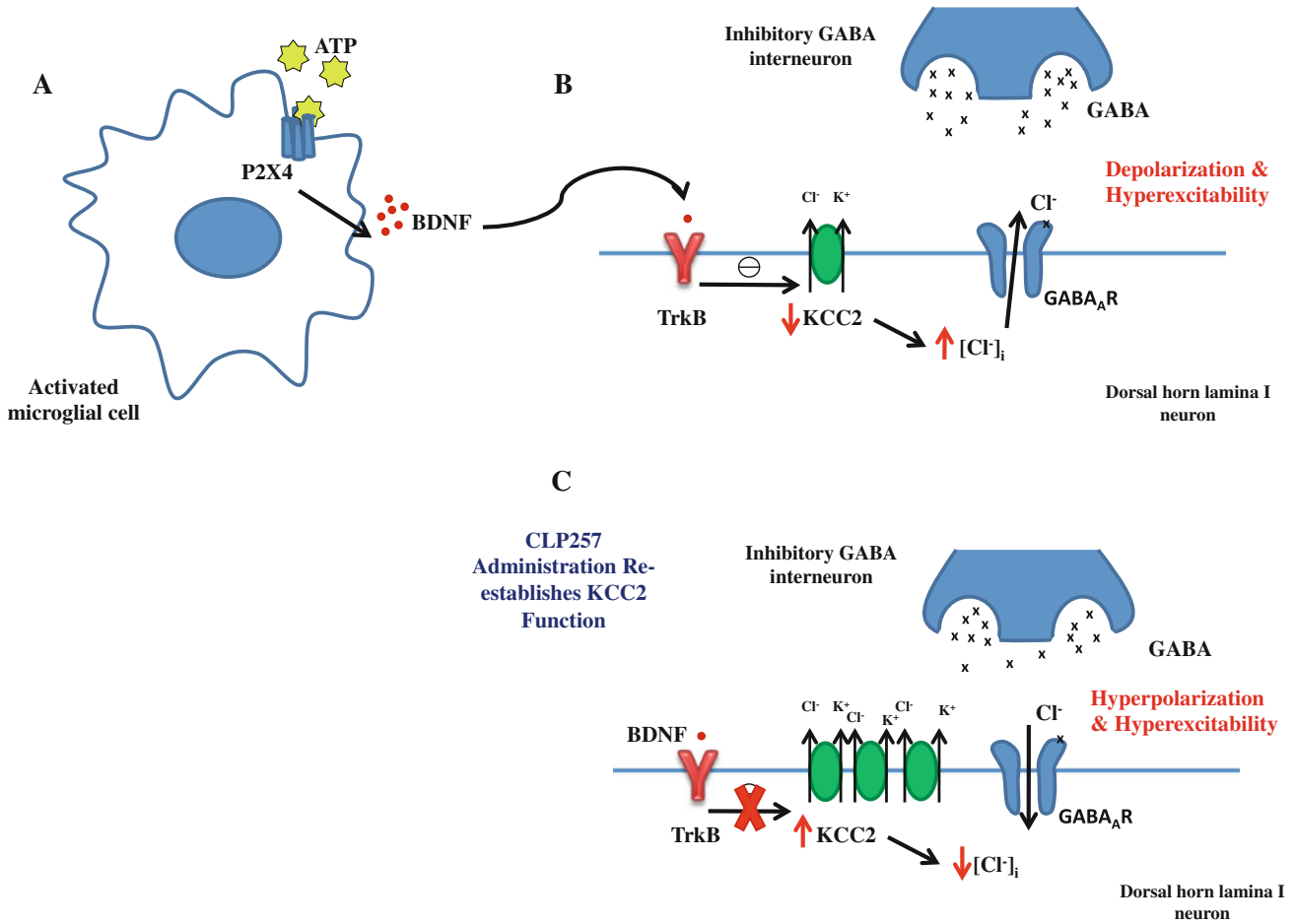

Figure Role of KCC2 in chronic pain. **A)** Binding of ATP to its purinergic receptor, P2X4, on activated microglia in the spinal cord causes the release of BDNF. **B)** The released BDNF binds to its TrkB receptor, leading to a decrease in KCC2. A lack of KCC2 at the plasma membrane causes an increase in **[Cl(-)]i**. This leads to a depolarizing shift whereby the binding of GABA released from inhibitory neurons is now depolarizing. This depolarizing shift causes hyperexcitability, which contributes to chronic pain. **C)** Administration of the new compound, CLP257, a chloride extrusion enhancer, has been shown to help re-establish KCC2 function. CLP257 is thought to do this by increases in plasma membrane levels of KCC2, which would decrease the high levels of **[Cl(-)]i** seen in chronic pain, meaning there would no longer be a depolarizing shift. GABAergic input would now be hyperpolarizing as normal, and there would no longer be any hyperexcitability. KCC2 = potassium-chloride exchanger 2; ATP = adenosine triphosphate; BDNF = brain-derived neurotrophic factor; TrkB = tyrosine kinase B; GABA = gamma-aminobutyric acid

extrusion enhancers may prove to be a successful therapeutic target for chronic pain.[84]

Additional contributors to the development of chronic pain

Bacteria and hypersensitivity

In chronic wounds, there is often a prolonged inflammatory response that can result in persistent pain.[85] Bacterial infections can further contribute to this enhanced pain state in the patient.[86] It had been previously thought that bacterial infections exacerbate the inflammatory response, causing an increase in pro-inflammatory mediators that irritate nerve endings. New research suggests, however, that bacteria can directly activate nociceptors.[87] This work showed that pain is correlated with bacterial load in mice infected with *Staphylococcus aureus*, bacteria responsible for many in-hospital wound infections.[87] The bacteria were also found in close contact with dermal nociceptors and mirrored the time course of hyperalgesia, suggesting a direct bacteria-neuron interaction.[87] This idea of a direct bacteria-nerve interaction was furthered when it was determined that host defences, such as adaptive immunity through T and B cells, were not required for pain.[87] Instead, the study found that bacterial molecules, such as N-formulated peptides and pore-forming toxin, α-hemolysin, act to induce calcium flux and action potentials in nociceptors.[87] This suggests that bacteria can act directly on nociceptors to cause pain. Interestingly, it was shown that increased hypersensitivity caused by bacteria may actually act to decrease immune responses.[87] This is contrary to the idea that hypersensitivity is usually associated with increased immune responses and pro-inflammatory cytokine release. Through the ablation of nociceptors, it was found that there was increased immune activity that may be mediated through different neuropeptides, such as CGRP, galanin, and somatostatin, which are known to have immunosuppressive qualities.[87] Research into the mechanisms by which bacterial infections can induce hypersensitivity provide new insight into an additional factor that can contribute to some forms of chronic pain. In addition, a new nervous-immune system interaction has also been revealed whereby pain acts to decrease the immune response. Further understanding into how different types of bacteria interact with nociceptors could lead to the development of better therapeutics to help those with increased hypersensitivity and pain after bacterial wound infections.

Sex differences in chronic pain

Sex differences between males and females also have an important role in chronic pain. Research into these differences has revealed interesting and clinically relevant differences that have increased our understanding of the underlying mechanisms of chronic pain. Many studies have shown that there is a slightly higher prevalence of chronic pain conditions in females.[88,89] Despite knowing this, the role of sex in the development of chronic pain is still poorly understood. Some research into this area has, however, revealed different roles of sex hormones in inflammatory pain through the use of the formalin test in both male and female mice and rats. Research in this area has shown that female rats and mice seem to be more sensitive to formalin pain compared with their male counterparts and that this occurs in the later more chronic phase of the formalin test.[90-93] Interestingly, it appears as though it is not the day-to-day changes in the estrous cycles of female animals that cause these differences, but rather, it is the underlying differences in testosterone and estrogen/progesterone in males and females.[90,94] It has been suggested that testosterone has a more protective role in this model of tonic inflammatory pain, while female sex hormones may act to increase inhibitory mechanisms in pain.[91,94,95] These findings are interesting as they suggest possible different underlying mechanisms for tonic inflammatory pain in males and females.

Other research in this area has also revealed a potential sex-related mechanism in glial cell activation through TLR4. As previously discussed, TLRs, such as TLR4, are essential in the activation of glial cells needed for the development of central sensitization. Mice with dysfunctional TLR4s show reduced allodynia and hyperalgesia in a nerve injury model.[72,96] Nevertheless, when the TLR4 gene itself is mutated, only male mice exhibit a reduction in pain sensitivity.[96,97] Furthermore, when TLR4 activity is manipulated using an antagonist in females, no change in pain sensitivity is observed.[97] This suggests that there may be different underlying mechanisms in glial activation mediating central sensitization in males and females. Understanding the underlying mechanistic differences between the sexes is important for the development of targeted therapeutics, as it is possible that only one of the sexes may benefit from a new drug if not tested first in both sexes. Further research in this area is needed for a better understanding of how sex differences can contribute to the development of chronic pain states.

Summary

Overall, a number of mechanisms are in place that can mediate the transition from acute to chronic pain. This transition can occur in the peripheral or central nervous

system, and it has become increasingly clear that the underlying mechanisms include more than just neuronal signalling pathways. Although chronic pain is a complex condition, research in this area has come a long way in understanding its underlying mechanisms. Improvements in understanding how neurons and immune cells interact have already begun to lead to the development of new therapeutic strategies like chloride extrusion enhancers.

Key points

- The transition from acute to chronic pain is complicated and involves more than just neurons.
- Changes in neuronal plasticity result in the development of different types of sensitization, such as peripheral and central sensitization, which lead to chronic pain states.
- Infiltration of immune cells into the dorsal horn of the spinal cord contributes to the development of chronic pain and is mediated by different chemoattractants.
- Glial cells play an important role in the development of pain, and their response is mediated by the release of different chemokines and cytokines.
- Other factors, such as sex differences and bacterial infection, can contribute to chronic pain in unique ways.

Competing interests None declared.

APPENDIX: Summary of abbreviations

Abbreviation	**Definition**
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ATP	Adenosine triphosphate
BDNF	Brain-derived neurotrophic factor
CaMKII	Calcium-calmodulin-dependent protein kinase II
cAMP	Cyclic adenosine monophosphate
CCL2/MCP-1	Chemokine (C-C motif) ligand 2
CCL3/MIP-1α	Chemokine (C-C motif) ligand 3
CGRP	Calcitonin gene-related peptide
EphB	EphrinB
ERBB2/3	v-erb-b2-erythnoblastic leukemia viral oncogene homolog 2/3
GDNF	Glial-derived neurotrophic factor
GPCR	G-protein-coupled receptors
IL-1β	Interleukin 1β
IL-6	Interleukin 6
KCC2	Potassium-chloride exchanger 2
LIF	Leukemia inhibitory factor
MAPK	Mitogen-activated protein kinase
mGluR	Metabotropic glutamate receptor
NGF	Nerve growth factor
NK	Neurokinin
NK1R	Neurokinin 1 receptors
NMDAR	N-methyl-D-aspartate receptors
pERK	Phosphorylated extracellular signal-regulated kinase
PGE_2	Prostaglandin E_2
PKA	Protein kinase A
PKC	Protein kinase C
P2X	Purinergic receptor
SP	Substance P
TKR	Tyrosine kinase receptors
TLR	Toll-like receptor
TNFα	Tumour necrosis factor-α
TrkB	Tyrosine kinase B
TRPV1	Transient receptor potential vanilloid receptor subtype 1

References

1. *Loeser, JD, Treede, RD*. The Kyoto protocol of IASP basic pain terminology. Pain 2008; 473-7.
2. *Moriarty O*, *McGuire BE*, *Finn DP*. The effect of pain on cognitive function: A review of clinical and preclinical research. Prog Neurobiol 2011; 93: 385-404.
3. *Institute of Medicine Committee on Advancing pain research, Care, and Education*. Relieving Pain in America: A blueprint for transforming prevention, care, education, and research. Nat Acad Press; 2011.
4. *Moulin DE*, *Clark AJ*, *Speechley M*, *Morley-Forster PK*. Chronic pain in Canada-Prevalence, treatment, impact, and the role of opioid analgesia. Pain Res Manag 2002; 7: 179-84.
5. *Sherman RA*, *Sherman CJ*, *Parker L*. Chronic phantom and stump pain among American veterans: results of a survey. Pain 1984; 18: 83-95.
6. *Woolf CJ*. Evidence for a central component of post-injury pain hypersensitivity. Nature 1983; 306: 686-8.
7. *Finnerup NB*, *Sørensen L*, *Biering-Sørensen F*, *Johannesen IL*, *Jensen TS*. Segmental hypersensitivity and spinothalamic function in spinal cord injury pain. Exp Neurol 2007; 207: 139-49.
8. *Woolf CJ*, *Wall PD*. Relative effectiveness of C primary afferent fibers of different origins in evoking a prolonged facilitation of the flexor reflex in the rat. J Neurosci 1986; 6: 1433-42.
9. *Detloff MR*, *Fisher LC*, *McGaughy V*, *Longbrake EE*, *Popovich PG*, *Basso DM*. Remote activation of microglia and pro-inflammatory cytokines predict the onset and severity of below-level neuropathic pain after spinal cord injury in rats. Exp Neurol 2008; 212: 337-47.
10. *Samad TA*, *Moore KA*, *Sapirstein A*, *et al*. Interleukin-1B-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature 2001; 410: 471-5.

11. *Ma QP*, *Woolf CJ*. Progressive tactile hypersensitivity: an inflammatory-induced incremental increase in the excitability of the spinal cord. Pain 1996; 67: 97-106.
12. *Shubayev VI*, *Angert M*, *Dolkas J*, *Campana WM*, *Palenscar K*, *Myers RR*. TNFα-induced MMP-9 promotes macrophage recruitment into injured peripheral nerve. Mol Cell Neurosci 2006; 31: 407-15.
13. *Ji SX*, *Zhuang ZY*, *Woolf CJ*, *Ji RR*. p38 mitogen-activated protein kinase is activated after a spinal nerve ligation in spinal cord microglia and dorsal root ganglion neurons and contributes to the generation of neuropathic pain. J Neurosci 2003; 23: 4017-22.
14. *Kim D*, *Kim MA*, *Cho IH*, *et al*. A critical role of toll-like receptor 2 in nerve injury-induced spinal cord glial cell activation and pain hypersensitivity. J Biol Chem 2007; 282: 14975-83.
15. *Allan SM*, *Tyrrell PJ*, *Rothwell NJ*. Interleukin-1 and neuronal injury. Nat Rev Immunol 2005; 5: 629-40.
16. *Guenther S*, *Reeh PW*, *Kress M*. Rises in $[Ca^{2+}]_i$ mediate capsaicin-and proton-induced heat sensitization of rat primary nociceptive neurons. Eur J Neurosci 1999; 11: 3143-50.
17. *Walder R*, *Radhakrishnan R*, *Loo L*, *et al*. TRPV1 is important for mechanical and heat sensitivity in uninjured animals and development of heat hypersensitivity after muscle inflammation. Pain 2012; 153: 1664-72.
18. *Chuang HH*, *Prescott ED*, *Kong H*, *et al*. Bradykinin and nerve growth factor release the caspsaicin receptor from PtdIns(4, 5)P2-mediated inhibition. Nature 2001; 411: 957-62.
19. *Gold MS*, *Levine JD*, *Correa AM*. Modulation of TTX-R INa by PKC and PKA and their role in PGE2-induced sensitization of rat sensory neurons in vitro. J Neurosci 1998; 18: 10345-55.
20. *Ji RR*, *Samad TA*, *Jin SX*, *Schmoll R*, *Woolf CJ*. P38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. Neuron 2002; 36: 57-68.
21. *Fischer MJ*, *Btesh J*, *McNaughton PA*. Disrupting sensitization of transient receptor potential vanilloid subtype 1 inhibits inflammatory hyperalgesia. J Neurosci 2013; 33: 7407-14.
22. *Banik RK*, *Brennan TJ*. Trpv1 mediates spontaneous firing and heat sensitization of cutaneous primary afferents after plantar incision. Pain 2009; 141: 41-51.
23. *Carreira EU*, *Carregaro V*, *Teixeira MM*, *et al*. Neutrophils recruited by CXCR1/2 signalling mediate post-incisional pain. Eur J Pain 2013; 17: 654-63.
24. *Cunha TM*, *Barsante MM*, *Guerrero AT*, *et al*. Treatment with DF 2162, a non-competitive allosteric inhibitor of CXCR1/2, diminishes neutrophil influx and inflammatory hypernociception in mice. Br J Pharmacol 2008; 154: 460-70.
25. *Cunha TM*, *Verri WA Jr*, *Schivo IR*, *et al*. Crucial role of neutrophils in the development of mechanical inflammatory hypernociception. J Leukoc Biol 2008; 83: 824-32.
26. *Ramon CD*, *Fernandes KS*, *Canetti C*, *Teixeira MM*, *Silva JS*, *Cunha FQ*. Neutrophil recruitment in immunized mice depends on MIP-2 inducing the sequential release of MIP-1alpha, TNFalpha and FTB(4). Eur J Immunol 2006; 36: 2025-34.
27. *Guerrero AT*, *Verri WA Jr*, *Cunha TM*, *et al*. Involvement of LTB4 in zymosan-induced joint nociception in mice: participation of neutrophils and PGE2. J Leukoc Biol 2008; 83: 122-30.
28. *Zhang J*, *De Koninck Y*. Spatial and temporal relationship between monocyte chemoattractant protein-1 expression and spinal glial activation following peripheral nerve injury. J Neurochem 2006; 97: 772-83.
29. *Perrin FE*, *Lacroix S*, *Avilés-Triqueros M*, *David S*. Involvement of monocyte chemoattractant protein-1, macrophage inflammatory protein-1alpha and interleukin-1beta in Wallerian degeneration. Brain 2005; 128: 854-66.
30. *Aggarwal BB*. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol 2003; 3: 745-56.
31. *Carroll SL*, *Miller ML*, *Frohnert PW*, *Kim SS*, *Corbett JA*. Expression of neuregulins and their putative receptors ErbB2 and ErbB3, is induced during Wallerian degeneration. J Neurosci 1997; 17: 1642-59.
32. *Zochodne DW*, *Levy D*, *Zwiers H*, *et al*. Evidence for nitric oxide and nitric oxide synthase activity in proximal stumps of transected peripheral nerves. Neurosci 1999; 91: 1515-27.
33. *Verge GM*, *Milligan ED*, *Maier SF*, *Watkins LR*, *Naeve GS*, *Foster AC*. Fractalkine (CX3CL1) and fractalkine receptors (CX3CR1) distribution in spinal cord and dorsal root ganglia under basal and neuropathic pain conditions. Eur J Neurosci 2004; 20: 1150-60.
34. *White FA*, *Sun J*, *Waters SM*, *et al*. Excitatory monocyte chemoattractant protein-1 signalling is unregulated in sensory neurons after chronic compression of the dorsal root ganglion. Proc Natl Acad Sci USA 2005; 102: 14092-7.
35. *Jin X*, *Gereau RW 4th*. Acute p38-mediated modulation of tetrodotoxin-resistant sodium channels in mouse sensory neurons by tumor necrosis factor-alpha. J Neurosci 2006; 26: 246-55.
36. *Zhuang ZY*, *Kawasaki Y*, *Tan PH*, *Wen YR*, *Huang J*, *Ji RR*. Role of the CXCR1/p38 MAPK pathway in spinal microglia for the development of neuropathic pain following nerve injury-induced cleavage of fractalkine. Brain Behav Immun 2007; 21: 642-51.
37. *Burnstock G*. Physiology and pathophysiology of purinergic neurotransmission. Physiol Rev 2007; 87: 659-797.
38. *Ji R*, *Befort K*, *Brenner GJ*, *Woolf CJ*. ERK MAP kinase activation in superficial spinal cord neurons induces prodynorphin and NK-1 upregulation and contributes to persistent inflammatory pain hypersensitivity. J Neursci 2002; 22: 478-85.
39. *Kim SS*, *Bae JC*, *Kim JY*, *et al*. Activation of p38 MAP kinase in the rat dorsal root ganglia and spinal cord following peripheral inflammation and nerve injury. Neuroreport 2002; 13: 2483-6.
40. *Piao ZG*, *Cho IH*, *Park CK*, *et al*. Activation of glia and microglia p38 MAPK in medullary dorsal horn contributes to tactile hypersensitivity following trigeminal sensory nerve injury. Pain 2006; 121: 219-31.
41. *Svensson CI*, *Marsala M*, *Westerlund A*, *et al*. Activation of p38 mitogen-activated protein kinase in spinal microglia is a critical link in inflammation-induced spinal pain processing. J Neurochem 2003; 86: 1534-44.
42. *Khakh BS*. Molecular physiology of P2X receptors and ATP signalling at synapses. Nat Rev Neurosci 2001; 2: 165-74.
43. *Pertovaara A*. The noradrenergic pain regulation system: A potential target for pain therapy. Eur J Pharmacol 2013; 716: 2-7.
44. *Woolf CJ*, *Salter MW*. Neuronal Plasticity: Increasing the Gain in Pain. Science 2000; 288: 1765-9.
45. *Woolf CJ*, *King AE*. Dynmaic alterations in the cutaneous mechanoreceptive fields of dorsal horn neurons in the rat spinal cord. J Neurosci 1990; 10: 2717-26.
46. *Simone DA*, *Sorkin LS*, *Oh U*, *et al*. Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. J Neurophysiol 1991; 66: 228-46.
47. *Guo W*, *Wei F*, *Zou S*, *et al*. Group I metabotropic glutamate receptor NMDA receptors coupling and signalling cascade mediate spinal dorsal horn NMDA receptors 2B tyrosine phosphorylation associated with inflammatory hyperalgesia. J Neurosci 2004; 24: 9161-73.
48. *Chen L*, *Huang LY*. Protein kinase C reduces Mg2+ block of NMDA-receptor channels as a mechanism of modulation. Nature 1992; 356: 521-3.
49. *Qui S, Chen T, Koga K et al*. An increase in synaptic NMDA receptors in the insular cortex contributes to neuropathic pain. Sci Signal 2013; 6: ra34.
50. *Afrah AW*, *Fiskå A*, *Gjerstad J*, *Gustafsson H*, *et al*. Spinal substance P release in vivo during the induction of long-term potentiation in dorsal horn neurons. Pain 2002; 96: 49-55.

51. *Khasabov SG*, *Rogers SD*, *Ghilardi JR*, *Peters CM*, *Mantyh PW*, *Simone DA*. Spinal neurons that possess the substance P receptor are required for the development of central sensitization. J Neurosci 2002; 22: 9086-98.
52. *Sun RQ*, *Lawand NB*, *Willis WD*. The role of calcitonin gene-related peptide (CGRP) in the generation and maintenance of mechanical allodynia and hyperalgesia in rats after intradermal injection of caspsaicin. Pain 2008; 104: 201-8.
53. *Balkowiec A*, *Katz DM*. Activity-dependent release of endogenous brain-derived neurotrophic factor from primary sensory neurons detected from ELISA in situ. J Neurosci 2000; 20: 7417-23.
54. *Pitcher MH*, *Ribeiro-da-Silva A*, *Coderre TJ*. Effects of inflammation on the ultrastructural localization of spinal dorsal horn group 1 metabotropic glutamate receptors. J Comp Neurol 2007; 505: 412-23.
55. *Fang L*, *Wu J*, *Lin Q*, *Willis WD*. Calcium-calmodulin-dependent protein kinase II contributes to spinal cord central sensitization. J Neurosci 2002; 22: 4196-204.
56. *Willis WD*. Role of neurotransmitters in sensitization of pain responses. Ann NY Acad Sci 2001; 933: 142-56.
57. *Carvalho AL*, *Durate CB*, *Carvalho AP*. Regulation of AMPA receptors by phosphorylation. Neurochem Res 2000; 25: 1245-55.
58. *Guo W*, *Zou S*, *Guan Y*, *et al*. Tyrosine phosphorylation of the NR2B subunit of the NMDA receptor in the spinal cord during the development and maintenance of inflammatory hyperalgesia. J Neurosci 2002; 22: 6208-17.
59. *Salter MW*, *Kalia LV*. Src kinases: A hub for NMDA receptor regulation. Nature Rev Neurosci 2004; 5: 317-28.
60. *Battaglia AA*, *Sehayek K*, *Grist J*, *McMahon S*, *Gavazzi I*. EphB receptors and ephrin-B ligands regulate spinal sensory connectivity and modulate pain processing. Nat Neurosci 2003; 6: 339-40.
61. *Hu HJ*, *Alter BJ*, *Carrasquillo Y*, *Qiu CS*, *Gereau RW 4th*. Metabotropic glutamate receptor 5 modulates nociceptive plasticity via extracellular signal-regulated kinase Kv4.2 signalling in spinal cord dorsal horn neurons. J Neurosci 2007; 27: 13181-91.
62. *Hu HJ*, *Carrasquillo Y*, *Karim F*, *et al*. The kv4.2 potassium channel subunit is required for pain plasticity. Neuron 2006; 50: 89-100.
63. *Xing J*, *Kornhauser JM*, *Xia Z*, *Thiele EA*, *Greenberg ME*. Nerve growth factor activates extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways to stimulate CREB serine 133 phosphorylation. Mol Cell Biol 1998; 18: 1946-55.
64. *Costandil L*, *Aguilera R*, *Goich M*, *et al*. Involvement of spinal cord BDNF in the generation and maintenance of chronic neuropathic pain in rats. Brain Res Bull 2011; 86: 454-9.
65. *Obata K*, *Noguchi K*. MAPK activation in nociceptive neurons and pain hypersensitivity. Life Sci 2004; 74: 2643-53.
66. *Costigan M*, *Befort K*, *Karchewski L*, *et al*. Replicate high-density rat genome oligonucleotide microarrays reveal hundreds of regulated genes in the dorsal root ganglion after peripheral nerve injury. BMC Neurosci 2002; 3: 16.
67. *Xing J*, *Ginty DD*, *Greenberg ME*. Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science 1996; 16: 959-63.
68. *Hunt SP*, *Pini A*, *Evan G*. Induction of c-fos-like protein in spinal cord neurons following sensory stimulation. Nature 1987; 328: 632-4.
69. *Mannion RJ*, *Costigan M*, *Decosterd I*, *et al*. Neurotrophins: peripherally and centrally acting modulators of tactile stimulus-induced inflammatory pain hypersensitivity. Proc Natl Acad Sci USA 1999; 96: 9385-90.
70. *Neumann S*, *Doubell TP*, *Leslie T*, *Woolf CJ*. Inflammatory pain hypersensitivity mediated by phenotypic switch in myelinated primary sensory neurons. Nature 1996; 384: 360-4.
71. *Noguchi K*, *Kawai Y*, *Fukuoka T*, *Senba E*, *Miki K*. Substance P induced by peripheral nerve injury in primary afferent sensory neurons and its effect on dorsal column nucleus neurons. J Neurosci 1995; 15: 7633-43.
72. *Tanga FY*, *Nutile-McMenemy N*, *DeLeo JA*. The CNS role of Toll-like receptors 4 in innate neuroimmunity and painful neuropathy. Proc Natl Acad Sci USA 2005; 102: 5856-61.
73. *Viviani B*, *Bartesaghi S*, *Gardoni F*, *et al*. Interleukin-1β enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. J Neurosci 2003; 24: 8692-700.
74. *Stellwagen D*, *Malenka RC*. Synaptic scaling mediated by glial TNF-α. Nature 2006; 440: 1054-9.
75. *Zhang RX*, *Li A*, *Liu B*, *et al*. Il-1ra alleviates inflammatory hyperalgesia through preventing phosphorylation of NMDA receptor NR-1 subunit in rats. Pain 2008; 135: 232-9.
76. *Kitto KF*, *Haley JE*, *Wilcox GL*. Involvement of nitric oxide in spinally mediated hyperalgesia in the mouse. Neurosci Lett 1992; 148: 1-5.
77. *Meller ST*, *Pechman PS*, *Gebhart GF*, *Maves TJ*. Nitric oxide mediates the thermal hyperalgesia produced in a model of neuropathic pain in the rat. Neurosci 1992; 50: 7-10.
78. *Nasu-Tada K*, *Koizumi S*, *Tsuda M*, *Kunifusa E*, *Inoue K*. Possible involvement of increase in spinal fibronectin following peripheral nerve injury in upregulation of microglia P2X4, a key molecule for mechanical allodynia. Glia 2006; 53: 769-75.
79. *Nakagawa T*, *Wakamatsu K*, *Zhang N*, *et al*. Intrathecal administration of ATP produces long-lasting allodynia in rats: differential mechanisms in the phase of the induction and maintenance. Neurosci 2007; 147: 445.
80. *Nakagawa T*, *Wakamatsu K*, *Maeda S*, *Shirakawa H*, *Kaneko S*. Differential contribution of spinal mitogen-activated protein kinases to the phase of long-lasting allodynia evoked by intrathecal administration of ATP in rats. Biol Pharm Bull 2008; 6: 1164-8.
81. *Trang T*, *Beggs S*, *Wan X*, *Salter MW*. P2X4-receptor-mediated synthesis and release of brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated protein kinase activation. J Neurosci 2009; 29: 3518-28.
82. *Coull JA, Beggs S, Boudreau D et al.* BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 2005; 438: 10-17-1021.
83. *Coull JA*, *Boudreau D*, *Bachand K*, *et al*. Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. Nature 2003; 424: 938-42.
84. *Gagnon M, Bergeron MJ, Lavertu G et al.,* Chloride extrusion enhancers as novel therapeutics for neurological diseases [published online ahead of print October 6 2013]. Nat Med. Doi:10.1038/nm.3356.
85. *White RJ*. Wound infection-associated pain. J Wound Care 2009; 18: 245-9.
86. *Gardner SE*, *Frantz RA*, *Doebbeling BN*. The validity of the clinical signs and symptoms used to identify localized chronic wound infection. Wound Repair Regen 2001; 3: 178-86.
87. *Chiu IM*, *Heesters BA*. *Ghasemlou et al*. Bacteria activate sensory neurons that modulate pain and inflammation. Nature 2013; 501: 52-7.
88. *Ruau D*, *Liu LY*, *Clark JD*, *Angst MS*, *Butte AJ*. Sex differences in reported pain across 11,000 patients captured in electronic medical records. J Pain 2012; 13: 228-34.
89. *Mogil JS*. Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. Nat Rev Neurosci 2012; 13: 859-66.

90. *Kim SJ*, *Calejesan AA*, *Li P*, *Wei F*, *Zhuo M*. Sex differences in late behavioural response to subcutaneous formalin injection in mice. Brain Res 1999; 829: 185-9.
91. *Gaumon I*, *Aresnault P*, *Marchand S*. The role of sex hormones on formalin-induced nociceptive responses. Brain Res 2002; 958: 139-45.
92. *Perissin L*, *Facchin P*, *Porro CA*. Tonic pain response in mice: effects of sex, season and time of day. Life Sci 2003; 72: 897-907.
93. *Aloisi AM*, *Ceccarelli I*, *Fiorenzani P*, *De Padova AM*, *Massafra C*. Testosterone affects formalin-induced responses differently in male and female rats. Neurosci Lett 2004; 361: 262-4.
94. *Vincler M*, *Maixner W*, *Vierck CJ*, *Light AR*. Estrous cycle modulation of nociceptive behaviours elicited by electrical stimulation and formalin. Pharmacol Biochem Behav 2001; 69: 315-24.
95. *Gaumon I*, *Arsenault P*, *Marchand S*. Specificity of female and male sex hormones on excitatory and inhibitory phase of formalin-induced nociceptive responses. Brain Res 2005; 1052: 105-11.
96. *Bettoni I*, *Comelli F*, *Rossini C*, *et al*. Glial TLR4 receptor as new target to treat neuropathic pain: efficacy of a new receptor antagonist in a model of peripheral nerve injury in mice. Glia 2008; 56: 1312-9.
97. *Sorge RE*, *LaCroix-Fralish MI*, *Tuttle AH*, *et al*. Spinal cord Toll-like receptor 4 mediates inflammatory and neuropathic hypersensitivity in male but not female mice. J Neurosci 2011; 31: 15450-4.