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## Neural circuits for pain: recent advances and current views

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## **Abstract**

The mammalian nervous system encodes many different forms of pain including those arising from short-term low-grade interactions with noxious thermal, chemical or mechanical sources to more serious forms of pain induced by trauma and disease. In this review, we highlight recent advances in understanding neural circuits that encode these forms of pain. Promising therapeutic strategies based on recent advances are also highlighted.

## Introduction

The ability to sense pain serves to protect us from harm and is thus an essential aspect of our well-being. Highlighting the importance of sensing pain, patients suffering from channelopathies that eliminate the ability to feel pain display a remarkably high early mortality largely due to self-mutilation and repetitive fractures (1). Pain is also of course a source of significant discomfort and humans have long sought ways to ameliorate pain as exemplified by the Ebers papyrus from ancient Egypt. As a concept, pain has evolved from archaic notions of demonic punishment to more contemporary views of biological circuit based origins for the sensation, an idea first proposed by the French philosopher Rene Descartes in the 17<sup>th</sup> century. The most recent definition of pain proposed by the International Association for the Study of Pain, unchanged since its first publication in 1979, defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”. The full biological complexity of pain and its underlying circuitry is not wholly conveyed by this definition. Pain stems from a rich and varied array of peripheral sensors that detect nociceptive stimuli produced by internal tissues and the external world. The information is subsequently distributed to a series of complex neural circuits in the spinal cord dorsal horn and then on to numerous brain regions, to produce a diverse set of emotions, actions and sensations (Figure 1). Pain also manifests with different qualities including stabbing or pricking, burning or aching, further highlighting the heterogeneity of the underlying neural circuitry. Lastly, longer lasting pain states produced by nerve and tissue damage, which are also heterogeneous in nature, provide ongoing awareness of the injured area. However, the pain can become chronic and debilitating if the tissue damage persists and in some conditions even after the wound has healed. Because of the high prevalence and lack of adequate treatment options,

unraveling the biological basis of persistent pain continues to be an area of intense study. The objective of this review is to provide an overview of pain circuitry with a special emphasis on recent significant advances in our understanding of pathological pain. We also discuss recent insights in the neural circuits for pain that have important therapeutic potential.

### **How is pain detected in the periphery?**

Pain is produced through the activation of nociceptive primary sensory neurons categorized into C, A $\delta$ , and to a lesser extent A $\beta$  classes, depending on their axon caliber, degree of myelination and conductivity properties as was first described by Nobel Laureate Herbert Gasser in the early 20<sup>th</sup> century. Nociceptors innervate the skin, deep tissues and internal organs and are tuned to detect a wide variety of noxious mechanical, thermal and chemical stimuli through the activation of modality-specific sensory transduction molecules. A number of these key molecules have been identified (for a comprehensive review, see (2)). Most if not all of the modalities are conveyed through the activation of more than one transducer, as was discovered in studies of heat transduction. Genetic deletion of the heat sensing transient receptor potential channel vanilloid 1 (TRPV1) severely impairs, but does not completely abolish thermal heat sensitivity in mice (3), revealing the existence of one or more additional heat sensors. Recent efforts have now identified TRPM3 (4) and the calcium-activated chloride channel ANO1 (5) as important contributors to the sensation of heat. Interestingly, as was observed with TRPV1, deletion of either channel alone strongly reduces, but does not completely eliminate noxious heat sensitivity. In contrast, ablation of TRPV1<sup>+</sup> fibers by intrathecal injection of the TRPV1 ligand capsaicin, the hot ingredient in chili peppers (6) or silencing the cells by selective uptake of the voltage-

gated sodium channel blocker QX-314, which inhibits nerve conduction (7) completely abolished noxious heat sensitivity, demonstrating that the fibers are sufficient to account for heat pain despite a more limited contribution from each channel. As TRPV1, TRPM3 and ANO1 show considerable overlap in their distribution, analyses of double and triple genetic deletion of the channels in mice may be required to fully understand at a cellular level how noxious heat is transduced. Cold is also transduced through multiple channels. Mice with a genetic deletion of the menthol-sensitive TRPM8, a critical transducer of innocuous cooling, show only partial avoidance of noxious cold temperatures, whereas selective ablation of TRPM8 expressing neurons in mice using diphtheria toxin completely abolish noxious cold sensitivity (8). Lastly, molecules that transduce mechanical nociception remain stubbornly elusive, as deletion of the leading candidate molecules in mice surprisingly produced little to no change in noxious mechanical pain sensitivity (discussed in more depth by (9, 10)).

### **Nociception in the periphery: more than just neurons**

Epithelial cells such as keratinocytes and Merkel cells directly interact with peripheral axon terminals and have been implicated in the modulation of sensory transduction. Recently, several elegant studies have demonstrated an active role for epithelial cells in tuning the response of sensory neurons. Merkel cells are the epidermal end organ of slowly adapting type-1 (SA1) mechanoreceptors and are involved in the detection of features such as edges and textures. Depolarization of the Merkel cells either by touch, which was shown to depend on the recently identified innocuous mechanotransduction channel, Peizo2 or by light-mediated activation of the exogenously expressed light-gated ion channel, channelrhodopsin, in Merkel cells directly excited the SA1 fibers through still unidentified neurotransmitter signaling mechanisms (11). Epithelial cells

were recently shown to also actively contribute to nociceptive transmission. Activation of channelrhodopsin or TRPV1 channels ectopically expressed by keratinocytes in mice induced action potential firing in sensory neurons, neuronal activity in the spinal cord and nocifensive behaviors (12, 13). Conversely, inhibition of the cells through activation of the light-sensitive chloride pump halorhodopsin (eNpHR3.0) inhibited the response of nociceptors and other primary sensory neurons in response to cutaneous stimulation (13). Studies that identify the salient signaling molecules or establish the extent to which epithelial cells contribute behaviorally to pain will be of interest.

### **Is pain a labeled line?**

Most nociceptors express various combinations of sensory transducers specific for different modalities (often heat and mechanical) and therefore are classified as “polymodal”. Recent large-scale efforts using single-cell RNA transcriptome analyses seek to better understand the molecular and functional logic of primary sensory neurons by parsing the transcriptome profiles of a hundreds of individual cells into groups defined by convergent gene expression patterns (14-16). These cluster analyses offer a richer picture of the diversity of nociceptors than previous molecular marker based classifications as well as access to a deeper understanding of the molecular constituents that give rise to the functional properties of sensory neurons (Figure 2). Importantly, while the precise relationships between the molecular constituents and the response properties of the neurons will require further study, the transcriptome data do show some predictive capacity: for example, the tyrosine hydroxylase (TH<sup>+</sup>) cluster expresses high levels of Piezo2, but not the heat sensors TRPV1, TRPM3 or ANO1, consistent with response of the cells to light touch, but not heat (17-19).

However, limits on the ability to predict the functional output of nociceptors based on their molecular and biophysical properties were also recently demonstrated in studies in which select polymodal populations, responsive to both heat and mechanical stimuli were ablated in mice. Diphtheria toxin-mediated ablation of nociceptors that express the Mas-related G-protein coupled receptor subtype D (Mrgprd) markedly reduced acute and persistent mechanical pain, but had no effect on thermal sensitivity (6). Similarly, ablation of the CGRP neurons, a population that partially overlaps with the heat sensors TRPV1, TRPM3 and to a lesser extent Ano1, predictably produced a profound loss of heat, but did not alter innocuous or noxious mechanical sensation (20). Additionally, loss of CGRP expressing sensory neurons, which are not responsive to cooling agents (21), profoundly increased tonic and evoked activity in spinal neurons associated with cold. This latter finding, mechanistically resembling the activation of dorsal horn itch circuits after silencing pain afferents (pain normally inhibits itch at the level of the spinal cord), further highlights the complexity of primary afferent coding as well as an important role for central processing in shaping the sensory percept.

### **Pain circuits of pathological pain**

Nerve and tissue damage produces dramatic changes in somatosensory circuits. While acute pain typically follows the activation of nociceptors, light touch activated neurons can be recruited into the nociceptive network to cause pain after injury. This pathological condition, termed mechanical allodynia, occurs in numerous peripheral neuropathies and central pain disorders, presenting in up to 50% of patients with neuropathic pain (for a comprehensive review see (22)). Still unknown is the identity of the light touch sensitive primary sensory population(s) that conveys mechanical



allodynia. A large number of primary afferents respond to light mechanical touch and are thus potential candidates (for a comprehensive review of light touch fibers see (23)).

While the identity of the mechanosensory neurons engaged during mechanical allodynia remains unclear, significant progress has been made in our understanding of innocuous sensory mechanotransduction (for a comprehensive review see (24)). As mentioned previously, innocuous tactile sensitivity was recently shown to be primarily dependent on the cation channel Piezo2 (25). In primary sensory neurons, the channel is almost exclusively expressed by low threshold mechanoreceptors and conditional deletion of Piezo2 in all sensory neurons in mice shows markedly reduced mechanical activation of low threshold mechanoreceptors as well as behavioral touch sensation, with no change in acute mechanical pain (26). Since low threshold mechanoreceptors convey mechanical allodynia, it was expected that Piezo2 would have an essential role in this type of pain. Surprisingly, mechanical allodynia induced by inflammation was unaffected in mice with a conditional deletion of Piezo2 in primary afferents. However, studies showing the cyclic AMP sensor Epac1 sensitizes Piezo2 after neuropathic injury indicate a potential role for the mechanotransducer in the allodynia induced by this type of injury (27). An effect of Piezo2 on mechanical allodynia induced by neuropathic, but not inflammatory injury would be consistent with a number of previous observations in which different types of injury engage distinct persistent pain circuits and mechanisms.

### **Integration in the spinal cord**

Primary sensory neurons innervate the six Rexed laminae of the spinal cord dorsal horn, the major locus for the integration of sensory input and descending supraspinal modulation. The central terminals of sensory neurons are somatotopically organized, forming ventro-dorsal oriented columns similar to the arrangement of the primary

somatosensory cortex. Most C- and A $\delta$ - nociceptive afferents form synaptic contacts in the superficial laminae (I-II) while low threshold A $\delta$  and A $\beta$  afferents generally project to deeper laminae (III-VI) (Figure 1). Accordingly, *in vivo* electrophysiological recordings in mammals have shown that the most superficial layers I and II respond mainly to noxious peripheral stimulation, while neurons in deep layers are more sensitive to touch. The dorsal horn is composed of a large number of excitatory (75%) and inhibitory (25%) interneuron populations as well as a smaller number of projection populations located in laminae I, III and IV-V. The projection neurons relay information to numerous supraspinal sites to give rise to both qualitative and affective aspects of the pain sensation. Recent studies of dorsal horn nociceptive circuits have focused largely on persistent pain and in particular mechanical allodynia because of its high prevalence and the need for better treatment options. (For comprehensive reviews of acute pain processing we refer the reader to (28, 29)).

### **Mechanical allodynia: the silent circuit in the spinal cord**

One of the principal theories to explain the basis for dorsal horn circuitry changes that underlie mechanical allodynia, originated with the gate control theory in which touch inhibits pain through a feed-forward inhibitory circuit within the superficial layers of the dorsal horn (30). In the case of mechanical allodynia, injury impairs the feed-forward inhibitory circuit, thus allowing light touch to access the nociceptive network. A seminal study providing support for this idea showed in spinal cord slices that low threshold A-fiber input is able to activate lamina I pain projection neurons through a polysynaptic network when inhibitory receptor antagonists are present (mimicking the injury-induced decrease in inhibition) (31). Importantly, the study established that the mechanical allodynia circuit, normally silent, is already in place under physiological conditions.

### **Circuits for mechanical allodynia: the excitatory neurons**

The circuit for mechanical allodynia was originally modeled as a dorsally-directed pathway that begins with excitatory interneurons at the laminae II-III border that express protein kinase C gamma (PKC $\gamma$ ) and ends with lamina I pain projection neurons (32) (Figure 3). Two intermediary populations in this pathway were recently identified through paired recordings, the transient central cells in inner lamina II and vertical cells in outer lamina II (33). Remarkably the dendrites of vertical cells extend deep into lamina III and receive input from most classes of primary afferents suggesting a potential role as integrators of the network (34). An expansion of the circuit into deeper laminae came from studies of the vesicular glutamate transporter 3 (VGLUT3). Seal and colleagues identified a population of excitatory interneurons in lamina III that transiently expresses VGLUT3 and receives almost exclusively low threshold input. Selective activation of the cells using a designer receptor strategy (DREADD), in which a modified receptor is engineered so it is only activated by an otherwise inert ligand, further demonstrated their important role in the transmission of touch as painful (35). Similarly, calretinin-expressing excitatory interneurons in inner lamina II were also identified as an essential element of the circuit. Importantly, functional analysis of the calretinin and PKC $\gamma$  populations using c-Fos analysis, an indicator of neural activity, revealed the existence of distinct excitatory microcircuits for mechanical allodynia, which are differentially engaged depending on whether the injury is inflammatory or neuropathic in nature(35).

### **Circuits for mechanical allodynia: the “gates”**

Inhibitory neurons are a fundamental element of the mechanical allodynia network in the spinal cord, forming “gates” that prevent the recruitment of low threshold fibers into the

nociceptive network under physiological conditions (Figure 3). In a heroic study using paired recordings in spinal cord slices, Lu and colleagues provide evidence that the PKC $\gamma$  interneurons, which receive A $\beta$ -fiber input, are normally under feed-forward glycinergic disynaptic inhibition (33). After neuropathic injury, which is known to impair inhibitory transmission in the dorsal horn, the authors show that A $\beta$ -fibers are able to drive action potentials in PKC $\gamma$  neurons and presumably the rest of the nociceptive network. Consistent with this finding, selective ablation of lamina III parvalbumin (PV)-expressing interneurons, which form contacts with the PKC $\gamma$  neurons, induces mechanical allodynia, indicating a role for PV interneurons in gating the allodynic pathway (36). Conversely, selective activation of the PV interneurons using the excitatory DREADD strategy reversed mechanical allodynia induced by nerve injury. In addition to PKC $\gamma$  neurons, PV interneurons are also proposed to act directly on the A $\beta$ -afferents innervating these neurons, suggesting alternative mechanisms for inhibitory control of the circuit (37). Qiufu Ma and colleagues demonstrated the involvement of a distinct population of inhibitory interneurons in the feed-forward gating of mechanical allodynia. Diphtheria toxin-induced ablation of dynorphin-expressing interneurons in the lamina II outer and at the lamina II-III border induced mechanical allodynia. The dynorphin gate was further demonstrated to control somatostatin-expressing interneurons, a major population of excitatory neurons that partially overlaps with PKC $\gamma$  neurons and potentially also vertical and transient central cells (38). Studies of the mechanical allodynia network are beginning to reveal not only individual elements, but also conceptual advances including the existence of distinct excitatory microcircuits and multiple distinct gates related to specific types of injuries.

## **Mechanisms of disinhibition underlying mechanical allodynia.**

Central sensitization responsible for mechanical allodynia produces various forms of disinhibition in the dorsal horn (for a comprehensive review see (39)). One well-described mechanism involves the injury-induced release of brain-derived neurotrophic factor, BDNF, which triggers down-regulation of the potassium chloride exchanger 2 (KCC2) in tropomyocin receptor kinase B- expressing pain transducing neurons, reducing the driving force for chloride and thus the strength of inhibitory transmission (40, 41). The mechanism of injury-induced BDNF release was recently shown to differ between males and females. Males require the activation of microglia, tissue resident macrophages of the central nervous system, while females instead require adaptive immune cells, possibly T-lymphocytes (42). Sex-differences are frequently observed in animal models of chronic pain and may provide insight into the many clinically observed sex differences in humans (for a more comprehensive review see (43)).

Investigation into the mechanisms underlying BDNF release in the dorsal horn of male rodents after injury have focused on ATP signaling through P2X4 receptors which requires microglia activation and on the involvement of chemokine signaling cascades that promote and maintain microglia activation (see (44)). A recent study in male mice reported that *de novo* production and release of colony-stimulating factor 1 (CSF1) by injured primary sensory neurons is required for the initial activation of the microglia as well as cell proliferation and self-renewal. Deletion of CSF1 specifically from primary sensory neurons or deletion of its downstream effector, the microglial membrane adaptor protein DAP12, was sufficient to markedly reduce microglial activation as well as the behavioral expression of mechanical allodynia induced by nerve injury (45).

## **Pain processing in the brain**

Human functional magnetic resonance imaging (fMRI) studies have demonstrated the coordinated activation of several brain areas in response to noxious somatic and visceral stimuli including the thalamus, the anterior cingulate cortex (ACC), the insular cortex (IS), primary and secondary sensory cortices (SI and SII) as well as the prefrontal cortex, basal ganglia (BG), cerebellum and amygdala. This network of brain regions involved in both sensory-discriminative and emotional-affective aspects of pain is termed the “pain matrix” (see (46)).

Several recent studies have questioned whether activation of the “pain matrix” specifically represents pain or is a more generalized system to detect salient events and state of the body. For example, the posterior insular cortex encodes nociceptive intensity and lesions of this brain area disrupt pain while direct stimulation of the area induces pain. However, the insular cortex has also been recognized as having a role in affective, cognitive and homeostatic functions for non-nociceptive sensory modalities. Recently, Mouraux and colleagues performed intracerebral recordings of the anterior and posterior insular cortex and reported that activity levels were similar in response to several modalities (visual, auditory, somatosensory) as well as to noxious and innocuous somatosensory stimuli, consistent with the idea that the insula is not specific for pain related information (47). Additionally, patients with loss-of-function mutations in the sodium channel Nav1.7 (SCN9) show congenital insensitivity to pain due a loss of noxious peripheral sensory drive (1). The response of patients and controls to normally noxious peripheral stimuli was assessed by fMRI. While both groups reported experiencing similar levels of sensation, with controls reporting the stimuli as painful and patients reporting no pain, the two groups nevertheless showed similar patterns and levels of activity within the pain matrix (48). The findings suggest that gross activity

measured in supraspinal structures may not reliably predict the pain experience. Rather pain related information is likely encoded by specific sub-regional patterns of activity throughout the pain matrix, which would require analysis by other methods and experimental designs allowing for causal inference. Recent efforts using machine-learning algorithms to assess by fMRI patterns of activity across the pain matrix are showing high predictive capacity for distinct forms of physical pain as well as social pain such as vicarious pain (49).

Complementing human brain imaging studies, work in rodents has focused on synaptic level mechanisms of pain processing and plasticity in regions of the pain matrix. These studies further emphasize the idea that individual brain areas have multiple functional roles. Anxiety, fear and emotional aspects of pain have been linked to the anterior cingulate cortex. A study by Koga and colleagues in mice identified a form of presynaptic long-term potentiation (pre-LTP) at thalamocortical synapses in the ACC induced by anxiety-evoking experiences and also chronic pain models (50). They further demonstrated that the pre-LTP represents the anxiety triggered by chronic pain. Co-existence of this form of pre-LTP with a previously identified form of postsynaptic LTP required for chronic pain (51), suggests an important role for these ACC synapses in the mutually enhancing interactions of anxiety and chronic pain.

Mechanisms of plasticity affecting motivation in models of persistent pain were also recently investigated. The nucleus accumbens has been implicated in subjective pain processing in humans and motivation in rodents. A recent study showed that the induction of persistent pain by either nerve injury or an inflammatory mediator impaired motivational behavior in mice measured using a progressive ratio operant task (nose pokes for reward) (52). The decrease in motivation was linked to enhanced release of the neuropeptide galanin in the nucleus accumbens core, which induced a form of long-

term depression (LTD) in dopamine receptor 2-expressing medium spiny neurons (D2-MSNs), thus reducing their activity. Importantly the injury-induced effects on D2-MSNs and motivated behavior were reversed by knockdown of the galanin receptor, GalR1 and by interfering with intracellular signaling pathways required for the LTD. Synaptic level investigations of pain-related mechanisms are providing important insights into the role of individual brain areas in the pain experience, including interactions with other brain functions, as well as opening potential new therapeutic avenues.

### **Blocking pain circuits at the periphery**

Recent studies have focused on the therapeutic potential of targeting voltage-gated sodium channels in sensory neurons to relieve pain. In addition to the Nav 1.7 loss-of-function mutations that cause congenital insensitivity to pain in humans, studies of Nav1.7 and Nav1.8 in mice also show a required role for the channels in many forms of acute and pathological pain (53, 54). A highly specific Nav1.7 inhibitor isolated from centipede venom,  $\mu$ -SLPTX-Ssm6a, when injected intraperitoneal in mice, shows remarkably potent analgesic effects on chemical-induced pain with an efficacy equivalent to or exceeding morphine (55). Similarly, monoclonal antibodies raised against Nav1.7, when injected into mice, effectively reduce mechanical allodynia and thermal hyperalgesia induced by inflammation and neuropathic injury (56). Given the promising therapeutic benefit of targeting Nav1.7 channels to relieve pain, several selective inhibitors are currently in phase II clinical trials.

Animal venoms that elicit pain are also particularly useful for identifying and characterizing therapeutically relevant nociceptive sensory molecules. MitTx, purified from the venom of the Texas coral snake *Micrurus tener tener* is a highly specific and strong potentiator of the acid-sensing channel, ASIC1. Injection of this peptide into the



hind paw of mice induces pronounced nocifensive behaviors (57). Conversely, central and peripheral delivery of the isopeptides mambalgin-1 and 2, purified from the venom of the *black mamba* snake, reverses mechanical allodynia and heat hyperalgesia induced by inflammatory agents by inhibiting ASIC channels, highlighting the therapeutic potential of this family of ion channels (58).

Recent advances in the *in vivo* application of optogenetic methods also provide attractive therapeutic opportunities for pain. One of the first studies to report the use of optogenetics to evaluate pain circuits *in vivo* targeted channelrhodopsin to Nav1.8-expressing primary sensory neurons in mice. As predicted, cutaneous light-mediated activation of the neurons elicited robust nocifensive behaviors (withdrawal, licking, jumping and vocalization) as well as conditioned place aversion (59). Testing opsins for therapeutic purposes, the inhibitory opsin NpHR was targeted to small diameter primary sensory neurons by injection of AAV6-NpHR into the sciatic nerve. Cutaneous light-mediated inhibition of the infected primary sensory neurons alleviated both inflammatory and neuropathic pain (60). The low penetration of light through the skin may be technically limiting, however, new implantable miniaturized optoelectronic systems for wireless control of primary sensory and spinal cord neurons are now available (61), offering a promising future for the treatment of pain through opsins.

Another innovative approach used to silence nociceptors targets the voltage-gated sodium channel blocker QX-314 to select populations, thereby inhibiting the conduction. Co-application of capsaicin, which permits entry of QX-314 through the cation channel TRPV1, to the plantar hind paw of mice, markedly reduced the response to heat and mechanical pressure (7). QX-314 has also been used to ameliorate persistent pain. In efforts to characterize the role of toll-like receptors in pain, Xu et al identified TLR5 as a receptor specific to A-fibers (62). Co-application of the TLR5 ligand

flagellin and QX-314 to the plantar hind paw of mice blocked A-fiber activity measured electrophysiologically *in vitro* and *in vivo*. Although the precise mechanism by which QX-314 enters the cells is still unclear, the approach successfully reversed mechanical allodynia and ongoing pain in a number of chronic neuropathic pain models in mice without producing obvious deficits in touch sensation.

Lastly, the use of gene therapy to restore the normal expression of dysregulated proteins in sensory neurons has become another attractive strategy to treat pain. The expression of the microRNA, miR-7a, is dramatically reduced after neuropathic injuries. Increasing its expression by intraganglionic injection of AAV6-miR-7a abolished mechanical allodynia and thermal hyperalgesia induced by nerve injury, although not by inflammation (63). In contrast, nerve injury significantly up-regulates the sodium channel Nav1.3. Intraganglionic injection of AAV5 expressing a small hairpin RNA against the channel reduced Nav1.3 expression and partially reversed nerve-injury induced mechanical allodynia (64).

### **Blocking pain circuits centrally**

Since mechanisms of disinhibition in the dorsal horn have a critical role in the expression of mechanical allodynia and spontaneous pain, therapeutic efforts have been directed toward the restoration of inhibitory tone. Administration of a small molecule enhancer of KCC2 activity (CLP290) to rodents restores inhibitory drive in dorsal horn neurons measured electrophysiologically in spinal cord slices and reverses behavioral evidence of mechanical allodynia induced by nerve injury. Similarly, overexpression of the co-transporter KCC2 by viral delivery through an intrathecal route in rodents reversed behavioral evidence of mechanical allodynia induced by peripheral nerve injury (Li et al., 2016). Humans with a mutation in the *HSN2*-containing splice

variant of the kinase WNK1 suffer from congenital insensitivity to pain. Kahle and colleagues reported that WNK1/HSN2 plays a role in nerve injury-induced mechanical allodynia by reducing KCC2 activity in lamina II neurons of the dorsal horn (65). Antagonizing WNK activity in rodents restored GABA-mediated inhibition of spinal cord lamina II neurons measured using electrophysiology in spinal cord slices and reversed the behavioral evidence of mechanical allodynia induced by peripheral nerve injury, but not by inflammatory injury.

Using an entirely different strategy based on the observation that GABAergic precursors from the medial ganglion eminence grafted into the forebrain correct neurological disorders of hyperexcitability such as epilepsy, Braz et al tested whether injecting the telecephalic GABAergic precursors directly into the dorsal horn of nerve-injured mice could restore inhibitory tone and reverse mechanical allodynia (66). Although the precursors maintained their cortical identity, they successfully integrated into the dorsal horn, forming local synaptic connections with primary sensory and spinal cord neurons. Importantly, mechanical thresholds were elevated to pre-injury levels by three weeks after transplantation. Interestingly, hypersensitivity induced by inflammation was unchanged after cell transplantation. The work demonstrates that strategies to reverse or compensate for injury-induced disinhibition in the dorsal horn are showing promise for the potential treatment of pain.

### **Conclusions and the road ahead**

Recent advances at the molecular, cellular and systems level are transforming our understanding of how the complex sensation of pain is encoded within the nervous system as well as providing exciting new avenues for therapeutic intervention. Several key aspects of the nociceptive network and the sensation of pain, however, remain to

be addressed. With respect to physiological sensory processing, major outstanding questions include: What is the identity of the noxious mechanotransducer(s)? What are the specific contributions of molecularly distinct primary sensory neurons to modality coding? What is the logic of nociceptor coding of pain? How do spinal cord circuits contribute to modality coding? What is the logic of the projection neuron populations? Which neuronal populations in the brain process the different aspects of pain? In terms of pathological pain, major outstanding questions include: which primary sensory neurons convey mechanical allodynia? What are the neuronal networks underlying mechanical allodynia induced by different types of injury? What are the neural networks for spontaneous pain? How does chronic pain manifest in the brain? Many cutting-edge techniques as mentioned in this review are currently being used to delineate the neural circuits for pain. Other techniques, such as delineating population codes using genetically encoded calcium or voltage sensors, are being adapted for use throughout the somatosensory system. Understanding the precise contributions of the neurons and molecules that underlie each form of pain will greatly facilitate therapeutic drug development.

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## **COMPETITIVE FINANCIAL INTEREST**

The authors declare no competing financial interests.

## FIGURE LEGEND

**Figure 1: Schematic describing the overall organization of somatosensory circuits.** Cutaneous sensory neurons (DRG) (blue; left) are activated by a variety of stimuli (mechanical, heat, cold, chemical, pruritogens) and project to the spinal cord dorsal horn (DH). In the DH (right), the central terminals of high threshold nociceptors (HT) are located in the most superficial laminae (I-II<sub>id</sub>) and lamina V. Low threshold mechanoreceptors (LTMR) preferentially end in the deep dorsal horn (II<sub>iv</sub>-V). The spinal cord is divided into ten laminae delineated by dotted lines (DH is I-VI) and is composed of numerous neuronal populations. Some identified populations are organized in longitudinal layers (only excitatory neurons are represented), tVGLUT3 neurons (orange cells) in lamina III-IV, PKC $\gamma$  (green cells) in lamina II<sub>iv</sub>, calretinin (blue cells) in II<sub>o</sub>-II<sub>id</sub>, vertical cells (yellow cells) in II<sub>o</sub> and projection neurons (red cells) in laminae I and IV-V. Projection neurons send information to the brainstem and thalamus that then send projections to several brain regions implicated in sensory-discriminative (upper middle, green) and emotional (upper middle red) sensory perception. ACC, anterior cingulate cortex; SI (II), primary (secondary) somatosensory cortex; PAG, periaqueductal gray area; PB, parabrachial nucleus; AMY, amygdala; PFC, prefrontal cortex; BG, basal ganglia; LTMR, low-threshold mechanoreceptor; HT, high-threshold; I, lamina I; II<sub>o</sub>, outer part of lamina II; II<sub>id</sub>, dorsal part of the inner lamina II; II<sub>iv</sub>, ventral part of inner lamina II; III, lamina III; IV, lamina IV; V, lamina V; VI, lamina VI; VII, lamina VII; VIII, lamina VIII; IX, lamina IX; X, lamina X.

**Figure 2: Schematic of the organization of primary sensory neurons.** The categories of myelinated and unmyelinated neurons (upper panel, middle) and their respective functional roles are derived from large-scale transcriptional analyses,

behavioral analyses and the literature. The lower panel shows the location of the central terminations of the primary sensory neuron categories. The schematic is based on the analyses of gene reporter mouse lines (PV, RET, TRKB, TH, MRGPRD, MRGPRA3, CGRP, TRPM8) and immunohistological analyses (TRKA, TRPV1, TAC1). Dotted lines represent the border between laminae indicated on the left. I, lamina I; II<sub>o</sub>, outer part of lamina II; II<sub>id</sub>, dorsal part of the inner lamina II; II<sub>iv</sub>, ventral part of inner lamina II; III, lamina III; IV, lamina IV.

**Figure 3: Schematic of the organization of the dorsal horn circuit for pain.**

Peripheral nociceptors (purple fiber) project onto excitatory interneurons in lamina II<sub>o</sub> (central cell, dark gray; vertical cells, yellow) and onto NK1R projection neurons in lamina I (red). Non-peptidergic afferents expressing MRGPRD (green fiber) project to lamina II<sub>id</sub> including to excitatory vertical cells with ventrally-directed elongated dendrites. Both primary afferents also contact inhibitory islet cells (elongated pink cells). Stimulation of nociceptive afferents activates excitatory central cells, vertical cells and NK1R projections neurons to mediate noxious pain. Inhibitory islet cells modulate this activity. Innocuous afferents (orange fiber) project onto excitatory interneurons expressing tVGLUT3 in lamina III (brown cell), PKC $\gamma$  in lamina II<sub>iv</sub> (green cell) and vertical cells in lamina II<sub>o</sub>. Myelinated afferents also contact PV inhibitory interneurons in lamina III (radial pink cell) and dynorphin (Dyn) inhibitory vertical cells in lamina II<sub>o</sub> (vertical pink cell). tVGLUT3 interneurons project onto excitatory vertical cell dendrites and intermediate excitatory interneurons in lamina III. Intermediate interneurons project onto PKC $\gamma$  and calretinin excitatory interneurons in lamina II<sub>iv</sub>. Inhibitory interneurons prevent A-fiber mediated activation of the nociceptive network through feed-forward circuits that act on PKC $\gamma$  interneurons, vertical cells and NK1R projection neurons. After

nerve injury, inhibition by PV and Dyn interneurons is reduced allowing A-fiber mediated activation of a dorsally-directed circuit that includes tVGLUT3 and PKC $\gamma$  neurons. After inflammatory injury, reduction in a still unknown mechanism of disinhibition allows A-fiber-mediated activation of a dorsally-directed circuit that includes tVGLUT3 and lamina II<sub>iv</sub> calretinin neurons. tVGLUT3, transient vesicular glutamate transporter 3; GABA, gamma-aminobutyric acid; Gly, glycine; PV, parvalbumin; PKC $\gamma$ , gamma isoform of protein kinase C; NK1R, neurokinin 1 receptor; I, lamina I; II<sub>o</sub>, outer part of lamina II; II<sub>id</sub>, dorsal part of the inner lamina II; II<sub>iv</sub>, ventral part of inner lamina II; III, lamina III.

## References

1. D. L. Bennett, C. G. Woods, Painful and painless channelopathies. *The Lancet. Neurology* **13**, 587-599 (2014).
2. D. Julius, J. R. Carlson, Editorial overview: molecular biology of sensation. *Current opinion in neurobiology* **34**, v-vi (2015).
3. M. J. Caterina *et al.*, Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* **288**, 306-313 (2000).
4. J. Vriens *et al.*, TRPM3 is a nociceptor channel involved in the detection of noxious heat. *Neuron* **70**, 482-494 (2011).
5. H. Cho *et al.*, The calcium-activated chloride channel anoctamin 1 acts as a heat sensor in nociceptive neurons. *Nature neuroscience* **15**, 1015-1021 (2012).
6. D. J. Cavanaugh *et al.*, Distinct subsets of unmyelinated primary sensory fibers mediate behavioral responses to noxious thermal and mechanical stimuli. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 9075-9080 (2009).
7. C. Brenneis *et al.*, Phenotyping the function of TRPV1-expressing sensory neurons by targeted axonal silencing. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **33**, 315-326 (2013).
8. W. M. Knowlton *et al.*, A sensory-labeled line for cold: TRPM8-expressing sensory neurons define the cellular basis for cold, cold pain, and cooling-mediated analgesia. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **33**, 2837-2848 (2013).
9. Y. Roudaut *et al.*, Touch sense: functional organization and molecular determinants of mechanosensitive receptors. *Channels* **6**, 234-245 (2012).

10. R. Sharif-Naeini, Contribution of mechanosensitive ion channels to somatosensation. *Progress in molecular biology and translational science* **131**, 53-71 (2015).
11. S. Maksimovic *et al.*, Epidermal Merkel cells are mechanosensory cells that tune mammalian touch receptors. *Nature* **509**, 617-621 (2014).
12. Z. Pang *et al.*, Selective keratinocyte stimulation is sufficient to evoke nociception in mice. *Pain* **156**, 656-665 (2015).
13. K. M. Baumbauer *et al.*, Keratinocytes can modulate and directly initiate nociceptive responses. *eLife* **4**, (2015).
14. D. Usoskin *et al.*, Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nature neuroscience* **18**, 145-153 (2015).
15. I. M. Chiu *et al.*, Transcriptional profiling at whole population and single cell levels reveals somatosensory neuron molecular diversity. *eLife* **3**, (2014).
16. C. L. Li *et al.*, Somatosensory neuron types identified by high-coverage single-cell RNA-sequencing and functional heterogeneity. *Cell research* **26**, 83-102 (2016).
17. M. C. Delfini *et al.*, TAFA4, a chemokine-like protein, modulates injury-induced mechanical and chemical pain hypersensitivity in mice. *Cell reports* **5**, 378-388 (2013).
18. L. Li *et al.*, The functional organization of cutaneous low-threshold mechanosensory neurons. *Cell* **147**, 1615-1627 (2011).
19. R. P. Seal *et al.*, Injury-induced mechanical hypersensitivity requires C-low threshold mechanoreceptors. *Nature* **462**, 651-655 (2009).
20. E. S. McCoy *et al.*, Peptidergic CGRP $\alpha$  primary sensory neurons encode heat and itch and tonically suppress sensitivity to cold. *Neuron* **78**, 138-151 (2013).
21. E. S. McCoy, B. Taylor-Blake, M. J. Zylka, CGRP $\alpha$ -expressing sensory neurons respond to stimuli that evoke sensations of pain and itch. *PloS one* **7**, e36355 (2012).
22. T. S. Jensen, N. B. Finnerup, Allodynia and hyperalgesia in neuropathic pain: clinical manifestations and mechanisms. *The Lancet. Neurology* **13**, 924-935 (2014).
23. V. E. Abraira, D. D. Ginty, The sensory neurons of touch. *Neuron* **79**, 618-639 (2013).
24. A. Zimmerman, L. Bai, D. D. Ginty, The gentle touch receptors of mammalian skin. *Science* **346**, 950-954 (2014).
25. B. Coste *et al.*, Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science* **330**, 55-60 (2010).
26. S. S. Ranade *et al.*, Piezo2 is the major transducer of mechanical forces for touch sensation in mice. *Nature* **516**, 121-125 (2014).
27. N. Eijkelkamp *et al.*, A role for Piezo2 in EPAC1-dependent mechanical allodynia. *Nature communications* **4**, 1682 (2013).
28. A. J. Todd, Neuronal circuitry for pain processing in the dorsal horn. *Nature reviews. Neuroscience* **11**, 823-836 (2010).
29. J. Braz, C. Solorzano, X. Wang, A. I. Basbaum, Transmitting pain and itch messages: a contemporary view of the spinal cord circuits that generate gate control. *Neuron* **82**, 522-536 (2014).
30. R. Melzack, P. D. Wall, Pain mechanisms: a new theory. *Science* **150**, 971-979 (1965).



31. C. Torsney, A. B. MacDermott, Disinhibition opens the gate to pathological pain signaling in superficial neurokinin 1 receptor-expressing neurons in rat spinal cord. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **26**, 1833-1843 (2006).
32. L. S. Mirauccourt, R. Dallel, D. L. Voisin, Glycine inhibitory dysfunction turns touch into pain through PKC $\gamma$  interneurons. *PloS one* **2**, e11116 (2007).
33. Y. Lu *et al.*, A feed-forward spinal cord glycinergic neural circuit gates mechanical allodynia. *The Journal of clinical investigation* **123**, 4050-4062 (2013).
34. T. Yasaka *et al.*, A putative relay circuit providing low-threshold mechanoreceptive input to lamina I projection neurons via vertical cells in lamina II of the rat dorsal horn. *Molecular pain* **10**, 3 (2014).
35. C. Peirs *et al.*, Dorsal Horn Circuits for Persistent Mechanical Pain. *Neuron* **87**, 797-812 (2015).
36. H. Petitjean *et al.*, Dorsal Horn Parvalbumin Neurons Are Gate-Keepers of Touch-Evoked Pain after Nerve Injury. *Cell reports* **13**, 1246-1257 (2015).
37. D. I. Hughes *et al.*, Morphological, neurochemical and electrophysiological features of parvalbumin-expressing cells: a likely source of axo-axonic inputs in the mouse spinal dorsal horn. *The Journal of physiology* **590**, 3927-3951 (2012).
38. B. Duan *et al.*, Identification of spinal circuits transmitting and gating mechanical pain. *Cell* **159**, 1417-1432 (2014).
39. H. U. Zeilhofer, H. Wildner, G. E. Yevenes, Fast synaptic inhibition in spinal sensory processing and pain control. *Physiological reviews* **92**, 193-235 (2012).
40. J. A. Coull *et al.*, BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* **438**, 1017-1021 (2005).
41. J. A. Coull *et al.*, Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature* **424**, 938-942 (2003).
42. R. E. Sorge *et al.*, Different immune cells mediate mechanical pain hypersensitivity in male and female mice. *Nature neuroscience* **18**, 1081-1083 (2015).
43. J. S. Mogil, Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. *Nature reviews. Neuroscience* **13**, 859-866 (2012).
44. S. Beggs, T. Trang, M. W. Salter, P2X4R+ microglia drive neuropathic pain. *Nature neuroscience* **15**, 1068-1073 (2012).
45. Z. Guan *et al.*, Injured sensory neuron-derived CSF1 induces microglial proliferation and DAP12-dependent pain. *Nature neuroscience* **19**, 94-101 (2016).
46. P. Schweinhardt, M. C. Bushnell, Pain imaging in health and disease--how far have we come? *The Journal of clinical investigation* **120**, 3788-3797 (2010).
47. G. Liberati *et al.*, Nociceptive Local Field Potentials Recorded from the Human Insula Are Not Specific for Nociception. *PLoS biology* **14**, e1002345 (2016).
48. T. V. Salomons, G. D. Iannetti, M. Liang, J. N. Wood, The "Pain Matrix" in Pain-Free Individuals. *JAMA neurology* **73**, 755-756 (2016).
49. A. Krishnan *et al.*, Somatic and vicarious pain are represented by dissociable multivariate brain patterns. *eLife* **5**, (2016).
50. K. Koga *et al.*, Coexistence of two forms of LTP in ACC provides a synaptic mechanism for the interactions between anxiety and chronic pain. *Neuron* **85**, 377-389 (2015).

51. H. Xu *et al.*, Presynaptic and postsynaptic amplifications of neuropathic pain in the anterior cingulate cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **28**, 7445-7453 (2008).
52. N. Schwartz *et al.*, Chronic pain. Decreased motivation during chronic pain requires long-term depression in the nucleus accumbens. *Science* **345**, 535-542 (2014).
53. M. S. Minett *et al.*, Pain without nociceptors? Nav1.7-independent pain mechanisms. *Cell reports* **6**, 301-312 (2014).
54. M. A. Nassar *et al.*, Nociceptor-specific gene deletion reveals a major role for Nav1.7 (PN1) in acute and inflammatory pain. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 12706-12711 (2004).
55. S. Yang *et al.*, Discovery of a selective NaV1.7 inhibitor from centipede venom with analgesic efficacy exceeding morphine in rodent pain models. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 17534-17539 (2013).
56. J. H. Lee *et al.*, A monoclonal antibody that targets a NaV1.7 channel voltage sensor for pain and itch relief. *Cell* **157**, 1393-1404 (2014).
57. C. J. Bohlen *et al.*, A heteromeric Texas coral snake toxin targets acid-sensing ion channels to produce pain. *Nature* **479**, 410-414 (2011).
58. S. Diochot *et al.*, Black mamba venom peptides target acid-sensing ion channels to abolish pain. *Nature* **490**, 552-555 (2012).
59. I. Daou *et al.*, Optogenetic Silencing of Nav1.8-Positive Afferents Alleviates Inflammatory and Neuropathic Pain. *eNeuro* **3**, (2016).
60. S. M. Iyer *et al.*, Virally mediated optogenetic excitation and inhibition of pain in freely moving nontransgenic mice. *Nature biotechnology* **32**, 274-278 (2014).
61. S. I. Park *et al.*, Soft, stretchable, fully implantable miniaturized optoelectronic systems for wireless optogenetics. *Nature biotechnology* **33**, 1280-1286 (2015).
62. Z. Z. Xu *et al.*, Inhibition of mechanical allodynia in neuropathic pain by TLR5-mediated A-fiber blockade. *Nature medicine* **21**, 1326-1331 (2015).
63. A. Sakai *et al.*, miR-7a alleviates the maintenance of neuropathic pain through regulation of neuronal excitability. *Brain : a journal of neurology* **136**, 2738-2750 (2013).
64. O. A. Samad *et al.*, Virus-mediated shRNA knockdown of Na(v)1.3 in rat dorsal root ganglion attenuates nerve injury-induced neuropathic pain. *Molecular therapy : the journal of the American Society of Gene Therapy* **21**, 49-56 (2013).
65. K. T. Kahle *et al.*, Inhibition of the kinase WNK1/HSN2 ameliorates neuropathic pain by restoring GABA inhibition. *Science signaling* **9**, ra32 (2016).
66. J. M. Braz *et al.*, Forebrain GABAergic neuron precursors integrate into adult spinal cord and reduce injury-induced neuropathic pain. *Neuron* **74**, 663-675 (2012).

