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Review

# Nociceptors—Noxious Stimulus Detectors

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*Clinical pain is a serious public health issue. Treatment of pain-related suffering requires knowledge of how pain signals are initially interpreted and subsequently transmitted and perpetuated. This review article is one of three reviews in this issue of* Neuron *that address our understanding of the pain process and possible solutions to the problem from both cellular- and systems-level viewpoints.*

In order to deal effectively with danger, it is imperative to know about it. This is what nociceptors do—these primary sensory neurons are specialized to detect intense stimuli and represent, therefore, the first line of defense against any potentially threatening or damaging environmental inputs. By sensing noxious stimuli and contributing to the necessary reactions to avoid them—rapid withdrawal and the experience of an intensely unpleasant or painful sensation, nociceptors are essential for the maintenance of the body's integrity. Although nociceptive pain is clearly an adaptive alarm system, persistent pain is maladaptive, essentially an ongoing false alarm. Here, we highlight the genesis of nociceptors during development and the intrinsic properties of nociceptors that enable them to transduce, conduct, and transmit nociceptive information and also discuss how their phenotypic plasticity contributes to clinical pain.

As a result of physiological experiments he conducted at the dawn of the 20th century, Charles Sherrington concluded, "There is considerable evidence that the skin is provided with a set of nerve endings whose specific office it is to be amenable to stimuli that do the skin injury, stimuli that in continuing to act would injure it still further" (Sherrington, 1903). He further stated that since "harmfulness is the characteristic of the stimuli by which [the nerve endings] are provocable…for physiological reference therefore they are preferably termed nocicipient" (Sherrington, 1903). A few years later Sherrington expanded his definition of a noxious stimulus as one with an intensity and quality sufficient to trigger reflex withdrawal, autonomic responses, and pain, collectively constituting what he called the nociceptive reaction. He also redefined the neural apparatus responsible for detecting a noxious stimulus as nociceptive nerves or nociceptors (Sherrington, 1906).

Implicit in this new term, coined 100 years ago, was that pain is a specific sensation with its own sensory machinery. This view was something that Descartes and von Frey had also argued in favor of, although in rather different ways. Sherrington's notion was directly counter to then widely held theories that pain resulted from a central summation in response to excessive sensory stimulation or that all nerve endings are similar and that certain patterns of activity produced by intense stimulation evoke pain. Fundamentally, this divergence reflected the competing specificity and pattern theories of pain that characterized much of 19th and early 20th century pain sensory biology. The debate reached a climax in the 1960s and 70s with Ed Perl arguing vigorously that pain is mediated by specialized high-threshold nociceptor sensory neurons (Bessou and Perl, 1969), and Pat Wall and Ron Melzack emphatically emphasizing central processing as generating pain (Melzack and Wall, 1965). It is now clear that this clash of the sensory titans was quite artificial. It is not an either/or situation; nociceptors are indeed the peripheral path to nociceptive pain, and altered central processing does contribute to pain hypersensitivity in patients. We can, however, now quietly relegate the view that sensory specificity is encoded by activity in nonspecialized primary sensory neurons to the garbage can of history and instead loudly celebrate the first century of the nociceptor, a specialized noxious stimulus detector. We also increasingly recognize that the nociceptor is highly modifiable in response to injury of its axon and on exposure to inflammation and that this plasticity is integral to its pain-generating functions and may reflect, moreover, a recapitulation of the signaling that determines its differentiation during development.

In interacting with the environment, living organisms have to recognize and react to harmful stimuli to avoid them. To do this, nociceptors have a high threshold and normally respond, as Sherrington clearly recognized, only to stimuli of sufficient energy to potentially or actually damage tissue. Some nociceptors are thinly myelinated

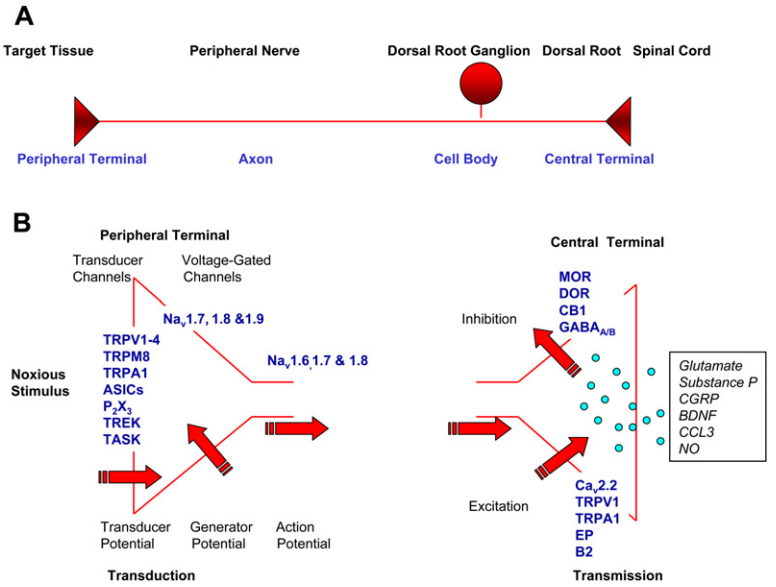


**Figure 1. The Nociceptor**

(A) The operational components of the nociceptor include a peripheral terminal that innervates target tissue and transduces noxious stimuli, an axon that conducts action potentials from the periphery to the central nervous system, a cell body in the dorsal root ganglion, and a central terminal where information is transferred to second order neurons at central synapses.

(B) Transduction is mediated by high-threshold transducer ion channels which depolarize the peripheral terminal activating voltage-dependent sodium channels. Transmission occurs in response to calcium influx at the central terminal releasing glutamate as well as multiple synaptic modulators and signaling molecules and is subject to both excitatory and inhibitory influences.

(Aδ-fibers) but most are unmyelinated (C fibers), and these slowly conducting afferents represent the majority of sensory neurons in the peripheral nervous system. Like all primary sensory neurons in the somatosensory system, they have their cell bodies in dorsal root or trigeminal ganglia, give rise to a single axon that bifurcates into a peripheral branch that innervates peripheral target tissue, and a central axon that enters the CNS to synapse on nociceptive second order neurons. The nociceptor in consequence has four major functional components, the peripheral terminal that transduces external stimuli and initiates action potentials, the axon that conducts action potentials, the cell body that controls the identity and integrity of the neuron, and the central terminal which forms the presynaptic element of the first synapse in the sensory pathway in the CNS (Figure 1). In addition to the contribution of the intrinsic properties of the neuron to its functional role, crucial too are the extrinsic signals that feed onto the neuron from targets, nerves, and the spinal cord that can produce profound phenotypic alterations.

Loss of nociceptor neurons in patients with hereditary sensory and autonomic neuropathy type 4, due to mutations in the nerve growth factor (NGF) TrkA receptor that result in a failure of nociceptor survival in the embryo (Verpoorten et al., 2006), produces congenital pain hyposensitivity, and starkly reveals the importance of nociceptors as an early warning device. These unfortunate individuals burn and chew their tongues and lips, and as a result of undetected damage, lose the tips of their fingers and damage joints. Ignorance of noxious stimuli is not bliss. A congenital indifference to pain without loss of nociceptor neurons has been shown recently to occur with loss of function mutations in the SCN9A gene encoding the alpha subunit of Na$_v$1.7 voltage-gated sodium channel (Cox et al., 2006; Goldberg et al., 2007).

In this review, we highlight recent insights into how nociceptors differentiate from progenitors during development to achieve the specialized nociceptor molecular phenotype, how they transduce noxious stimuli and transfer input to the CNS, and how some of the adaptive and maladaptive functional and phenotypic changes that occur in nociceptors produce spontaneous pain and pain hypersensitivity.

## Development and Differentiation of Nociceptors

### *Nociceptor Genesis*

Nociceptors develop from those neural crest stem cells that migrate from the dorsal part of the neural tube and form late during neurogenesis, whereas neurons born earlier become proprioceptors or low-threshold mechanoceptors (Anderson, 2000; Fitzgerald, 2005; Lawson and Biscoe, 1979; Marmigere and Ernfors, 2007). All newly formed embryonic nociceptors express the TrkA nerve growth factor receptor (Marmigere and Ernfors, 2007). However, the transcription factors that determine nociceptor cell fate remain unclear. Formation of most TrkA$^+$ neurons is dependent on the proneural transcription factor *Neurogenin1* (*Ngn1*) (Ma et al., 1998, 1999). Ngn1 activity is, however, not specific for nociceptors—it's also required for formation of TrkB$^+$ and TrkC$^+$ low-threshold mechanoceptors (Ma et al., 1998, 1999). The homeobox gene *Brn3a* and the zinc finger gene *Klf7* are required for the maintenance, but not the initiation of *TrkA* expression, but these transcription factors are also like Ngn1, expressed in both developing nociceptors and nonnociceptors (Lei et al., 2006). The Runx1 runt domain transcription factor is however, expressed exclusively in TrkA$^+$ neurons at early embryonic stages (Chen et al., 2006; Kramer et al., 2006; Levanon et al., 2002; Marmigere et al., 2006; Theriault et al., 2005; Yoshikawa et al., 2007), but because its expression is initiated some time after the onset of TrkA expression, it is unlikely to be involved in early nociceptor cell fate determination (Chen et al., 2006).

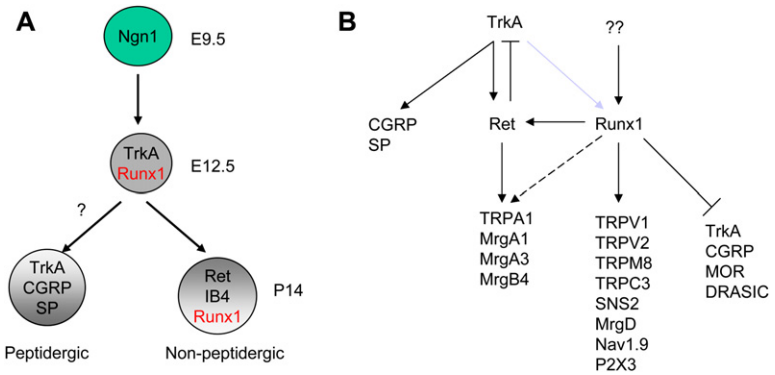


**Figure 2. Nociceptor Development**

(A) Progressive segregation of peptidergic versus nonpeptidergic nociceptors. Note that Runx1 expression is extinguished in prospective TrkA$^+$ peptidergic neurons, but the signals that trigger Runx1 downregulation in these neurons remain elusive ("?").

(B) An interaction network that controls expression of nociceptor-specific molecules. The purple arrow indicates that TrkA signaling is required to maintain Runx1 expression at perinatal stages, but it remains unknown if TrkA signaling is directly involved in this process. The signals that control the initiation of Runx1 expression are also unknown ("??"). The dashed arrow indicates possibility that Runx1 has a direct role in controlling expression of TRPA1, MrgA1, A3 and B4, although Runx1 could activate these genes by regulating Ret.

### *Segregation of Peptidergic versus Nonpeptidergic Nociceptors*

Following sensory neurogenesis, prospective nociceptors undergo two distinct differentiation pathways that lead to the formation of two major classes of nociceptors, peptidergic and nonpeptidergic nociceptors. These two sets of nociceptors express distinct repertoires of ion channels and receptors and innervate distinct peripheral and central targets (Braz et al., 2005; Chen et al., 2006; Snider and McMahon, 1998; Zylka et al., 2005). During the perinatal and postnatal period, about half of developing nociceptors switch off TrkA and begin to express Ret, the transmembrane signaling component of the receptor for glial cell-derived growth factor (GDNF) and other GDNF-related growth factors. These neurons become the nonpeptidergic nociceptors, most of which bind isolectin B4 (IB4$^+$). The remaining nociceptors retain TrkA (a few also coexpress Ret) and develop into the peptidergic class of nociceptors that express CGRP and SP and do not bind IB4 (IB4$^-$) (Bennett et al., 1996; Molliver et al., 1997).

The dynamic expression of Runx1 appears to be an important player in this process (Figure 2; Chen et al., 2006; Kramer et al., 2006; Yoshikawa et al., 2007). Early embryonic nociceptors share a similar molecular identity, coexpressing both TrkA and Runx1 (Chen et al., 2006). During the period when nociceptor segregation occurs, Runx1's expression is extinguished in prospective TrkA$^+$ peptidergic cells but persists in nonpeptidergic neurons (Chen et al., 2006). Conditional knockout of *Runx1* in the DRG transforms these nonpeptidergic cells to a TrkA$^+$ CGRP$^+$ identity, and in this situation most nociceptors develop as peptidergic nociceptors (Chen et al., 2006; Yoshikawa et al., 2007). Conversely, constitutive expression of Runx1 in all nociceptors is sufficient to suppress embryonic peptidergic differentiation (Kramer et al., 2006). Runx1 also coordinates afferent targeting to the spinal cord; in mice that lack *Runx1* prospective IB4$^+$ nonpeptidergic afferents adopt the projection pattern typical of peptidergic afferents (Chen et al., 2006). These observations suggest that persistent Runx1 expression promotes the Ret$^+$ nonpeptidergic cell fate, whereas loss of Runx1 is essential for peptidergic differentiation.

Several recent studies suggest that Runx1 and TrkA/Ret signaling pathways form a complex interaction loop for establishing nonpeptidergic nociceptor cell fate (Ibanez and Ernfors, 2007 ; Luo et al., 2007). TrkA-signaling is required to activate Ret, partly it appears by maintaining Runx1 expression at perinatal stages (Luo et al., 2007). Raf kinases acting downstream of TrkA or other signaling molecules are required to maintain Ret expression by controlling expression of the Runx1 cofactor protein, CBF-β (Zhong et al., 2006). Finally, Ret signaling acts to suppress TrkA expression in prospective nonpeptidergic neurons (Luo et al., 2007; Figure 2).

However, despite progress in teasing out the determinants of nociceptor specification, several issues remain to be resolved. Because both TrkA and Ret are required for afferents to innervate peripheral targets (Luo et al., 2007; Patel et al., 2000), a loss of either TrkA or Ret signaling prevents nociceptors from receiving other target-derived signals. Consequently, it is not known if TrkA signaling directly or indirectly controls expression of Runx1, CBF-β, and Ret, or if Ret signaling is directly involved in TrkA suppression. In addition, while TrkA signaling is required to maintain Runx1 expression at embryonic stages, Runx1 expression is extinguished from TrkA$^+$ peptidergic nociceptors during perinatal/postnatal development, and we need to determine, therefore, if TrkA signaling switches from activating to suppressing Runx1 expression at different developmental stages or if a peripheral innervation defect in the absence of TrkA signaling indirectly extinguishes Runx1 expression. A further problem is that the intrinsic transcription factors that establish peptidergic nociceptor cell fate still remain elusive. Although Runx1 suppresses TrkA expression during postnatal development (Chen et al., 2006), it is capable of activating TrkA expression after ectopic expression in the neural tube and migratory neural crest cells (Marmigere et al., 2006). Runx1 can, therefore, exert opposing activities depending on the cellular context. It will be extremely interesting to establish if changes in context-dependent

transcriptional activities contribute to the phenotypic switches in nociceptors that occur in pathological conditions (see below).

### *Regulation of Nociceptive Ion Channels and Receptors*

The mature nociceptor expresses dozens of ion channels and receptors, and the correct establishment of their expression is essential for nociceptors to detect specific noxious stimuli (see below, "The Differentiated Nociceptor"). There are two notable features about the developmental control of sensory channels/receptor expression. First, many sensory channels/receptors are expressed in only a partially overlapping or mutually exclusive manner, including TRP class thermal/chemical receptors and Mrg class G protein-coupled receptors (Dong et al., 2001; Hjerling-Leffler et al., 2007; Story et al., 2003; Zylka et al., 2003). Second, the emergence of individual sensory channels/receptors is subject to complex temporal control. For example, expression of three TRP channels, TRPV1, TRPM8, and TRPA1, is initiated at E12.5, E16.5, and P0, respectively (Hjerling-Leffler et al., 2007), while TRPA1 expression in peptidergic nociceptors is established at P0 and nonpeptidergic nociceptors at P14, respectively (Hjerling-Leffler et al., 2007).

The complex expression pattern of sensory channels and receptors is established through a series of hierarchical controls. TrkA signaling is required to establish the molecular and functional identity of nociceptors (Lewin, 1996; Luo et al., 2007; Patel et al., 2000; Ritter et al., 1991). *Runx1* is required to activate expression of a large fraction of nociceptor-specific ion channels and receptors (Chen et al., 2006). All known sensory receptors associated with nonpeptidergic nociceptors are, for example, eliminated in *Runx1* conditional knockout mice, including the TRP channel TRPC3, the ATP-gated P2X3 receptor, the sodium channel Nav1.9, and a dozen Mrg-class GPCRs (Chen et al., 2006). Transient Runx1 expression in peptidergic nociceptors is also required for expression of TRP thermal/chemical receptors, including TRPM8, TRPA1, TRPV1, and TRPV2. Runx1, however, is dispensable for or even suppresses the expression of a separate group of ion channels/receptors, including acid-sensing ion channels (ASIC1-3), the opioid receptor MOR, and the sodium channel Nav1.8/SNS (Chen et al., 2006). In other words, nociceptor sensory channels/receptors can be divided into Runx1-dependent and Runx1-independent subgroups (Figure 2). Runx1-dependent genes are further divided into three groups: (1) Ret-dependent, (2) Ret-independent and TrkA-dependent, and (3) Ret-independent and TrkA-independent (Luo et al., 2007). Runx1 is selectively required for thermal but not mechanical pain sensitivity in vivo (Chen et al., 2006), supporting the idea that specification of nociceptive mechanical and thermal sensitivity is subject to separate genetic control.

In summary, intrinsic factors, such as Runx1, interface with target-derived signals like NGF and GDNF to establish nociceptor diversity. The close interaction between extrinsic signals and intrinsic factors must also contribute to the capacity of nociceptors to considerable plasticity in pathological conditions (see below).

## The Differentiated Nociceptor

### *Peripheral Terminal*

The peripheral terminal of the mature nociceptor is its raison d'etre. This is where noxious stimuli are detected and transduced into inward "generator" currents that, if sufficiently large, begin to drive action potentials along the axon to the CNS and in this way set in train the events that ultimately lead to a conscious awareness of the noxious stimulus, pain. The sensory specificity of the nociceptor is established by expression of ion channels tuned to respond with a high threshold only to particular features of the mechanical, thermal, and chemical environment (Ramsey et al., 2006). The high threshold of these transducers differentiates nociceptors from sensory neurons that respond to innocuous stimuli by virtue of expressing tranducers with low thresholds. The identification and characterization of nociceptor transducers over the past decade, starting with TRPV1 by David Julius and colleagues (Caterina et al., 1999), has been one of the great success stories of sensory biology and now includes TRP, ASIC, and potassium and ligand-gated ion channels.

Initially it appeared that the function of nociceptors would be easily defined by identifying nociceptor-specific transducers, characterizing their properties in heterologous expression systems, and studying the phenotype of mice with null mutations of these genes. Not too surprisingly, it is rather more complicated. While TRPV1, for example, initially looked from its threshold, which coincides with that of heat pain (~42°C), as if it might be responsible alone for this sensation (Caterina et al., 1999), thermal sensitivity in the warm-hot range turns out to be mediated by multiple TRP channels—TRPV1, TRPV2, TRPV3, and TRPV4 (Dhaka et al., 2006)—that all express a particular C-terminal domain that confers this sensitivity (Brauchi et al., 2006). The extent to which all these TRPs are expressed in heat-responsive nociceptor neurons remains uncertain. Heat-evoked activity in nociceptors may also be modulated by coexpression of heat-sensitive potassium channels like TREK-1 (Alloui et al., 2006) whose activity is reduced by increased heat.

While all now agree that cool stimuli are sensed by the TRPM8 channel (Bautista et al., 2007; Colburn et al., 2007; Dhaka et al., 2007), which has a different C-terminal domain from heat sensitive TRPs (McKemy et al., 2002), there is some uncertainty over which channel(s), TRPM8, TRPA1, or another channel, detect intense cold, the cold that produces a "burning" pain (Bandell et al., 2004;Jordt et al., 2004), since TRPM8 null mice still respond to intense cold (Dhaka et al., 2007; Bautista et al., 2007). Knockouts do not always produce identical phenotypes and therefore do not provide direct answers but sometimes pose more questions, as in the case of TRPA1 null mice (Bautista et al., 2006; Kwan et al., 2006). The contribution of TRPA1 to cold sensitivity of DRG neurons in vitro could be indirect; cold increases $[Ca^{2+}]_i$, and $Ca^{2+}$ directly

activates TRPA1 via an EF hand domain (Zurborg et al., 2007). Interestingly, while tactile sensibility and motor function deteriorate in the cold, pain perception persists, and this is achieved by expression in nociceptor peripheral terminals of the TTX-R Nav1.8 voltage-gated sodium channel, whose inactivation, unlike TTX-S channels, is cold resistant (Zimmermann et al., 2007).

There is even more uncertainty about noxious mechanical transduction with several competing but unvalidated candidates for the high-threshold "pinch" mechanotransducer that include TRPs, ASICs, and potassium channels (Hu et al., 2006). A null mutation of stomatin-like protein 3 (SLP3), a mammalian mec-2 homolog expressed in sensory neurons, leads to loss of mechanosensitivity in low-threshold Aβ and high threshold Aδ fibers, but not in C fiber polymodal nociceptors (Wetzel et al., 2007). While SLP3 may be, therefore, an essential subunit of some mechanotransducers, possibly with ASIC channels, the identity of the C nociceptor mechanotransducer still remains elusive. In *C. elegans*, mutations of trpa-1, the ortholog of TRPA1, result in defects in mechanosensory behaviors (Kindt et al., 2007), but the role of this channel to mechanosensation in vertebrates is still uncertain.

Many TRP channels are sensitive to a gourmet collection of pungent chemicals from chili, garlic, peppermint, mustard, horseradish, oregano, savory, clove, and thyme (Macpherson et al., 2005; Xu et al., 2006), as well as less pleasantly to environmental irritants such as acrolein, a Word War I chemical weapon and component of cigarette smoke (Bautista et al., 2006), and to some spider toxins (Siemens et al., 2006). A remarkable finding is that mustard oil and other TPA1-activating irritants produce a direct reversible covalent modification of reactive cystines in the N-terminal region of the channel to activate it (Macpherson et al., 2007; Hinman et al., 2006). In addition to this capacity to detect multiple extrinsic chemical signals, endogenous ligands like the endocannabinoid anandamide activate TRP channels (van der Stelt et al., 2005), and this and other fatty acid amides like N-acyl-taurine (Saghatelian et al., 2006), may enable the channels to detect change in tissues independent of external stimuli.

While each transducer channel was originally thought to identify its own "adequate" stimulus, heteromultimers of TRP channels are possible (Liapi and Wood, 2005), and the presence of these in sensory neurons and the transduction properties of such complexes, if they exist, need to be explored. Moreover, TRP splice variants may act as endogenous TRP inhibitors, regulating sensitivity (Vos et al., 2006; Wang et al., 2004). What is certain though, is that there is a major coupling between GPCRs and some TRP channels in the membrane. TRPA1, for example, acts as a receptor-operated channel for bradykinin, generating calcium influx in response to activation of the B2 receptor (Bautista et al., 2006). This type of coupling may be true for other inflammatory mediators/chemokines both with TRPA1 and other TRP channels and provides a means for GPCRs and receptor tyrosine kinases to depolarize the terminal. As a result, although the fundamental role of nociceptors is to detect potentially tissue-damaging stimuli, if damage has occurred this can also be detected by release of bradykinin and other ligands whose receptors are coupled to TRP receptors.

TRPA1 appears to be a major integrator of diverse chemical (wasabi, mustard, raw garlic, and bradykinin) and perhaps even of thermal and mechanical noxious stimuli as well (Bandell et al., 2004, 2006; Kwan et al., 2006) and possesses at the molecular level a feature characteristic of many nociceptors that they are polymodal and respond to several different kinds of input. TRPV1 is also polymodal, detecting heat and multiple chemical (capsaicin, proton, spider toxins) stimuli (Dhaka et al., 2006). Nevertheless, there is also nociceptor specificity spanning diverse ranges of thresholds and sensitivities to exogenous and endogenous stimuli. Some nociceptors have quite low thresholds with maximal responses in the noxious range, not unlike the TRPV3 and TRPV4 channels; others have thresholds so high that they do not normally respond to noninjurious stimuli, similar to TRPV2, and these are called sleeping nociceptors because they "wake" and become responsive only in the presence of inflammation.

### Peripheral Sensitization

Peripheral sensitization represents a form of stimulus-evoked functional plasticity of the nociceptor. The stimulus in this situation is a set of inflammatory mediators released from injured and inflammatory cells that sensitize it, reducing threshold and increasing responsiveness (Figure 3). Essentially at the site of injury/inflammation and as a result of the change in the chemical milieu produced by disruption of cells, degranulation of mast cells, secretion by inflammatory cells, and induction of enzymes like cyclooxygenase-2, nociceptors change from being exclusively noxious stimulus detectors to detectors also of innocuous inputs. As a result, low-intensity stimuli gain access to the nociceptive pathway and begin to produce pain. A broad range of sensitizers do this, including kinins, amines, prostanoids, growth factors, chemokines, and cytokines, which with protons and ATP make up an "inflammatory soup" that is "tasted" by the nociceptor terminal and as a result changes it. Several new ingredients of the soup have been identified, including the TGFβ member activin (Xu and Hall, 2006), TNFα (Jin and Gereau, 2006), the chemokine CCL3 (Zhang et al., 2005a), prokineticins (Vellani et al., 2006), proteases that activate protease-activated GPCR receptors (Grant et al., 2007; Dai et al., 2007), and GDNF (Malin et al., 2006). The large numbers of sensitizing agents acting in parallel makes interruption of their effects a fairly poor option to treat inflammatory pain. Nevertheless, treatment that blocks PGE2 synthesis consequent on local induction of COX-2 has been the basis of most nonsteroidal anti-inflammatory mild analgesics. Several other approaches targeting specific sensitizers are under development, such as anti-NGF antibodies, but these are likely always to have a ceiling effect, reducing but not eliminating pain, since other sensitizers will still be available. More

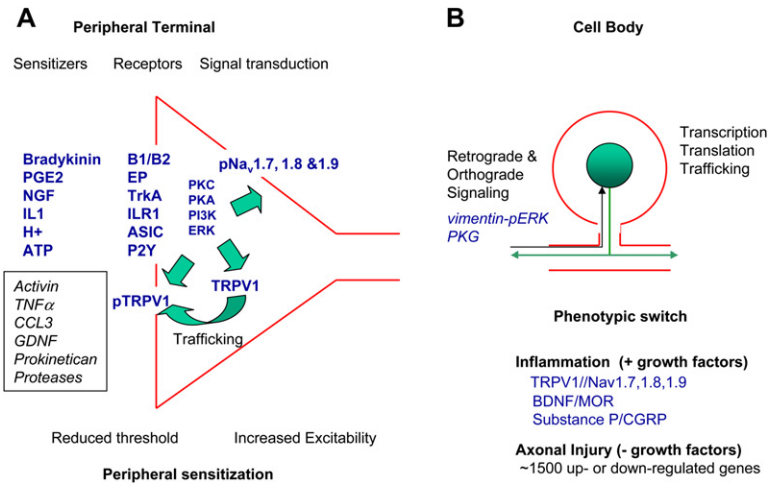


**Figure 3. Nociceptor Plasticity**

(A) Peripheral sensitization involves a lowering of the threshold of the nociceptor in response to inflammatory sensitizers that activate, via diverse signal transduction pathways in the peripheral terminal, alterations in the trafficking and properties of transducer and sodium channels, largely as a result of phosphorylation.

(B) Phenotypic switches occur in nociceptors in response to inflammation and axonal injury by virtue of exposure to retrogradely transported signal molecules or absence of target-derived signals.

effective analgesics for local inflammatory pain may well be ones that can act downstream of the convergence of multiple different sensitizers on the effectors of the increased excitability.

Nociceptor sensitizers produce their effects on binding to their receptors on the nociceptor membrane by activation of multiple intracellular signal transduction pathways in the peripheral terminal that include PKC (Hucho et al., 2005), PKA (Varga et al., 2006), PI3K (Malik-Hall et al., 2005), the MAP kinases ERK and p38 (Jin and Gereau, 2006; Mizushima et al., 2007), as well as JNK (Doya et al., 2005). Downstream of these cascades, the effectors of peripheral sensitization are mainly phosphorylation of TRP and voltage-gated sodium channels, altering threshold and kinetics (Figure 3). Endogenous PIP2 in the nociceptor membrane inhibits TRPV1, leading to the suggestion that activation of PLC$\gamma$ by TrkA by metabolizing PIP2 relieves TRPV1 from inhibition to produce sensitization (Chuang et al., 2001). Alternatively TrkA may activate a signaling pathway involving PI3 kinase early and Src kinase as the downstream element that phosphorylates TRPV1 (Zhang et al., 2005b). Insertion of TRPV1 into the membrane from intracellular stores may represent another mode, apart from changing threshold, of increasing transduction sensitivity (Zhang et al., 2005b). From multiple knockout studies, it appears that both TRPV1 and TRPA1 have a considerable contribution to peripheral sensitization (Bautista et al., 2006; Caterina et al., 2000; Davis et al., 2000; Kwan et al., 2006), as do the VGSCs, Nav1.8, Nav1.7, and Nav1.9 (Amaya et al., 2006; Kerr et al., 2001; Nassar et al., 2004, 2005, 2006). TRP receptor antagonists and sodium channel selective blockers may be a useful approach, therefore, for reducing peripheral sensitization and thereby inflammatory pain (Jarvis et al., 2007).

Mutations in the intracellular linker parts of the Na$_v$1.7 voltage-gated sodium channel and in its S4 segment increase the excitability of nociceptors and produce inherited erythermalgia, a condition where warm stimuli provoke episodic burning pain (Choi et al., 2006). This channelopathy indicates the crucial contribution of sodium channels not only to action potential conduction, but also to the threshold and responsiveness of nociceptors. Some patients with postherpetic neuralgia develop burning pain with a reduced thermal threshold that has been considered to be the result of an ''irritable nociceptor'' (Fields et al., 1998). Whether this reflects a herpes zoster-induced acquired alteration in Na$_v$1.7 distribution due to altered trafficking or function, resulting from post-translational changes is an intriguing possibility. Recently, individuals homozygous for nonsense mutations in Na$_v$1.7 that lead to a complete loss of function when expressed heterologously have been found to have an inability to feel pain, without any loss of innocuous sensitivity (Cox et al., 2006; Goldberg et al., 2007). Given expression in nociceptor neurons of several tetrodotoxin-sensitive (Na$_v$1.6, Na$_v$1.7) and resistant (Na$_v$1.8 and Na$_v$1.9) sodium channels, the complete loss of pain sensitivity is remarkable. Global loss of Na$_v$1.7 is lethal in mice, and a spatial knockout restricted to nociceptors produces no major change in nociception (Nassar et al., 2004), indicating that null mutations in mice and men produce very different phenotypes! It is possible in humans that Na$_v$1.7 plays a key role in producing the generator potential that amplifies transducer-mediated depolarizations to initiate action potentials, in the action potential itself, or in preventing branch point blockade.

### *Phenotypic Switches*

The cell bodies of nociceptor neurons in the dorsal root ganglion need to keep themselves informed about the status of their peripheral terminals. Peripheral inflammation, in addition to driving peripheral sensitization, produces retrograde signals in nociceptor neurons that increase in the soma the transcription of neuropeptides, BDNF, and sodium channels, as well as increasing the translation of TRP channels to augment both central transmission and peripheral sensitization (Ji et al., 2002; Mannion et al., 1999; Neumann et al., 1996; Figure 3). Furthermore, inflammation via NGF increases expression of mu-opiod

receptors enhancing sensitivity to opioids (Puehler et al., 2004). Transport of NGF-activated Trk receptors and perhaps those of other growth factors from the periphery appear to be a major means whereby nociceptor cell bodies change in response to peripheral inflammation (Delcroix et al., 2003). In general terms, inflammation tends to cause an expansion or increase in the expression of specific nociceptive ion channels/receptors. The increase in expression, while functionally significant, is quite limited in scope, and the underlying transcriptional mechanisms are unclear and may involve axonal transport of transcripts and local translation.

If the peripheral axon of nociceptors is severed, disrupting contact of the cell body with its terminal and in this way with the peripheral target, profound changes in transcription occur. Negative signals such as a loss of target-derived growth factors and positive signals like retrograde protein kinase G (Sung et al., 2006) or a vimentin-dependent translocation of activated ERK (Perlson et al., 2006) drive activation of multiple signal-transduction pathways in the cell body that alter transcription in >1000 genes (Costigan et al., 2002;Xiao et al., 2002). Some of these represent attempts by the neuron to survive the major physical and metabolic insult inherent in the injury, others are attempts for the axon to regrow, but many axotomy-induced transcriptional changes are maladaptive and produce alterations in function that can drive neuropathic pain. Injured neurons lose some normal nociceptor features with, for example, a downregulation of TRP and sodium channels, while at the same time gaining a new molecular identity. One example of this is the upregulation in DRG neurons of enzymes involved in the synthesis and recycling of tetrahydrobiopterin (BH4) after peripheral axonal injury (Tegeder et al., 2006). BH4 is an essential cofactor for aromatic amine hydroxylases and nitric oxide synthases, and its increase drives NO production in DRG neurons, which in turn increases calcium influx in the neurons. Inhibition of BH4 synthesis produces analgesia, while BH4 produces pain-like behavior. Intriguingly, a human variant of the rate-limiting enzyme in the BH4 pathway, GTP cyclohydrolase, which produces less BH4 in response to stressors, is associated with less chronic pain in patients after surgery (Tegeder et al., 2006). Although injured DRG neurons show a major phenotypic shift, so do their spared noninjured neighbors, and indeed it is these cells that may drive much spontaneous pain.

### Spontaneous Activity and Pain

Peripheral sensitization produces a state of heightened sensitivity to peripheral stimuli that are either normally innocuous or are noxious but now produce exaggerated or prolonged effects both represent stimulus-dependent pains. A common aspect of clinical pain conditions, however, is pain in the absence of any readily identifiable stimulus, stimulus-independent pain. Spontaneous pain may arise from signal molecules continuously released after tissue damage or inflammation that act on nociceptor peripheral terminals to either drive a depolarization sufficient to initiate action potentials or produce a reduction in threshold such that normal ambient temperatures or the pulsation of blood vessels now activate what had been high-threshold thermo- and mechanonociceptors. After peripheral inflammation and peripheral nerve lesions, spontaneous foot lifting, a surrogate of spontaneous pain, correlates with spontaneous activity in C fibers but not with allodynia, implying that spontaneous pain is driven from the periphery and differs mechanistically from stimulus-evoked pain (Djouhri et al., 2006).

The nociceptor is designed to initiate activity only at its peripheral terminal. Any action potentials that originate from the axon or cell body represent pathological ectopic firing and produce sensory inflow in the absence of sensory stimuli or ongoing peripheral inflammation. Ectopic activity can be generated by pacemaker-like spontaneous depolarizations resulting from an abnormal hyperexcitability of the membrane that occurs after peripheral nerve injury due to alterations in ion channel expression and trafficking (Liu et al., 2002). Although injured DRG neurons display ectopic activity, increasingly it is recognized that neighboring intact fibers are, after partial peripheral nerve injury, an even larger source of such spontaneous activity (Djouhri et al., 2006). One explanation for this is that the intact fibers are exposed to signal molecules produced by deafferented Schwann cells, such as TNF$\alpha$. Several channels may contribute to ectopic action potential initiation including a hyperpolarization-activated, cation-nonselective, cyclic nucleotide-modulated channel (Lee et al., 2005), a Ca-activated chloride current (Hilaire et al., 2005), as well as potassium and sodium channels. Sodium channels seem obvious candidates both because of their intrinsic properties and because sodium channel blockers produce analgesia in some patients with neuropathic pain. However, while antisense knockdown has implicated Nav1.8 in rodent models of neuropathic pain (Gold et al., 2003), surprisingly knockouts of Nav1.8, 1.9, 1.7 and 1.3 have no change in the neuropathic pain phenotype (Amaya et al., 2006; Nassar et al., 2004, 2005, 2006; Priest et al., 2005). Selective sodium channel blockers will be required to resolve whether these channels are the major drivers of spontaneous activity after nerve injury and represent the best target for treating the severe lancinating or shock-like pain these patients experience (Jarvis et al., 2007).

### Presynaptic Terminal

The central terminals of nociceptors are located in the superficial dorsal horn of the spinal cord for somatic neurons and in the spinal nucleus of the trigeminal for those innervating the face (Figure 1). These terminals drive synaptic input to second-order neurons, transferring information carried by action potentials about the intensity and duration of peripheral noxious stimuli. The rostrocadual and mediolateral topography of the central terminal reflects the spatial location of the peripheral terminal, while their dorsoventral location reflects the identity of the nociceptor as a result of the coordination between specification and axonal guidance during development. Unlike low-threshold primary sensory neurons that use glutamate as their

sole transmitter, nociceptors variously use glutamate, neuropeptides, and proteins like BDNF as transmitters/synaptic modulators and in consequence evoke fast and slow excitatory postsynaptic potentials that show considerable spatial and temporal summation.

Transmitter release is regulated by multiple factors that control or modulate calcium influx in response to the invasion of the terminal by action potentials to activate the vesicle-release machinery. The major voltage-gated calcium channel in nociceptors is Cav2.2, and alternative splicing of the mRNA of the channel results in a structurally and functionally distinct N-type calcium channel splice isoform in subsets of nociceptors (Castiglioni et al., 2006). The alternative spliced variant of Cav2.2 uniquely is sensitive to a G protein-coupled receptor voltage-dependent and voltage-independent inhibition (Raingo et al., 2007). The N-type channel blocker ziconatide, a conotoxin, produces analgesia, but its side-effect profile necessitates intrathecal delivery; use-dependent Cav2.2 blockers may have a greater therapeutic index, as may those that have greater selectivity for the nociceptor-specific splice isoform. In addition to the pore-forming $\alpha$ subunit, calcium channels have accessory subunits including the $\alpha 2\delta$ subunit, which is the binding site for gabapentin and pregabalin, drugs with efficacy in neuropathic pain (Field et al., 2006). It is not clear though, exactly how these drugs act to produce analgesia; they are not calcium-channel blockers, but there are suggestions that they may under some circumstances reduce transmitter release. The $\alpha 2\delta$ subunit is upregulated in nociceptor terminals after peripheral axonal injury and represents an example of a target induced in particular disease conditions. Part of pain therapy needs to be, therefore, the matching of drugs for those situations that their targets are present and contributing to the pain.

Transmitter-modulated reductions in transmitter release from nociceptors is a prominent control mechanism in nociception, increasing or decreasing access of nociceptor input to the CNS, and includes endogenous opioids acting on mu- and delta-opiate receptors, GABA on $GABA_B$ receptors, and endogenous cannabinoids on CB1 receptors, a regular den of receptors for drugs of abuse (Figure 1). The level of these receptors changes dynamically with in the case of the mu-opiate receptor, an increase after inflammation (Puehler et al., 2004) and a decrease after axonal injury (Kohno et al., 2005). The delta-opiate receptor (DOR) has another form of regulation. Very little of the receptor is normally inserted in the nociceptor terminal membrane; most is located in the membrane of peptide-containing, large dense core vesicles. Following nociceptor activation, fusion of the vesicles to the terminal membrane during synaptic release due to stimulus-triggered exocytosis introduces the receptor to the terminal in a manner that depends on an interaction between DOR and preprotachykinin (Guan et al., 2005). DOR-mediated analgesia requires then prior activation of the nociceptor—pain before analgesia. Surprisingly, deletion of CB1 only in nociceptors has revealed that cannabinoids exert their analgesic action primarily on CB1 receptors expressed on the peripheral terminals of nociceptors (Agarwal et al., 2007), which may enable development of peripherally acting cannabinoid analgesics without central side effects.

Balanced against the braking influences on the central terminal are those that act to increase transmitter release, including PGE2 (Vasko, 1995) and bradykinin (Wang et al., 2005). In this respect, the central terminal of the nociceptor resembles its peripheral terminal. PGE2 is produced following induction of COX-2 in dorsal horn neurons in response to peripheral inflammation (Samad et al., 2001), while bradykinin is released in the spinal cord in within minutes of nociceptor input (Wang et al., 2005), and both act via their G protein-coupled receptors to increase transmitter release. Bradykinin increases calcium influx via the TRPA1 channel (Bautista et al., 2006), which may act therefore as a regulator of transmitter release (Kosugi et al., 2007). It turns out that a major component of the analgesic action of TRPV1 antagonists is surprisingly on the central and not on the peripheral terminal of nociceptors (Cui et al., 2006). This raises the question as to what ligands or stimuli drive the receptor here. It is obviously not heat, and fatty acid amides are likely endogenous ligand candidates. In addition to regulating the flow of information by transmitter release, nociceptor neurons also produce chemokine signals after axonal injury that activate microglia in the dorsal horn to contribute to alterations in sensory processing in the spinal cord (Verge et al., 2004).

## Conclusion

The nociceptor neuron is highly specialized to respond only to noxious stimuli and communicate this information accurately to the CNS. It is however, not a static detector; the functional and chemical plasticity of the receptor ensures that its threshold and responsiveness, as well as the efficacy of its synaptic contacts, are regulated to reflect changes produced by activity, inflammation, and axonal injury. Heightened pain sensitivity can contribute to healing by helping avoid contact with the damaged body part until repair has occurred. Persistent alterations in nociceptors that drive pain in the absence of noxious stimuli or inflamed or damaged tissue represent, by contrast, a pathological change in the nociceptive system, and successful treatment of such pain requires restoring the nociceptor to its normal function—silent except in the presence of imminent danger. This requires understanding its normal function and how it changes, particularly in response to axonal injury, something that we have made considerable progress in achieving since Sherrington first recognized the existence of these sensory neurons a century ago.

## REFERENCES

Agarwal, N., Pacher, P., Tegeder, I., Amaya, F., Constantin, C.E., Brenner, G.J., Rubino, T., Michalski, C.W., Marsicano, G., Monory, K., et al.

(2007). Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. Nat.Neurosci. *10*, 870–879.

Alloui, A., Zimmermann, K., Mamet, J., Duprat, F., Noel, J., Chemin, J., Guy, N., Blondeau, N., Voilley, N., Rubat-Coudert, C., et al. (2006). TREK-1, a K+ channel involved in polymodal pain perception. EMBO J. *25*, 2368–2376.

Amaya, F., Wang, H., Costigan, M., Allchorne, A., Hatcher, J.P., Egerton, J., Stean, T., Morisett, V., Grose, D., Gunthorpe, M.J., et al. (2006). The voltage-gated sodium channel $Na_{v1.9}$ is an effector of peripheral inflammatory pain hypersensitivity. J. Neurosci. *26*, 12852–12860.

Anderson, D.J. (2000). Genes, lineages and the neural crest: a speculative review. Philos. Trans. R. Soc. Lond. B Biol. Sci. *355*, 953–964.

Bandell, M., Story, G.M., Hwang, S.W., Viswanath, V., Eid, S.R., Petrus, M.J., Earley, T.J., and Patapoutian, A. (2004). Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron *41*, 849–857.

Bautista, D.M., Jordt, S.E., Nikai, T., Tsuruda, P.R., Read, A.J., Poblete, J., Yamoah, E.N., Basbaum, A.I., and Julius, D. (2006). TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell *124*, 1269–1282.

Bautista, D.M., Siemens, J., Glazer, J.M., Tsuruda, P.R., Basbaum, A.I., Stucky, C.L., Jordt, S.E., and Julius, D. (2007). The menthol receptor TRPM8 is the principal detector of environmental cold. Nature *448*, 204–208.

Bennett, D.L.H., Averill, S., Priestley, J.V., and McMahon, S.B. (1996). Postnatal changes in the expression of trkA high affinity NGF receptor in primary sensory neurones. Eur. J. Neurosci. *8*, 2204–2208.

Bessou, P., and Perl, E.R. (1969). Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli. J. Neurophysiol. *32*, 1025–1043.

Brauchi, S., Orta, G., Salazar, M., Rosenmann, E., and Latorre, R. (2006). A hot-sensing cold receptor: C-terminal domain determines thermosensation in transient receptor potential channels. J. Neurosci. *26*, 4835–4840.

Braz, J.M., Nassar, M.A., Wood, J.N., and Basbaum, A.I. (2005). Parallel "pain" pathways arise from subpopulations of primary afferent nociceptor. Neuron *47*, 787–793.

Castiglioni, A.J., Raingo, J., and Lipscombe, D. (2006). Alternative splicing in the C-terminus of CaV2.2 controls expression and gating of N-type calcium channels. J. Physiol. *576*, 119–134.

Caterina, M.J., Rosen, T.A., Tominaga, M., Brake, A.J., and Julius, D. (1999). A capsaicin-receptor homologue with a high threshold for noxious heat. Nature *398*, 436–441.

Caterina, M.J., Leffler, A., Malmberg, A.B., Martin, W.J., Trafton, J., Petersen-Zeitz, K.R., Koltzenburg, M., Basbaum, A.I., and Julius, D. (2000). Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science *288*, 306–313.

Chen, C.L., Broom, D.C., Liu, Y., de Nooij, J.C., Li, Z., Cen, C., Samad, O.A., Jessell, T.M., Woolf, C.J., and Ma, Q. (2006). Runx1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain. Neuron *49*, 365–377.

Choi, J.S., Dib-Hajj, S.D., and Waxman, S.G. (2006). Inherited erythermalgia. Limb pain from an S4 charge-neutral Na channelopathy. Neurology *67*, 1563–1567.

Chuang, H.H., Prescott, E.D., Kong, H., Shields, S., Jordt, S.E., Basbaum, A.I., Chao, M.V., and Julius, D. (2001). Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature *411*, 957–962.

Colburn, R.W., Lubin, M.L., Stone, D.J., Jr., Wang, Y., Lawrence, D., D'Andrea, M.R., Brandt, M.R., Liu, Y., Flores, C.M., and Qin, N. (2007). Attenuated cold sensitivity in TRPM8 null mice. Neuron *54*, 379–386.

Costigan, M., Befort, K., Karchewski, L., Griffin, R.S., D'Urso, D., Allchorne, A., Sitarski, J., Mannion, J.W., Pratt, R.E., and Woolf, C.J. (2002). Replicate high-density rat genome oligonucleotide microarrays reveal hundreds of regulated genes in the dorsal root ganglion after peripheral nerve injury. BMC Neurosci. *3*, 16.

Cox, J.J., Reimann, F., Nicholas, A.K., Thornton, G., Roberts, E., Springell, K., Karbani, G., Jafri, H., Mannan, J., Raashid, Y., et al. (2006). An SCN9A channelopathy causes congenital inability to experience pain. Nature *444*, 894–898.

Cui, M., Honore, P., Zhong, C., Gauvin, D., Mikusa, J., Hernandez, G., Chandran, P., Gomtsyan, A., Brown, B., Bayburt, E.K., et al. (2006). TRPV1 receptors in the CNS play a key role in broad-spectrum analgesia of TRPV1 antagonists. J. Neurosci. *26*, 9385–9393.

Dai, Y., Wang, S., Tominaga, M., Yamamoto, S., Fukuoka, T., Higashi, T., Kobayashi, K., Obata, K., Yamanaka, H., and Noguchi, K. (2007). Sensitization of TRPA1 by PAR2 contributes to the sensation of inflammatory pain. J. Clin. Invest. *117*, 1979–1987.

Davis, J.B., Gray, J., Gunthorpe, M.J., Hatcher, J.P., Davey, P.T., Overend, P., Harries, M.H., Latcham, J., Clapham, C., Atkinson, K., et al. (2000). Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature *405*, 183–187.

Delcroix, J.D., Valletta, J.S., Wu, C., Hunt, S.J., Kowal, A.S., and Mobley, W.C. (2003). NGF signaling in sensory neurons: Evidence that early endosomes carry NGF retrograde signals. Neuron *39*, 69–84.

Dhaka, A., Viswanath, V., and Patapoutian, A. (2006). Trp ion channels and temperature sensation. Annu. Rev. Neurosci. *29*, 135–161.

Dhaka, A., Murray, A.N., Mathur, J., Earley, T.J., Petrus, M.J., and Patapoutian, A. (2007). TRPM8 is required for cold sensation in mice. Neuron *54*, 371–378.

Djouhri, L., Koutsikou, S., Fang, X., McMullan, S., and Lawson, S.N. (2006). Spontaneous pain, both neuropathic and inflammatory, is related to frequency of spontaneous firing in intact C fiber nociceptors. J. Neurosci. *26*, 1281–1292.

Dong, X., Han, S., Zylka, M.J., Simon, M.I., and Anderson, D.J. (2001). A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. Cell *106*, 619–632.

Doya, H., Ohtori, S., Fujitani, M., Saito, T., Hata, K., Ino, H., Takahashi, K., Moriya, H., and Yamashita, T. (2005). c-Jun N-terminal kinase activation in dorsal root ganglion contributes to pain hypersensitivity. Biochem. Biophys. Res. Commun. *335*, 132–138.

Field, M.J., Cox, P.J., Stott, E., Melrose, H., Offord, J., Su, T.Z., Bramwell, S., Corradini, L., England, S., Winks, J., Kinloch, R.A., et al. (2006). Identification of the {alpha}2-{delta}-1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of pregabalin. Proc. Natl. Acad. Sci. USA *103*, 17537–17542.

Fields, H.L., Rowbotham, M., and Baron, R. (1998). Postherpetic neuralgia: irritable nociceptors and deafferentation. Neurobiol. Dis. *5*, 209–227.

Fitzgerald, M. (2005). The development of nociceptive circuits. Nat. Rev. Neurosci. *6*, 507–520.

Gold, M.S., Weinreich, D., Kim, C.S., Wang, R., Treanor, J., Porreca, F., and Lai, J. (2003). Redistribution of Na(V)1.8 in uninjured axons enables neuropathic pain. J. Neurosci. *23*, 158–166.

Goldberg, Y.P., MacFarlane, J., MacDonald, M.L., Thompson, J., Dube, M.P., Mattice, M., Fraser, R., Young, C., Hossain, S., Pape, T., et al. (2007). Loss-of-function mutations in the Nav1.7 gene underlie congenital indifference to pain in multiple human populations. Clin. Genet. *71*, 311–319.

Grant, A.D., Cottrell, G.S., Amadesi, S., Trevisani, M., Nicoletti, P., Materazzi, S., Altier, C., Cenac, N., Zamponi, G.W., Bautista-Cruz, F., et al. (2007). Protease-activated receptor 2 sensitizes the transient receptor potential vanilloid 4 ion channel to cause mechanical hyperalgesia in mice. J. Physiol. *578*, 715–733.

Guan, J.S., Xu, Z.Z., Gao, H., He, S.Q., Ma, G.Q., Sun, T., Wang, L.H., Zhang, Z.N., Lena, I., Kitchen, I., et al. (2005). Interaction with vesicle luminal protachykinin regulates surface expression of delta-opioid receptors and opioid analgesia. Cell *122*, 619–631.

Hjerling-Leffler, J., Alqatari, M., Ernfors, P., and Koltzenburg, M. (2007). Emergence of functional sensory subtypes as defined by transient receptor potential channel expression. J. Neurosci. *27*, 2435–2443.

Hilaire, C., Campo, B., Andre, S., Valmier, J., and Scamps, F. (2005). K(+) current regulates calcium-activated chloride current-induced after depolarization in axotomized sensory neurons. Eur. J. Neurosci. *22*, 1073–1080.

Hinman, A., Chuang, H.H., Bautista, D.M., and Julius, D. (2006). TRP channel activation by reversible covalent modification. Proc. Natl. Acad. Sci. USA *103*, 19564–19568.

Hu, J., Milenkovic, N., and Lewin, G.R. (2006). The high threshold mechanotransducer: a status report. Pain *120*, 3–7.

Hucho, T.B., Dina, O.A., and Levine, J.D. (2005). Epac mediates a cAMP-to-PKC signaling in inflammatory pain: an isolectin B4(+) neuron-specific mechanism. J. Neurosci. *25*, 6119–6126.

Ibanez, C.F., and Ernfors, P. (2007). Hierarchical control of sensory neuron development by neurotrophic factors. Neuron *54*, 673–675.

Jarvis, M.F., Honore, P., Shieh, C.C., Chapman, M., Joshi, S., Zhang, X.F., Kort, M., Carroll, W., Marron, B., Atkinson, R., Thomas, J., et al. (2007). A-803467, a potent and selective Nav1.8 sodium channel blocker, attenuates neuropathic and inflammatory pain in the rat. Proc. Natl. Acad. Sci. USA *104*, 8520–8525.

Ji, R.R., Samad, T.A., Jin, S.X., Schmoll, R., and Woolf, C.J. (2002). p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. Neuron *36*, 57–68.

Jin, X., and Gereau, R.W. (2006). Acute p38-mediated modulation of tetrodotoxin-resistant sodium channels in mouse sensory neurons by tumor necrosis factor-alpha. J. Neurosci. *26*, 246–255.

Jordt, S.E., Bautista, D.M., Chuang, H.H., McKemy, D.D., Zygmunt, P.M., Hogestatt, E.D., Meng, I.D., and Julius, D. (2004). Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature *427*, 260–265.

Kerr, B.J., Souslova, V., McMahon, S.B., and Wood, J.N. (2001). A role for the TTX-resistant sodium channel Nav 1.8 in NGF-induced hyperalgesia, but not neuropathic pain. Neuroreport *12*, 3077–3080.

Kindt, K.S., Viswanath, V., Macpherson, L., Quast, K., Hu, H., Patapoutian, A., and Schafer, W.R. (2007). Caenorhabditis elegans TRPA-1 functions in mechanosensation. Nat. Neurosci. *10*, 568–577.

Kohno, T., Ji, R.R., Ito, N., Allchorne, A.J., Befort, K., Karchewski, L.A., and Woolf, C.J. (2005). Peripheral axonal injury results in reduced mu opioid receptor pre- and post-synaptic action in the spinal cord. Pain *117*, 77–87.

Kosugi, M., Nakatsuka, T., Fujita, T., Kuroda, Y., and Kumamoto, E. (2007). Activation of TRPA1 channel facilitates excitatory synaptic transmission in substantia gelatinosa neurons of the adult rat spinal cord. J. Neurosci. *27*, 4443–4451.

Kramer, I., Sigrist, M., de Nooij, J.C., Taniuchi, I., Jessell, T.M., and Arber, S. (2006). A role for Runx transcription factor signaling in dorsal root ganglion sensory neuron diversification. Neuron *49*, 379–393.

Kwan, K.Y., Allchorne, A.J., Vollrath, M.A., Christensen, A.P., Zhang, D.S., Woolf, C.J., and Corey, D.P. (2006). TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron *50*, 277–289.

Lawson, S.N., and Biscoe, T.J. (1979). Development of mouse dorsal root ganglia: an autoradiographic and quantitative study. J. Neurocytol. *8*, 265–274.

Lee, D.H., Chang, L., Sorkin, L.S., and Chaplan, S.R. (2005). Hyperpolarization-activated, cation-nonselective, cyclic nucleotide-modulated channel blockade alleviates mechanical allodynia and suppresses ectopic discharge in spinal nerve ligated rats. J. Pain *6*, 417–424.

Levanon, D., Bettoun, D., Harris-Cerruti, C., Woolf, E., Negreanu, V., Eilam, R., Bernstein, Y., Goldenberg, D., Xiao, C., Fliegauf, M., et al. (2002). The Runx3 transcription factor regulates development and survival of TrkC dorsal root ganglia neurons. EMBO J. *21*, 3454–3463.

Lei, L., Zhou, J., Lin, L., and Parada, L.F. (2006). Brn3a and Klf7 cooperate to control TrkA expression in sensory neurons. Dev. Biol. *300*, 758–769.

Lewin, G.R. (1996). Neurotrophins and the specification of neuronal phenotype. Philos. Trans. R. Soc. Lond. B Biol. Sci. *351*, 405–411.

Liapi, A., and Wood, J.N. (2005). Extensive co-localization and heteromultimer formation of the vanilloid receptor-like protein TRPV2 and the capsaicin receptor TRPV1 in the adult rat cerebral cortex. Eur. J. Neurosci. *22*, 825–834.

Liu, C.N., Devor, M., Waxman, S.G., and Kocsis, J.D. (2002). Subthreshold oscillations induced by spinal nerve injury in dissociated muscle and cutaneous afferents of mouse DRG. J. Neurophysiol. *87*, 2009–2017.

Luo, W., Wickramasinghe, S.R., Savitt, J.M., Griffin, J.W., Dawson, T.M., and Ginty, D.D. (2007). A hierarchical NGF signaling cascade controls Ret-dependent and Ret-independent events during development of nonpeptidergic DRG neurons. Neuron *54*, 739–754.

Ma, Q., Chen, Z., del Barco Barrentes, I., de la Pompa, J.L., and Anderson, D.J. (1998). neurogenin1 is essential for the determination of neuronal precursors for proximal cranial sensory ganglia. Neuron *20*, 469–482.

Ma, Q., Fode, C., Guillemot, F., and Anderson, D.J. (1999). Neurogenin1 and neurogenin2 control two distinct waves of neurogenesis in developing dorsal root ganglia. Genes Dev. *13*, 1717–1728.

Macpherson, L.J., Geierstanger, B.H., Viswanath, V., Bandell, M., Eid, S.R., Hwang, S., and Patapoutian, A. (2005). The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. Curr. Biol. *15*, 929–934.

Macpherson, L.J., Dubin, A.E., Evans, M.J., Marr, F., Schultz, P.G., Cravatt, B.F., and Patapoutian, A. (2007). Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. Nature *445*, 541–545.

Malik-Hall, M., Dina, O.A., and Levine, J.D. (2005). Primary afferent nociceptor mechanisms mediating NGF-induced mechanical hyperalgesia. Eur. J. Neurosci. *21*, 3387–3394.

Malin, S.A., Molliver, D.C., Koerber, H.R., Cornuet, P., Frye, R., Albers, K.M., and Davis, B.M. (2006). Glial cell line-derived neurotrophic factor family members sensitize nociceptors in vitro and produce thermal hyperalgesia in vivo. J. Neurosci. *26*, 8588–8599.

Mannion, R.J., Costigan, M., Decosterd, I., Amaya, F., Ma, Q.P., Holstege, J.C., Ji, R.R., Acheson, A., Lindsay, R.M., Wilkinson, G.A., and Woolf, C.J. (1999). Neurotrophins: peripherally and centrally acting modulators of tactile stimulus-induced inflammatory pain hypersensitivity. Proc. Natl. Acad. Sci. USA *96*, 9385–9390.

Marmigere, F., and Ernfors, P. (2007). Specification and connectivity of neuronal subtypes in the sensory lineage. Nat. Rev. Neurosci. *8*, 114–127.

Marmigere, F., Montelius, A., Wegner, M., Groner, Y., Reichardt, L.F., and Ernfors, P. (2006). The Runx1/AML1 transcription factor selectively regulates development and survival of TrkA nociceptive sensory neurons. Nat. Neurosci. *9*, 180–187.

McKemy, D.D., Neuhausser, W.M., and Julius, D. (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature *416*, 52–58.

Melzack, R., and Wall, P.D. (1965). Pain mechanisms: a new theory. Science *150*, 971–979.

Mizushima, T., Obata, K., Katsura, H., Sakurai, J., Kobayashi, K., Yamanaka, H., Dai, Y., Fukuoka, T., Mashimo, T., and Noguchi, K. (2007). Intensity-dependent activation of extracellular signal-regulated protein kinase 5 in sensory neurons contributes to pain hypersensitivity. J. Pharmacol. Exp. Ther. *321*, 28–34.

Molliver, D.C., Wright, D.E., Leitner, M.L., Parsadanian, A.S., Doster, K., Wen, D., Yan, Q., and Snider, W.D. (1997). IB4-binding DRG neurons switch from NGF to GDNF dependence in early postnatal life. Neuron *19*, 849–861.

Nassar, M.A., Stirling, L.C., Forlani, G., Baker, M.D., Matthews, E.A., Dickenson, A.H., and Wood, J.N. (2004). Nociceptor-specific gene deletion reveals a major role for Nav1.7 (PN1) in acute and inflammatory pain. Proc. Natl. Acad. Sci. USA *101*, 12706–12711.

Nassar, M.A., Levato, A., Stirling, L.C., and Wood, J.N. (2005). Neuropathic pain develops normally in mice lacking both Nav1.7 and Nav1.8. Mol. Pain *1*, 24.

Nassar, M.A., Baker, M.D., Levato, A., Ingram, R., Mallucci, G., McMahon, S.B., and Wood, J.N. (2006). Nerve injury induces robust allodynia and ectopic discharges in Nav1.3 null mutant mice. Mol. Pain *2*, 33.

Neumann, S., Doubell, T.P., Leslie, T.A., and Woolf, C.J. (1996). Inflammatory pain hypersensitivity mediated by phenotypic switch in myelinated primary sensory neurones. Nature *384*, 360–364.

Patel, T.D., Jackman, A., Rice, F.L., Kucera, J., and Snider, W.D. (2000). Development of sensory neurons in the absence of NGF/TrkA signaling in vivo. Neuron *25*, 345–357.

Perlson, E., Michaelevski, I., Kowalsman, N., Ben Yaakov, K., Shaked, M., Seger, R., Eisenstein, M., and Fainzilber, M. (2006). Vimentin binding to phosphorylated Erk sterically hinders enzymatic dephosphorylation of the kinase. J. Mol. Biol. *364*, 938–944.

Priest, B.T., Murphy, B.A., Lindia, J.A., Diaz, C., Abbadie, C., Ritter, A.M., Liberator, P., Iyer, L.M., Kash, S.F., Kohler, M.G., Kaczorowski, G.J., et al. (2005). Contribution of the tetrodotoxin-resistant voltage-gated sodium channel NaV1.9 to sensory transmission and nociceptive behavior. Proc. Natl. Acad. Sci. USA *102*, 9382–9387.

Puehler, W., Zollner, C., Brack, A., Shaqura, M.A., Krause, H., Schafer, M., and Stein, C. (2004). Rapid upregulation of mu opioid receptor mRNA in dorsal root ganglia in response to peripheral inflammation depends on neuronal conduction. Neuroscience *129*, 473–479.

Raingo, J., Castiglioni, A.J., and Lipscombe, D. (2007). Alternative splicing controls G protein-dependent inhibition of N-type calcium channels in nociceptors. Nat. Neurosci. *10*, 285–292.

Ramsey, I.S., Delling, M., and Clapham, D.E. (2006). An introduction to TRP channels. Annu. Rev. Physiol. *68*, 619–647.

Ritter, A.M., Lewin, G.R., Kremer, N.E., and Mendell, L.M. (1991). Requirement for nerve growth factor in the development of myelinated nociceptors in vivo. Nature *350*, 500–502.

Saghatelian, A., McKinney, M.K., Bandell, M., Patapoutian, A., and Cravatt, B.F. (2006). A FAAH-regulated class of N-acyl taurines that activates TRP ion channels. Biochemistry *45*, 9007–9015.

Samad, T.A., Moore, K.A., Sapirstein, A., Billet, S., Allchorne, A., Poole, S., Bonventre, J.V., and Woolf, C.J. (2001). Interleukin-1β-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature *410*, 471–475.

Sherrington, C.S. (1903). Qualitative differences of spinal reflex corresponding with qualitative difference of cutaneous stimulus. J. Physiol. *30*, 39–46.

Sherrington, C.S. (1906). The Integrative Action of the Nervous System (New York: Scribner).

Siemens, J., Zhou, S., Piskorowski, R., Nikai, T., Lumpkin, E.A., Basbaum, A.I., King, D., and Julius, D. (2006). Spider toxins activate the capsaicin receptor to produce inflammatory pain. Nature *444*, 208–212.

Snider, W.D., and McMahon, S.B. (1998). Tackling pain at the source: new ideas about nociceptors. Neuron *20*, 629–632.

Story, G.M., Peier, A.M., Reeve, A.J., Eid, S.R., Mosbacher, J., Hricik, T.R., Earley, T.J., Hergarden, A.C., Andersson, D.A., Hwang, S.W., et al. (2003). ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell *112*, 819–829.

Sung, Y.J., Chiu, D.T., and Ambron, R.T. (2006). Activation and retrograde transport of protein kinase G in rat nociceptive neurons after nerve injury and inflammation. Neuroscience *141*, 697–709.

Tegeder, I., Costigan, M., Griffin, R.S., Abele, A., Belfer, I., Schmidt, H., Ehnert, C., Nejim, J., Marian, C., Scholz, J., et al. (2006). GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence. Nat. Med. *12*, 1269–1277.

Theriault, F.M., Nuthall, H.N., Dong, Z., Lo, R., Barnabe-Heider, F., Miller, F.D., and Stifani, S. (2005). Role for Runx1 in the proliferation and neuronal differentiation of selected progenitor cells in the mammalian nervous system. J. Neurosci. *25*, 2050–2061.

van der Stelt, M., Trevisani, M., Vellani, V., De Petrocellis, L., Schiano, M.A., Campi, B., McNaughton, P., Geppetti, P., and Di Marzo, V. (2005). Anandamide acts as an intracellular messenger amplifying Ca2+ influx via TRPV1 channels. EMBO J. *24*, 3026–3037.

Varga, A., Bolcskei, K., Szoke, E., Almasi, R., Czeh, G., Szolcsanyi, J., and Petho, G. (2006). Relative roles of protein kinase A and protein kinase C in modulation of transient receptor potential vanilloid type 1 receptor responsiveness in rat sensory neurons in vitro and peripheral nociceptors in vivo. Neuroscience *140*, 645–657.

Vasko, M.R. (1995). Prostaglandin-induced neuropeptide release from spinal cord. Prog. Brain Res. *104*, 367–380.

Vellani, V., Colucci, M., Lattanzi, R., Giannini, E., Negri, L., Melchiorri, P., and McNaughton, P.A. (2006). Sensitization of transient receptor potential vanilloid 1 by the prokineticin receptor agonist Bv8. J. Neurosci. *26*, 5109–5116.

Verge, G.M., Milligan, E.D., Maier, S.F., Watkins, L.R., Naeve, G.S., and Foster, A.C. (2004). Fractalkine (CX3CL1) and fractalkine receptor (CX3CR1) distribution in spinal cord and dorsal root ganglia under basal and neuropathic pain conditions. Eur. J. Neurosci. *20*, 1150–1160.

Verpoorten, N., Claeys, K.G., Deprez, L., Jacobs, A., Van, G.V., Lagae, L., Arts, W.F., De Meirleir, L., Keymolen, K., Ceuterick-de Groote, C., De Jonghe, P., et al. (2006). Novel frameshift and splice site mutations in the neurotrophic tyrosine kinase receptor type 1 gene (NTRK1) associated with hereditary sensory neuropathy type IV. Neuromuscul. Disord. *16*, 19–25.

Vos, M.H., Neelands, T.R., McDonald, H.A., Choi, W., Kroeger, P.E., Puttfarcken, P.S., Faltynek, C.R., Moreland, R.B., and Han, P. (2006). TRPV1b overexpression negatively regulates TRPV1 responsiveness to capsaicin, heat and low pH in HEK293 cells. J. Neurochem. *99*, 1088–1102.

Wang, C., Hu, H.Z., Colton, C.K., Wood, J.D., and Zhu, M.X. (2004). An alternative splicing product of the murine trpv1 gene dominant negatively modulates the activity of TRPV1 channels. J. Biol. Chem. *279*, 37423–37430.

Wang, H., Kohno, T., Amaya, F., Brenner, G.J., Ito, N., Allchorne, A., Ji, R.R., and Woolf, C.J. (2005). Bradykinin produces pain hypersensitivity by potentiating spinal cord glutamatergic synaptic transmission. J. Neurosci. *25*, 7986–7992.

Wetzel, C., Hu, J., Riethmacher, D., Benckendorff, A., Harder, L., Eilers, A., Moshourab, R., Kozlenkov, A., Labuz, D., Caspani, O., et al. (2007). A stomatin-domain protein essential for touch sensation in the mouse. Nature *445*, 206–209.

Xiao, H.S., Huang, Q.H., Zhang, F.X., Bao, L., Lu, Y.J., Guo, C., Yang, L., Huang, W.J., Fu, G., Xu, S.H., Cheng, X.P., et al. (2002). Identification of gene expression profile of dorsal root ganglion in the rat peripheral axotomy model of neuropathic pain. Proc. Natl. Acad. Sci. USA *99*, 8360–8365.

Xu, P., and Hall, A.K. (2006). The role of activin in neuropeptide induction and pain sensation. Dev. Biol. *299*, 303–309.

Xu, H., Delling, M., Jun, J.C., and Clapham, D.E. (2006). Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP channels. Nat. Neurosci. *9*, 628–635.

Yoshikawa, M., Senzaki, K., Yokomizo, T., Takahashi, S., Ozaki, S., and Shiga, T. (2007). Runx1 selectively regulates cell fate specification and axonal projections of dorsal root ganglion neurons. Dev. Biol. *303*, 663–674.

Zimmermann, K., Leffler, A., Babes, A., Cendan, C.M., Carr, R.W., Kobayashi, J., Nau, C., Wood, J.N., and Reeh, P.W. (2007). Sensory neuron sodium channel Nav1.8 is essential for pain at low temperatures. Nature *447*, 856–859.

Zhang, N., Inan, S., Cowan, A., Sun, R., Wang, J.M., Rogers, T.J., Caterina, M., and Oppenheim, J.J. (2005a). A proinflammatory chemokine, CCL3, sensitizes the heat- and capsaicin-gated ion channel TRPV1. Proc. Natl. Acad. Sci. USA *102*, 4536–4541.

Zhang, X., Huang, J., and McNaughton, P.A. (2005b). NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J. *24*, 4211–4223.

Zhong, J., Pevny, L., and Snider, W.D. (2006). "Runx"ing towards sensory differentiation. Neuron *49*, 325–327.

Zurborg, S., Yurgionas, B., Jira, J.A., Caspani, O., and Heppenstall, P.A. (2007). Direct activation of the ion channel TRPA1 by Ca2+. Nat. Neurosci. *10*, 277–279.

Zylka, M.J., Dong, X., Southwell, A.L., and Anderson, D.J. (2003). Atypical expansion in mice of the sensory neuron-specific Mrg G protein-coupled receptor family. Proc. Natl. Acad. Sci. USA *100*, 10043–10048.

Zylka, M.J., Rice, F.L., and Anderson, D.J. (2005). Topographically distinct epidermal nociceptive circuits revealed by axonal tracers targeted to Mrgprd. Neuron *45*, 17–25.