The development and modulation of nociceptive circuitry
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Nociceptive circuitry processes the signals evoked by activating specialized peripheral sensory receptors for pain perception. Recent studies show that the neuronal phenotypes in the dorsal root ganglia and spinal dorsal horn are determined by distinct sets of transcription factors during development. Anatomical analyses with genetic approaches demonstrate that each subset of nociceptive sensory neurons has topographically distinct circuits at both spinal and brain levels. Moreover, the sensitivity of primary afferents can be rapidly regulated not only by phosphorylation of receptors, ion channels and associated regulatory proteins but also by stimulus-induced cell surface expression of G-protein-coupled receptors. In chronic pain states the molecular characteristics of spinal nociceptive circuits are altered, enabling normal peripheral stimuli to induce pain hypersensitivity.

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\section*{Introduction}
The perception of pain is initiated by signaling the presence of noxious stimuli through specialized small-diameter neurons of the dorsal root ganglion (DRG; see glossary), which give rise to thinly myelinated (A\textsubscript{δ}-fibers) or unmyelinated (C-fibers; see glossary) afferent fibers. Two major subsets of small DRG neurons have been identified. One subset contains nerve growth factor (NGF) receptor TrkA. These NGF-sensitive small DRG neurons express the neuropeptides calcitonin gene-related peptide (CGRP) and substance P. Their central afferents terminate in lamina I, outer lamina II (II\textsubscript{o}), and the dorsal part of the inner lamina II (II\textsubscript{i}) of the spinal cord. About 40\% of neurons within this subset also contain glial cell line-derived neurotrophic factor (GDNF) family receptor (GFR) \(\alpha_3\) and Ret receptor tyrosine kinase (Ret), suggesting that they are sensitive to both NGF and artemin. The other subset is non-peptidergic and expresses cell surface glycoconjugates, which can be identified with isocitelin B4 (IB4) binding. The neurons in this subset express GFRA1 or GFRA2 together with Ret and are, therefore, sensitive to GDNF and neurturin. Central IB4-positive fibers innervate lamina III of the spinal cord. In the dorsal horn of spinal cord, the central terminals of afferents make synapses either with the projection neurons in lamina I or IV or with spinal relay neurons that express gamma-aminobutyrate (GABA), opioid peptides and other modulators. The processed signals are then transmitted to higher levels of the brain to contribute to pain perception.

During the past two years, researchers have made several major advances in studies on the mechanisms of development and modulation of nociceptive circuitry (see glossary). Several transcription factors have been found to determine the phenotypes of small DRG neurons and neurons in the dorsal horn of the spinal cord during development. In adults, the circuitry can be acutely or chronically modulated in response to nociceptive stimuli. After injury or inflammation of peripheral tissues, nociceptors are sensitized by the stimulation of pronociceptive mediators (see glossary) that are released by the damaged cells and tissues. There is mounting evidence that the modulation of phosphorylation of receptors, ion channels and associated regulatory proteins leads to the alteration of intrinsic properties or cell surface expression of ion channels in DRG neurons and spinal dorsal horn neurons. In addition to this evidence, it has been found that nociceptive stimuli can modulate the neuronal sensitivity to neurotransmitters or neuromodulators by inducing rapid insertion of G-protein-coupled receptors into the plasma membrane. In chronic pain states, the phenotypic modification occurs in spinal nociceptive circuits, enabling neurotrophic factors and other signaling molecules to induce pain hypersensitivity. These recent advances indicate that the nociceptive circuitry is altered in response to either acute or chronic noxious stimuli by modulation of its molecular and cellular machinery. In the present review, we focus on the recent findings regarding the specification of the spinal nociceptive circuitry during development and the modulation of this circuitry in pain states.

\section*{Specification of nociceptive circuitry}
\textbf{Genetic control of the development of spinal nociceptive circuits}
Several transcription factors control the phenotypes of DRG neurons during development. At early stages of embryonic development, the neuronal determination gene neurogenin1 (Ngn1) is required for the formation...
of TrkA-expressing sensory precursors, whereas Ngn2 is required for the generation of TrkB- or TrkC-expressing precursors (Figure 1). Ngn2 function as differentiation factors that regulate the expression of NeuroD, a differentiation factor that can cause premature differentiation of neural precursor cells. Expression of both Ngn1 and the homeobox gene Brn3a is, then, required for survival of most sensory neurons [1]. The Kruppel-like zinc finger transcription factor 7 (Klf7) is required for expression of TrkA [2]. It is also known that the Runt-related transcription factors (Runxs), Runx1/PEBP2αB (polymavirus enhancer binding factor 2 αB subunit)/AML1 (acute myeloid leukemia 1 protein), Runx2/PEBP2α/AML3 and Runx3/PEBP2αC/AML2, interact with a common cofactor PEBP2β to control a variety of developmental processes. Both Runx1 and Runx3 are expressed in DRGs [3]. Runx1 expression is restricted to TrkA-expressing sensory precursors of small DRG neurons [4,5,6], whereas Runx3 is involved in the differentiation of proprioceptive TrkC-expressing large DRG neurons [6,7] (Figure 1). During postnatal development the persistent expression of Runx1 marks the subset of small DRG neurons that undergoes the developmental transition from expressing TrkA to expressing Ret. Runx1 expression is extinguished in small DRG neurons that maintain TrkA expression. Moreover, Runx1 activates expression of several transient receptor potential cation channels (TRPs) such as TRPV1 and TRPA1, purinergic receptor P2X3, and Mas-related G-protein-coupled receptors (Mrgprs), whereas Runx1 is required to suppress the expression of neuropeptides CGRP and substance P.

In the dorsal horn of the embryonic spinal cord, the Tlx-class homeobox genes Tlx3 and Tlx1 determine whether neurons will have an excitatory glutamatergic or inhibitory GABAergic cell fate [8]. The expression of Lbx1, another homeobox gene, specifies default GABAergic differentiation in dorsal horn neurons [9]. Tlx3 antagonizes Lbx1, which in turn enables a subset of Lbx1-expressing neurons to differentiate into glutamatergic neurons. These findings suggest that the specification of spinal nociceptive circuits is determined by distinct sets of transcription factors during development. It will be interesting to find out whether the mechanisms for phenotypic modification of the spinal circuits in chronic pain states are also induced by transcription factors.

Genetic approaches to analyzing topographical nociceptive circuits

The topographical nociceptive circuits are traditionally analyzed with neural tracing methods and immunostaining. Zylka et al. [10] investigated non-peptidergic sensory neurons using genetically encoded axonal tracers (which were expressed from the Mrgpr member D locus). They found that these fibers terminated in a special layer of epidermis, the stratum granulosum, and that the termination zone was distinct from that innervated by peptidergic fibers. Furthermore, using the transneuronal transport of a genetically expressed lectin tracer, wheat germ agglutinin, in voltage-gated sodium channel 1.8 (Nav 1.8)-expressing non-peptidergic small DRG neurons, Braz et al. [11] identified an independent nociceptive circuit: non-peptidergic afferents → lamina II neurons → lamina V projection neurons → amygdala, hypothalamus, bed nucleus of the stria terminalis and globus pallidus. This circuit is parallel to the lamina I-based ascending pathway: peptidergic afferents → lamina I projection neurons → thalamus and brainstem parabrachial nuclei.
Thus, it appears that peptidergic and non-peptidergic peripheral innervation and ascending pathways are principally segregated. These findings demonstrate the potential of genetic approaches in the analysis of anatomic modulation of nociceptive circuits during development and in chronic pain states.

**Modulation of nociceptive circuitry**

**Stimulus-induced cell surface expression of G-protein-coupled receptors**

Both genetic deletion of the δ-opioid receptor (DOR) in the mouse and single nucleotide polymorphisms in the human DOR gene cause abnormal pain sensitivity [12,13], indicating an important role in pain modulation. In peptidergic small DRG neurons, DORs are mainly localized in the cytoplasm and are often associated with large dense-core vesicles (LDCVs) [14**]. In contrast to the limited number of surface DORs, μ-opioid receptors (MORs) are primarily localized on the cell surface. Pro-tachykinin, the precursor protein of the pronociceptive neuropeptide substance P, is responsible for sorting of DORs into LDCVs at the trans-Golgi network through physical interaction with DOR. The LDCV-associated DORs are inserted into the plasma membrane by
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The schematic shows the model of LDCV-mediated neurotransmission and modulation in the terminals of peptidergic nociceptive afferents. (a) In the steady state, LDCVs containing SP, CGRP and BDNF are stored in the terminals. The membrane of the LDCVs contains DORs, 5-HT1D and 5-HT7 receptors, whereas the plasma membrane of the neuron contains μ-opioid receptors and other subtypes of 5-HT receptors. (b) Nociceptive stimuli trigger Ca\textsuperscript{2+} influx that induces exocytosis of LDCVs and surface expression of LDCV-associated receptors, leading to a rapid change in the neuronal sensitivity to receptor-specific ligands. (c) Following peripheral inflammation, both the expression and the secretion of SP, CGRP and BDNF are enhanced in small DRG neurons. The increase in SP might enhance the sorting of DORs into LDCVs. Thus, peripheral inflammation could facilitate both the biogenesis and the exocytosis of the LDCVs that contain SP, CGRP, BDNF and DOR. Following peripheral nerve injury, the expression of BDNF and neuropeptides GAL, VIP and PACAP is upregulated in small DRG neurons, whereas SP expression is downregulated. The reduction in SP might decrease the number of DORs sorted into LDCVs, whereas the expression of a large number of receptors and channels is markedly regulated. Thus, the majority of LDCVs in the afferent terminals of injured small DRG neurons contain different peptides and, possibly, different receptors. Exocytosis of LDCVs would further modulate the plasma membrane that has been modified by changing expression of surface receptors and channels. Abbreviations: 5-HT7, serotonin 7 receptor; 5-HT1D, serotonin 1D receptor; BDNF, brain-derived neurotrophic factor; CGRP, calcitonin gene-related peptide; DOR, δ-opioid receptor; GAL, galanin; GPCR, G-protein-coupled receptor; LDCV, large dense-core vesicle; PACAP, pituitary adenylate cyclase-activating polypeptide; SP, substance P; VIP, vasoactive intestinal polypeptide.

Recent studies also show that serotonin 1D receptor (5-HT1D) and serotonin 7 receptor (5-HT7) are associated with LDCVs in C-fibers [21,22] (Figure 2). Moreover, in ~40% of the small DRG neurons that contain the Y2 receptor for neuropeptide Y, the Y2 receptor is found in vesicles. By contrast, in another subpopulation of small DRG neurons the Y1 receptor for neuropeptide Y is exclusively localized on the cell surface [23]. These results suggest that certain receptor subtypes of a neurotransmitter or neuromodulator can be specifically sorted into the regulated secretory pathway (see glossary) for stimulus-induced insertion, whereas other subtypes are sorted into the constitutive secretory pathway (see glossary) for spontaneous insertion. Following the exocytosis of LDCVs in response to nociceptive stimuli, the newly inserted receptors would induce a rapid modulation of the sensitivity of sensory neurons to the receptor ligands, either by interacting with the receptors that exist in the plasma membrane or by modulating the activity of downstream signaling molecules. Further studies will be required to elucidate the physiological and pharmacological functions of stimulus-induced surface insertion of G-protein-coupled receptors in pain modulation.

Pathological modification of nociceptive circuitry

Peripheral nerve injury induces marked changes in the gene expression profiles in both the DRGs and the spinal dorsal horn [24]. Several studies suggest that neuropathic pain (see glossary) might also be related to molecular changes in uninjured DRG neurons, in addition to its correlation with the molecular changes in injured neurons [25–28]. The ectopic firing in injured DRG neurons [29]...
might cause release of bioactive molecules that function at uninjured neurons to regulate gene expression. Therefore, further analysis on gene regulation in the uninjured DRG neurons would contribute greatly to our understanding of the mechanisms of spontaneous pain. Experiments with genetically mutated mice show that lysophosphatidic acid receptor [30], pituitary adenylate cyclase-activating polypeptide [32], interleukin 1 [33] and brain-derived neurotrophic factor (BDNF) [34] are involved in the pathogenesis of neuropathic pain, whereas Na,1.9 [35], neuronal nitric oxide synthase [36] and acid-sensing ion channel 3 [37] are involved in inflammatory pain. The mechanisms of action of BDNF and AMPA-type glutamate receptors are discussed in the following subsections.

The role of BDNF in pain hypersensitivity
In C-fiber terminals, BDNF is present in CGRP-containing LDCVs [38] and can be released by nociceptive stimulation. In transfected pheochromocytoma cells and hippocampal neurons, sortilin, a VPS10 domain-containing protein, controls sorting of BDNF into LDCVs [39]. The BDNF released from C-fibers would probably act at its high-affinity receptor TrkB, which is expressed in spinal dorsal horn neurons, including the spinothalamic projection neurons [40–42]. In ~10% of DRG neurons, BDNF is co-expressed with TrkB [38]. Thus, BDNF is active at both presynaptic and postsynaptic TrkB receptors in the superficial dorsal horn (see glossary).

Following peripheral nerve injury, BDNF is upregulated in DRG neurons [43]. BDNF heterozygous knockout mice exhibit a significant suppression of nerve injury-induced pain responses [34]. In parallel to increased levels of BDNF, complementary DNA (cDNA) array analysis confirms an earlier finding that TrkB is also markedly upregulated in the spinal dorsal horn after peripheral nerve injury [44]. Furthermore, it is suggested that BDNF released from ATP-stimulated microglial cells causes a shift in transmembrane anion gradient in lamina I projection neurons, which contributes to neuronal disinhibition and nerve injury-induced tactile alldynia (see glossary) [45*]. However, no cellular evidence for the expression and regulation of BDNF in spinal glia has been found to date. Taken together, these findings suggest that BDNF is an important mediator in pain hypersensitivity.

Modulation of AMPA receptor composition
The spinal glutamatergic system has been implicated in the pathogenesis of both inflammatory and neuropathic pain. AMPA receptors are assembled from four subunits, GluR1, 2, 3 and 4. Incorporation of GluR2 into heteromeric AMPA receptors reduces the permeability of the receptor channel to Ca2+, and modifies current rectification and macroscopic channel conductance. GluR2 is almost universally present in AMPA receptor-containing synapses throughout spinal laminae [46], which suggests that most AMPA receptors in the spinal dorsal horn have low permeability to Ca2+. By contrast, GluR1 is present at postsynaptic AMPA receptors in the dendrites that make synaptic contact with afferents in laminae I–II. It has been shown that activation of presynaptic AMPA receptors causes inhibition of glutamate release from the central terminals of primary afferent fibers, possibly through primary afferent depolarization [47]. However, the presynaptic localization of AMPA receptors in primary afferent terminals remains to be shown, although GluR1, 2 and 3 can be seen with immunostaining in the cell bodies of DRG neurons [47].

Recent studies show that changes in AMPA receptor composition lead to modulation of activity-dependent synaptic processing of nociceptive signaling. In spinal neurons of GluR1-deficient mice, a decrease in the number of Ca2+-permeable AMPA receptors is accompanied by a reduction in acute inflammatory hyperalgesia [48], whereas in GluR2-deficient mice an increase in spinal Ca2+-permeable AMPA receptors enhances long-lasting inflammatory hyperalgesia. Furthermore, in spinal neurons the synaptic targeting of NMDA receptors is dependent on the presence of synaptic AMPA receptors that are linked to NMDA receptors through an interaction involving the transmembrane coupling molecule stargazin and a MAGUK (membrane-associated guanylate kinase) class of cytoplasmic anchoring molecule, such as PSD-95 [49]. Expression of GluR1, 2, 3 and 4 is upregulated in the dorsal spinal cord following peripheral nerve injury [44,50]. Thus, it is interesting to see whether these changes could alter AMPA receptor composition at the synapses in the spinal laminae and contribute to the processing of neuropathic pain.

Conclusions
Recent advances suggest that the phenotypes of nociceptive DRG neurons and dorsal horn neurons are determined by distinct sets of transcription factors. The specification of these neurons includes controlling distribution and redistribution of receptor and ion channels and their regulatory molecules. Stimulus-induced surface expression of receptors and ion channels appears to be a mechanism that rapidly modulates the neuronal sensitivity in response to nociceptive stimuli. However, the role of the stimulus-induced surface insertion of receptors and channels in dynamic regulation of the interactions among membrane proteins and in the transmission of nociceptive signals remains to be further investigated. Chronic nerve injury and inflammation could lead to the reprogramming of phenotypes of neurons in the circuitry, and therefore distort the normal stimulus-response characteristics of the circuitry. In the future, it will be important to link the functional analysis of the regulated molecules at
molecular and cellular levels to pain behaviors and pharmacological evaluation.

**Update**

The TRP cation channels transduce mechanical, thermal and pain-related inflammatory signals. Using TRPA1 gene knockout mice, Bautista et al. [51**] demonstrated that the TRPA1 channel plays a central role in the pain response to endogenous inflammatory mediators, such as Bradykinin, and to a diverse array of volatile irritants. The authors also showed that the TRPA1 channel was not required for the initial detection of noxious cold. Thus, the TRPA1 channel is an important component of the transduction machinery that activates nociceptors in response to endogenous and environmental irritants or pronociceptive agents that elicit inflammatory pain.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


Using Runx1 knockout mice, the authors demonstrate that the persistent expression of Runx1 marks a subset of small DRG neurons that undergo the developmental transition from TrkA expression to Ret expression. This study also shows that Runx1 is required to activate or suppress the expression of a large cohort of ion channels, receptors and neuropeptides that are involved in pain modulation.


Using Tlx1, Tlx3 or Pax2 knockout mice, the authors show that Tlx1 and Tlx3 serve as post-mitotic selector genes that determine glutamatergic versus GABAergic cell fates in the embryonic dorsal spinal cord.


Using transneuronal transport of a genetically expressed lectin tracer, wheat germ agglutinin, in the non-peptidergic subpopulation of Na+1.8-expressing small DRG neurons, the authors demonstrate that in the spinal cord the lamina II neurons targeted by non-peptidergic afferents contact lamina V projection neurons. These neurons predominantly project to neurons in amygdala, hypothalamus, bed nucleus of the stria terminalis, and globus pallidus.


The authors reveal that DORs are sorted into neuropeptide-containing LDCVs by direct physical interaction with protachykinin. This mechanism is essential for DOR-mediated spinal analgesia and the development of morphine tolerance. The spinal opioid and tachykinin systems are directly linked by protachykinin-DOR interaction.


23. Brumovsky P, Stanic D, Shuster S, Herzog H, Villar M, Hökfelt T: Neuropeptide Y2 receptor protein is present in peptidergic...
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29. Sukhotinsky I, Ben-Dor E, Raber P, Devor M: Contribution of AMPA receptors to synaptic NMDA receptors is mediated by stargazin and AMPA receptor-dependent clustering of synaptic NMDA receptors in injured and intact primary afferent neurons and presynaptic inhibition of glutamate release. J Neurosci 2005, 25:335-349.


42. This study clearly demonstrates that the TRPA1 channel plays an essential role in neurogenic inflammatory pain, but not in the initial detection of cold or sound. The TRPA1 channel is an important downstream target of pronociceptive agents, such as Bradykinin, that produce nociceptor excitation through their actions on the phospholipase C (PLC) signaling pathway.