Nociceptors: the sensors of the pain pathway

Adrienne E. Dubin, Ardem Patapoutian


Specialized peripheral sensory neurons known as nociceptors alert us to potentially damaging stimuli at the skin by detecting extremes in temperature and pressure and injury-related chemicals, and transducing these stimuli into long-ranging electrical signals that are relayed to higher brain centers. The activation of functionally distinct cutaneous nociceptor populations and the processing of information they convey provide a rich diversity of pain qualities. Current work in this field is providing researchers with a more thorough understanding of nociceptor cell biology at molecular and systems levels and insight that will allow the targeted design of novel pain therapeutics.

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Specialized peripheral sensory neurons known as nociceptors alert us to potentially damaging stimuli at the skin by detecting extremes in temperature and pressure and injury-related chemicals, and transducing these stimuli into long-ranging electrical signals that are relayed to higher brain centers. The activation of functionally distinct cutaneous nociceptor populations and the processing of information they convey provide a rich diversity of pain qualities. Current work in this field is providing researchers with a more thorough understanding of nociceptor cell biology at molecular and systems levels and insight that will allow the targeted design of novel pain therapeutics.

Introduction

Pain, as a submodality of somatic sensation, has been defined as a "complex constellation of unpleasant sensory, emotional and cognitive experiences provoked by real or perceived tissue damage and manifested by certain autonomic, psychological, and behavioral reactions" (1). The benefit of these unpleasant sensations, however, is underscored by extreme cases: patients lacking the ability to perceive pain due to hereditary neuropathies often maintain unrealized infections, self-mutilate, and have curtailed life spans (2). Normally, nociception (see Glossary, Sidebar 1) and the perception of pain are evoked only at pressures and temperatures extreme enough to potentially injure tissues and by toxic molecules and inflammatory mediators. These high threshold physical and noxious chemical stimuli are detected by specialized peripheral sensory neurons (nociceptors). This is in contrast to the high sensitivity of visual, auditory, olfactory, taste, and somatosensory organs to their adequate stimuli. Pain is described as having different qualities and temporal features depending on the modality and locality of the stimulus, respectively: first pain is described as lancinating, stabbing, or pricking; second pain is more pervasive and includes burning, throbbing, cramping, and aching and recruits sustained affective components with descriptors such as "sickening" (3). The intensity of these global reactions underscores the importance of avoiding damaging situations for survival and maintaining homeostasis. As opposed to the relatively more objective nature of other senses, pain is highly individual and subjective (4, 5) and the translation of nociception into pain perception can be curtailed by stress or exacerbated by anticipation (6).

Here, we review the nociceptive aspect of pain perception, focusing on nociceptors innervating the skin and subserving exteroception of noxious stimuli. Discussion of the similarities and differences among cutaneous, visceral, muscle, and joint nociception can be found elsewhere (7–9). We provide an overview of how noxious stimuli are detected, encoded, and conveyed to the CNS. Since recent reviews have described in detail the molecules involved in detecting noxious stimuli (10–13) and contributing to protective mechanisms mediating enhanced pain at the site of injury (14), we take an integrative approach that highlights recently discovered cellular transduction/conduction mechanisms in the context of different nociceptor fiber types identified in vivo and ex vivo. We further discuss innovations using genetic and pharmacological tools that begin to address how particular nociceptor populations contribute to the perception of specific pain qualities. Since maladaptive changes in normal physiological mechanisms underlie a variety of pathologies leading to chronic pain, a thorough understanding of nociception is required to identify the interventions most likely to provide therapeutic benefit.

Anatomy and physiology of cutaneous nociception

Significant insights into the cellular and molecular basis of cutaneous nociception have been realized from studies on conscious humans and surrogate animal models (15, 16), although we are far from understanding the cell biology of pain perception. Advances are hampered by the difficulties inherent in studying neuronal processes in humans, cellular changes in nociceptors induced by invasive methods, the inability to record directly from the tiny structures where transduction of noxious stimuli occurs, and the uncertainty in model systems that an animal’s behavior is due to its perception of pain (15, 17). Although the morphology of sensory nociceptive nerve endings is highly conserved in animals from rodents to humans (5, 9, 17–19), cutaneous nociceptors are an extremely heterogeneous group of neurons housed in peripheral sensory ganglia located just outside the CNS that transduce external noxious stimuli in the skin, up to meters away from their cell bodies.

Minimally invasive extracellular single unit recordings from nerve fibers in peripheral nerves (microneurography) and skin nerve preparations in mammals (20) and microneurography combined with psychophysical measurements in human subjects (15, 16, 21) have revealed the existence of distinct classes of nociceptor activated by noxious stimuli. Adequate stimuli include temperature extremes (>~40°C–45°C or <~15°C), intense pressure, and chemicals signaling potential or actual tissue damage. Nociceptors are generally electrically silent (12) and transmit all-or-none action potentials only when stimulated. However, nociceptor activity does not per se lead to the perception of pain. The latter requires peripheral information to reach higher centers and normally depends on the frequency of action potentials in primary afferents, temporal summation of pre- and postsynaptic signals, and central influences (7).

The speed of transmission is directly correlated to the diameter of axons of sensory neurons and whether or not they are myelinated. Most nociceptors have small diameter unmyelinated axons (C-fibers) (12) bundled in fascicles surrounded by Schwann cells and support conduction velocities of 0.4–1.4 m/s (22) (Figure 1). Initial fast-onset pain is mediated by A-fiber nociceptors whose axons are myelinated and support conduction velocities of approximately 5–30 m/s (most in the slower Aδ range) (22). Nociceptive fibers have been

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classified (23) on the basis of their conduction velocity and sensitivity and threshold to noxious mechanical (M), heat (H), and cold (C) (Tables 1 and 2) (9, 23). Units responding to thermal, mechanical, and chemical stimuli (polymodal) are the most common C-fiber type observed in fiber recordings (C-MH, C-MC, C-MHC) (7, 9, 24) (Table 1). The expression of differential repertoires of transduction molecules (particularly chemical sensors) confers a rich functional heterogeneity upon this class. The use of electrical stimulation to search for receptive fields, rather than mechanical stimulation, identified nociceptors normally insensitive to mechanical and heat stimulation (silent; C-MH,H) that become sensitive to noxious mechanical or temperature only after being sensitized by inflammatory mediators (25, 26). C-fibers responsive to noxious heat (C-H; ~10% of C-nociceptors) play a major role in heat sensation (see below). A-fiber nociceptors are predominately heat- and or mechanosensitive (A-MH, A-H, A-M) (19, 27) (Table 2), however, sensitivity to noxious cold is also observed (27–29). Determining the contribution of each of these fiber types to pain...
perception requires an understanding of the molecular mechanisms underlying the detection of particular stimulus modalities and nociceptor connectivity in central circuits.

Noxious stimuli are transduced into electrical signals in free “unencapsulated” nerve endings that have branched from the main axon and terminate in the wall of arterioles and surrounding connective tissue, and may innervate distinct regions in the dermis and epidermis (17, 30). The endings are ensheathed by Schwann cells except at the end bulb and at mitochondria- and vesicle-rich varicosities (17). A-fibers lose their myelin sheath and the unmyelinated A-fiber branches cluster in separated small spots within a small area, the anatomical substrate for their receptive field (17). In contrast, specialized nonneuronal structures conferring high sensitivity to light touch, stretch, vibration, and hair movement are innervated by low threshold A-fibers (11). Nociceptive endings are in the vicinity of keratinocytes, mast cells, and Langerhans cells, indicating the capacity of peripheral sensory endings to monitor the status of the skin (31). Nociceptors, like other primary somatosensory neurons, are pseudounipolar (Figure 1): a single process emanates from the cell body in the dorsal root ganglion (DRG) or trigeminal ganglion (TG) and bifurcates, sending a peripheral axon to innervate the skin and a central axon to synapse on second-order neurons in the dorsal horn of the spinal cord or the trigeminal subnucleus caudalis (Vc) (13), respectively (Figure 1A). In this way, propagating electrical signals between periphery and spinal cord (or brainstem) follow a direct axonal pathway, thus reducing the risk of conduction failure (32). Nociceptors are excitatory neurons and release glutamate as their primary neurotransmitter as well as other components including peptides (e.g., substance P, calcitonin gene-related peptide [CGRP], somatostatin) important in both central synaptic signaling and efferent signaling in the skin (13). Invasion of action potentials into the nociceptor soma via the short stem axon (32) can lead to biochemical changes (e.g., phosphorylation and activation of MAPK superfamily of signaling pathways) that ultimately alter gene expression and functional phenotype (33, 34). Although it is thought that direct communication between the soma of primary sensory neurons does not occur, vesicle exocytosis is observed in dissociated soma and may influence associated Schwann cells and possibly nearby neurons (35, 36). The central axon of DRG neurons enters the spinal cord via the dorsal root and sprouts branches that innervate multiple spinal segments in the rostral and caudal direction as well as the segment associated with the particular DRG and dorsal column nuclei of the caudal medulla (7). They terminate predominantly in laminae I, II, and V of the dorsal horn on relay neurons and local interneurons important for signal modification (13, 37, 38) (Figure 1, B and C). The relay neurons project to the medulla, mesencephalon, and thalamus, which in turn project to somatosensory and anterior cingulate cortices to drive sensory-discriminative and affective-cognitive aspects of pain, respectively (38). Local inhibitory and excitatory interneurons in the dorsal horn as well as descending inhibitory and facilitatory pathways originating in the brain modulate the transmission of nociceptive signals, thus contributing to the prioritization of pain perception relative to other competing behavioral needs and homeostatic demands (39).

The cell body (soma) has served as an extremely useful model to study molecules and modulatory mechanisms mediating transduction of noxious stimuli, transmission of electrical signals to the CNS, and release of neurotransmitters and neuropeptides.
at central and peripheral terminals (40, 41). The soma expresses many molecular entities that are expressed in free nerve endings, central terminals, and axon (13). However, data from whole-cell soma recordings have been shown in a few cases to be at odds with behavioral or peripheral physiological data (e.g., heat transduction, refs. 42–44; and proton responsiveness, ref. 45). Although the underlying differences in these cases may be due to differential distribution of transduction molecules, it is also possible that nonneuronal peripheral components are required in vivo and lacking in dissociated neuronal cultures. This underscores the importance of corroborating results from cultured neurons with behavior and/or acute preparations retaining intact terminal fields. Labeling with retrograde dyes injected into the target tissue has enabled characterization of functional attributes of the soma of nociceptors innervating those tissues. The heterogeneity of functional phenotypes observed in isolated sensory cell bodies (46, 47) appears to reflect the variability observed in cutaneous nociceptor fiber types observed in studies in which recordings from fiber or soma during receptive field stimulation are combined with subsequent nociceptor labeling to identify terminal morphology (48, 49) and the expression of nocisensors (50), markers, and peptides (48) (Table 1). Nociceptors differentially express a variety of anatomical and biochemical markers (e.g., the expression of versican, the binding partner for the lectin B4 [IB4]; ref. 51), however the functional significance of these markers, especially given striking species differences (49, 52), are unknown. Here, we will address how the functional heterogeneity of the nociceptor has an impact on the perception of pain.

### Table 1

<table>
<thead>
<tr>
<th>Mechanical sensitivity</th>
<th>Mechanical insensitivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C-MH</strong></td>
<td><strong>C-M</strong></td>
<td><strong>C-H</strong></td>
</tr>
<tr>
<td>Percentage observed</td>
<td>35–approaching 100</td>
<td>10–15</td>
</tr>
<tr>
<td>CV (m/s)</td>
<td>0.8–1.0 (human, monkey);</td>
<td>0.84 (human);</td>
</tr>
<tr>
<td></td>
<td>−0.5 (mouse)</td>
<td>−0.5 (mouse);</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.35 (mouse);</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sensitive C-fibers</td>
</tr>
<tr>
<td>Correlation of heat response and PR</td>
<td>Yes/repeated stimulation sensitizes (hairy, not glabrous)</td>
<td>NA</td>
</tr>
<tr>
<td>Heat threshold</td>
<td>39°C–51°C</td>
<td>NA</td>
</tr>
<tr>
<td>Correlation of mechanical response to punctuate stimulation and PR</td>
<td>Static: no; accelerating: yes</td>
<td>Accelerating: yes</td>
</tr>
<tr>
<td>Mechanical threshold</td>
<td>30 mN (human);</td>
<td>30 mN (human);</td>
</tr>
<tr>
<td></td>
<td>25 mN (monkey);</td>
<td>18 mN (mouse)</td>
</tr>
<tr>
<td>Correlation of tonic pinch response to tonic pinch and PR</td>
<td>Yes; initial pain; adapting</td>
<td>Yes; initial pain; adapting</td>
</tr>
<tr>
<td>Electrical stimulation of axon → flare</td>
<td>No: uninjured skin Yes: UV-B–treated skin</td>
<td>No: uninjured skin</td>
</tr>
<tr>
<td>Known chemical activators</td>
<td>Cap → TRPV1 (transient);</td>
<td>AITC → TRPA1 (36%)</td>
</tr>
<tr>
<td>Effect of injury/inflammation</td>
<td>Nonuniform changes in activity after burn: hypoalgesia (glabrous); sensitization (hairy)</td>
<td>Unknown</td>
</tr>
<tr>
<td>Comments</td>
<td>Heat responses independent of TRPV1; may provide neural code for magnitude judgments of heat pain; contribute to stimulus localization</td>
<td>Responsible for TRPV1-heat hyperalgesia; not capable of precise localization of stimulus</td>
</tr>
</tbody>
</table>

Classification of nociceptors on the basis of mechanical sensitivity varies among investigators using different cut-off values and methods. For clarity, cold-sensitive fibers are not included. Cap, capsaicin; CV, conduction velocity; PR, human subject pain ratings; +, immunoreactive.
membrane conductance changes and electrogenic pump activity. Since the electrochemical gradients for sodium (Na$^+$) and chloride (Cl$^-$) (+) are more negative than the resting potential, closure of sodium channels and activation of potassium (K$^+$) channels (which give transient responses to capsaicin (57), are not included in this table. HTM, high threshold mechanoreceptor.

### Table 2
Major heat and/or mechanosensitive nociceptor A-fiber classes

<table>
<thead>
<tr>
<th>Percentage observed</th>
<th>CV (m/s)</th>
<th>Correlation of heat response and PR</th>
<th>Heat threshold</th>
<th>Correlation of mechanical response to punctuate stimulation and PR</th>
<th>Mechanical threshold</th>
<th>Known chemical activators</th>
<th>Effect of injury/inflammation</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-MH II</td>
<td>–10 (hairy skin)</td>
<td>11, 15 (monkey)</td>
<td>Yes; response is transient (rapid onset [&lt;1 s], early peak discharge [&lt;1 s], adapting); fatigue to repeated stimuli</td>
<td>43°C–47°C</td>
<td>Yes but with threshold offset</td>
<td>11–15 bar (monkey)</td>
<td>Cap → TRPV1</td>
<td>Burn: heat sensitization, hyperalgesia in glabrous skin; may mediate 2° punctate mechanohyperalgesia</td>
</tr>
<tr>
<td>A-MH I</td>
<td>–20 (hairy skin)</td>
<td>11, 25 (monkey)</td>
<td>Yes: long latency to respond and late peak discharge; shows sensitization</td>
<td>&gt;53°C</td>
<td>YES</td>
<td>3.7–5 bar (monkey)</td>
<td>Cap insensitive</td>
<td>Burn: heat sensitization, hyperalgesia in glabrous skin; may mediate 2° punctate mechanohyperalgesia</td>
</tr>
<tr>
<td>A-M</td>
<td>15–50</td>
<td>14 (monkey)</td>
<td>NA</td>
<td>NA</td>
<td>8.2 bar (monkey); 25 mN (mouse)</td>
<td>50, 55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For clarity, A-H and A-MH ("MIA," ref. 26), which give long-lasting responses to capsaicin (57), are not included in this table. HTM, high threshold mechanoreceptor.

### The response of nociceptors to noxious stimuli
Close proximity of distal extremities to a hot or cold surface, intense pressure or squeezing, and irritating chemicals can result in a sub-second somatotopic withdrawal response. Activation of nociceptors requires that adequate stimuli depolarize peripheral terminals (producing a receptor potential) with sufficient amplitude and duration. This ensures that despite any attenuation and slowing of the receptor potential by passive propagation between the sites of transduction and action potential generation, information such as stimulus intensity will be encoded in the resulting train of impulses. Although the distance to action potential initiation is not known for fibers innervating the skin, action potential generation has been proposed to be at or near the site of transduction in Aδ cold fibers innervating the guinea pig cornea (53). In this model, action potentials can be generated at differing distances from the terminal ending depending on the extent of depolarization of the fiber and resulting inactivation of voltage-gated channels involved in conduction (53). Theoretically, the depolarizing receptor potential can be accomplished by multiple membrane conductance changes and electrogenic pump activity. Since the electrochemical gradients for sodium (Na$^+$), calcium (Ca$^{2+}$), and chloride (Cl$^-$) (37) are more positive than the resting membrane potential in sensory neurons, the opening of ion channels permeable to these ions will cause the membrane potential to shift in the positive direction (depolarize). Since the electrochemical gradient for potassium (K$^+$) is more negative than resting potential, closure of active potassium channels not only depolarizes the membrane potential but amplifies current-induced voltage fluctuations due to the resulting increase in membrane resistance (54).

The identification of the molecules that mediate or contribute to heat-, cold-, mechanical-, and chemical-induced generator potentials has been achieved by careful characterization of currents in native cells, the use of novel approaches to identify genes encoding channels and receptors, genome sequencing and bioinformatics, chemical tools (e.g., calcium dyes, pharmacological agents), and innovative genetic approaches. Although significant progress has been made in the study of heat and chemical transduction, many pieces of the puzzle are still missing (Table 3 and Figure 2).

### Transduction of noxious heat
At least 5 classes of nociceptor reveal an increase in activity dependent on the intensity of the heat stimulus beyond the threshold for pain perception (~40°C–45°C) into the noxious range (9, 24). Microneurography studies on conscious human subjects have addressed whether activity in a particular fiber correlates with the psychological rating of pain. Under normal conditions, the activity in only a subset of heat-responsive fibers correlates to the degree of pain perceived (C-MH, A-MH type I, A-MH type II; Tables 1 and 2). A-fibers (A-MH Type II innervating hairy skin) responding to temperatures slightly cooler than the perceptual pain threshold for heat are proposed to mediate first pain in humans (55). These fibers rapidly activate, adapt during prolonged heat stimulation, fatigue between heat stimuli, and are sensitive to capsaicin (55–57), a selective agonist of the mammalian heat-activated nonselective cation (NSC) channel transient receptor potential VI (TRPV1) (58) (Table 3). In humans and monkeys, slowly developing second pain is evoked by activation of C-fibers and A-MH type I–fibers...
(9, 24), the latter requiring longer exposures to noxious temperatures to achieve maximal firing rates (Tables 1 and 2). Heat-induced C-MH fiber activity in primates correlates with human pain perception in the absence of injury (9). In human glabrous skin, however, neither first pain (56) nor A-MH type II–fiber innervation is observed (59). Investigation of noxious heat transduction in animal models is complicated because the conservation of these pathways is unknown.

Although human C-MH polymodal nociceptors are activated in a temperature range (39°C–51°C) similar to recombinant TRPV1 (58) and have been reported to be transiently activated by capsaicin (60), genetic deletion of TRPV1 in mice only partially reduces noxious heat sensitivity in behavioral assays (42, 43) and has no effect on heat responsiveness of the C-fibers tested (42, 44, 49). This suggests that, at least in mice, heat sensors other than TRPV1 are the major contributors to acute heat-induced pain. Cultures of rodent sensory ganglia indicate expression of TRPV1 in approximately 50% of neurons (61) and 75% of small-medium diameter neurons (43), similar to the proportion of C-MH fibers in a skin-nerve-sensory ganglion–spinal cord preparation (49).

Although functionally characterized mouse C-MH neurons were not immunoreactive for TRPV1 (50), neurons with low expression levels may have been missed. Importantly, every identified mouse C-H neuron (~10% of the population) revealed TRPV1-like immunoreactivity (50) and no functionally identified C-H nociceptors were observed in TRPV1-deficient animals. This result cannot be explained by a phenotypic switch to C-MH fibers in these mice since mechanical sensitivity of the C-fiber population was unchanged (50). Furthermore, noxious acute heat responses evoked in rat C-MH saphenous fibers were not decreased by ruthenium red at concentrations that block the capsaicin response (45).

In humans, the prolonged time course of the strong C-H response observed by application of the receptive field matched the duration of the perceived pain (60) and is consistent with the results of rodent studies (50). Disparities regarding the contribution of TRPV1 to fiber subtype functionality (especially C-MH fibers) may depend on species, stimulation techniques, tissue preparation, and sensitivity of the assay. However, it is clear that acute heat responses are mediated by mechanisms in addition to TRPV1 activation, at least in mouse. In the context of tissue injury, however, polymodal C-MH fibers are certainly important in TRPV1-mediated thermal hyperalgesia, indicating that inflammation upregulates the contribution of TRPV1 to heat-evoked nociceptive behaviors in mouse (see below). TRPV2 (Table 3) has been suggested to mediate, at least in part, the receptor potential of A-MH type I–fibers, since it is activated by temperatures greater than 52°C in recombinant expression studies (62); however, the lack of selective pharmacological tools for manipulation of TRPV2 activity has hindered rigorous evaluation of this hypothesis. Transducers of heat stimuli into the noxious range other than TRPV1 and TRPV2 (45, 49) include other TRPV channels (TRPV3 and TRPV4) that have been reported to exist on sensory neurons (10) as well as constitutively active K+ channels (KCNK2) inhibited by heat (63) (Table 3). Although TRPV3 undergoes dramatic sensitization to repeated heat stimuli, TRPV3 does not appear to be involved in agonist sensitization of C-fiber heat responses (44). Nocisensors directly activated by heat are also expressed on resident skin cells (e.g., TRPV3 and TRPV4 on keratinocytes; refs. 10 and 31) and cause the release of allogenic substances that indirectly influence nociceptor firing (64) (Table 3).

Transduction of noxious cold

Identifying the mechanisms used by nociceptors to transduce noxious cold has lagged behind progress in understanding heat transduction mechanisms (65). The intensity of cold pain in humans increases linearly with stimulus intensity between about 20°C and 0°C. The threshold for pain perception to cold is much less precise than that for heat, but is about 15°C (66). There is tremendous variability in threshold for cutaneous cold-evoked fiber activity observed in mammals in part due to the rate of cooling (approximately +30°C to ~18°C; refs. 27, 66–68). Homeostatic processes engaged during in vivo studies (e.g., vasculature changes) and potential tissue damage occurring at subfreezing temperatures are likely to indirectly influence nociceptor responsiveness. Furthermore, measuring cold nociceptive behavior in animals has proven to be challenging, perhaps due to the prolonged exposure time in most assays (68).

Cooling the skin to 4°C activates A- and C-fibers sensitive to innocuous cooling and cold-sensitive nociceptors (27–29, 67, 69), consistent with the presence of two populations of cold-sensitive neurons observed in culture (70–72). Cool-sensitive nonnociceptive afferents are spontaneously active at normal skin temperature and their excitability increases with decreasing temperature (53, 67). The menthol-activated NSC channel TRPM8 (10) is responsible for the detection of innocuous cooling (69, 73, 74) and contributes to spontaneous firing (69) in mice. The effective range for TRPM8-mediated cold coding extends from just below skin temperature into the noxious range (10°C–15°C and below; ref. 10). Although mouse studies have yielded conflicting results regarding the requirement of TRPM8 in behavioral responses to noxious stimuli, more recent work using a novel cold-plate assay convincingly demonstrates a role for mouse TRPM8 in sensing noxious cold (75). Furthermore, the analgesic effects of cold temperature (17°C) were lost in mice lacking TRPM8 in the context of formalin-induced inflammation (74). Whether TRPM8-mediated analgesia is dependent on peripheral and/or central sites of action is unknown but may be addressed now that TRPM8-expressing neurons and their peripheral and central fibers can be visualized by GFP expression driven by the TRPM8 promoter (76, 77). Importantly, studies in humans and mice reveal species differences in pathways sensing innocuous cold: A-fiber block completely suppresses the cold response in humans (78), yet the majority of TRPM8-expressing fibers responsible for innocuous cold transduction in mice have small diameters (76, 77).

Noxious cold stimuli activate NSC currents and calcium influx (10, 79) and decrease K+ channel activity (80) and Na+K−ATPase function (65) (Table 3). The temporal dissociation of the qualities of pain/ache vs. prickle/heat to noxious cold (3°C) suggest underlying differences in transduction mechanisms or information processing (66). The cation channel TRPA1 (10) has been proposed to play a role in this process because it has a threshold near 17°C, is expressed in nociceptors together with TRPV1 (10), and is required for cold sensation in mice (81, 82). Human genetic studies have suggested TRPA1 contributes to variation in cold-pain sensitivity (5). Although TRPA1 may respond to cold indirectly through cold-induced intracellular calcium release (10), slow temperature ramps can activate TRPA1 in excised patches in the absence of calcium (82, 83). However, the contribution of TRPA1 to cold sensation is debated since TRPA1 activation was not observed in a heterologous expression system or cultured TG neurons to which relatively short cold stimuli to 5°C were applied (84), and not all
## Table 3
Proposed ion channel sensors of cutaneous noxious stimuli

<table>
<thead>
<tr>
<th>Noxious stimulus</th>
<th>Transduction channel</th>
<th>Evidence for role in acute nociception</th>
<th>Role in hyperalgesia</th>
<th>Comments</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat ≥ 43°C</td>
<td>TRPV1 ≥ 43°C (unsensitized), specific receptor for sub-μM Cap</td>
<td>Chemical activation (Cap, reserpinization): heat nociception by activating C-fiber fibers (mouse); reduced central wide dynamic range neuron response to 49°C in null mice</td>
<td>Major contributor to thermal (heat) after carrageenan or CFA-induced inflammation; heat threshold decreased by inflammatory mediators (e.g., BK, NGF-mediated phosphorylation via P–p38); after burn, heat hyperalgesia reduced in KO; upregulation and enhanced activity in inflammation</td>
<td>Only heat receptor that releases CGRP and substance P in periphery when activated; Cap induces predominantly stinging and burning pain</td>
<td>10, 34, 42–44, 50, 134</td>
</tr>
<tr>
<td></td>
<td>TRPV2 ≥ 52°C, recombinant</td>
<td>No evidence for a loss of heat sensitive fibers in mice lacking TRPV2 together with TRPV1</td>
<td>Null mice reveal no deficits in thermal (heat) or mechanical hyperalgesia induced by CFA or BK and formalin-induced behaviors</td>
<td>Proposed to mediate heat response in A-MH type II-fibers (human/primate)</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>TRPV3: threshold 32°C–39°C into noxious range</td>
<td>Heat: increased withdrawal latency on hot plate ≥ 50°C</td>
<td>Null mice reveal no deficits in thermal (heat) or mechanical hyperalgesia induced by CFA or BK and formalin-induced behaviors</td>
<td>Reveals strong sensitization by repeated heating; mediates ATP release from keratinocytes and PGE2 release from keratinocytes overexpressing TRPV3</td>
<td>64, 135–138</td>
</tr>
<tr>
<td></td>
<td>TRPV4: threshold 27°C–34°C into noxious range</td>
<td>No contribution to 50°C-induced spike frequency at 40°C; no phenotype on hot plate to 50°C</td>
<td>Thermal (heat): slight increase in withdrawal latency at 45°C–46°C; activated by concerted action of components in inflammatory soup</td>
<td>Protein has been detected in DRG that depends on an intact TRPV4 gene; high expression in keratinocytes</td>
<td>10, 67, 139–142</td>
</tr>
<tr>
<td></td>
<td>KCNK2 (TREK-1) 30°C–45°C</td>
<td>Null mice reveal enhanced heat-dependent (30°C–45°C) saphenous C-fiber activity; decreased thermal (46°C–50°C) tail withdrawal latency; higher percentage of heat-sensitive/TRPV1-negative fibers</td>
<td>Recombinant channel activity inhibited by H+, LPA; inflammation increased heat (46°C) (but not cold) hyperalgesia in C-MH fibers in null mice; LPA and proteins inhibit channel activity</td>
<td>2-pore potassium channel reduced activity causes increased excitability; contributes to suppression of heat activation of C-MH fibers; negatively regulated by Gαs and Gq protein–coupled receptors, such as PGE2, serotonin, and glutamate</td>
<td>63, 143, 144</td>
</tr>
<tr>
<td></td>
<td>KCNK4 (TRAAK)</td>
<td>Null mice reveal: higher percentage of heat-sensitive/TRPV1-negative fibers and heat hyperalgesia</td>
<td>Null mice reveal heat (46°C) hyperalgesia after carrageenan</td>
<td>Two pore potassium channel</td>
<td>144</td>
</tr>
<tr>
<td>Cold</td>
<td>TRPM8: threshold near 26°C and active into the noxious range</td>
<td>Severe deficit in cooling-induced behavior and sensory neuronal responses in null mice; contributes to avoidance of moderately low temperature and cold hypersensitivity</td>
<td>Contributes to cold allodynia in CFA model of inflammation</td>
<td>Predominant detector of innocuous cold in vivo; responsible for the analgesic effect produced by cold or chemical cooling compounds</td>
<td>10, 69, 73–75, 145</td>
</tr>
<tr>
<td></td>
<td>TRPA1; moderately specific receptor for mid-μM AITC</td>
<td>Contributes to nociceptive behaviors on 0°C cold plate and acetone-induced nociceptive behaviors; and cold hypersensitivity at 10°C</td>
<td>Contributes to CFA-induced cold hyperalgesia and BK-induced hyperalgesia; null mice reveal increased threshold to punctate mechanical stimuli (von Frey) and reduced inflammatory hyperalgesia</td>
<td>Controversial (see text); does not contribute to heat hyperalgesia during CFA-induced inflammation</td>
<td>10, 75, 81, 82, 85, 146–148</td>
</tr>
<tr>
<td></td>
<td>KCNK4 (TRAAK) Together with KCNK2 (but not alone) contributes to nociceptive cold-induced C-fiber activity (lowered threshold) and behavior</td>
<td>Null mice reveal inflammation-induced mechanical (von Frey) hyperalgesia and reduced PGE2-induced pain</td>
<td>Hypertonic (10%) saline-induced pain reduced in null mice</td>
<td>Combination of TREK-1 and TRAAK important</td>
<td>144</td>
</tr>
<tr>
<td>Intense punctate and/or pinch pressure</td>
<td>KCNK2 (TREK-1)</td>
<td>Contributes to von Frey punctate mechanical–induced pain, but not pressure-induced pain</td>
<td>Contributes to punctate von Frey–induced pain; together with KCNK2 contributes to osmotic pain</td>
<td>Combination of TREK-1 and TRAAK important</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>KCNK4 (TRAAK)</td>
<td>Contributes to punctate von Frey–induced pain; together with KCNK2 contributes to osmotic pain</td>
<td>Null mice reveal heat-induced hyperalgesia (von Frey) after carrageenan and CFA injection in null mice</td>
<td>Mechanical hyperalgesia</td>
<td>142, 149</td>
</tr>
<tr>
<td></td>
<td>TRPV4</td>
<td>TRPV4 KO: reduced response to tail clamp</td>
<td></td>
<td>Although activated by hypotonicity, there is lack of consensus for a role of TRPV4 in nociceptive mechanical stimulus transduction; protein has been detected in DRG that depends on an intact TRPV4 gene</td>
<td>142, 149</td>
</tr>
<tr>
<td></td>
<td>TRPA1</td>
<td>Cutaneous nociceptor fibers from TRPA1-null mice exhibit lower firing rates in response to mechanical stimuli</td>
<td>Mechanical hyperalgesia BK-induced reduction in mechanical withdrawal threshold requires TRPA1</td>
<td>A direct effect of mechanical stimuli on mammalian TRPA1 has not been demonstrated</td>
<td>81, 147, 148</td>
</tr>
<tr>
<td></td>
<td>TRPV1</td>
<td>Null mice have normal threshold for punctuate and pinch stimuli</td>
<td>After burn, mechanical hyperalgesia reduced in KO; BK-induced reduction in mechanical withdrawal threshold required TRPV1; no defects in mechanical sensitivity after AITC or CFA injection in null mice</td>
<td>Role in mechanical hyperalgesia</td>
<td>42, 85, 134</td>
</tr>
<tr>
<td>Kv4.3</td>
<td>Mechanical hyperalgesia after i.t. antisense ODN injection (rat lumbar DRG); no effect on heat response</td>
<td></td>
<td></td>
<td>Reduced activity causes increased hyperalgesia</td>
<td>150</td>
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<td></td>
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<td>150</td>
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</tbody>
</table>

NGF, nerve growth factor; ND, not determined; i.t., intrathecal.
Mouse lines constitutively lacking TRPA1 reveal a cold phenotype in behavioral and neural assays (85). However, two independent studies recently demonstrated a role of TRPA1 in noxious cold sensitivity (75, 82). TRPA1 is established as a general sensor for noxious irritating electrophilic compounds (including allyl isothiocyanate [mustard oil] [AITC] and cinnamaldehyde, the active pungent ingredients in hot mustard and cinnamon, respectively; refs. 84 and 86) and is sensitized by inflammatory mediators (87). These electrophilic agonists open an integral channel pore by covalent binding to the intracellular N terminus of the channel protein.
(88, 89). Importantly, endogenous reactive chemicals are also effective agonists of TRPA1 (90). How TRPA1-expressing neurons might mediate the burning sensation of hot mustard (AITC) may be explained by anatomical and psychophysical results: AITC is a strong chemical activator of a subset of TRPV1-expressing neurons, and activity in peripheral fibers transmitting information about cold stimuli in the presence of an A-fiber block (presumably C-fibers) evoke burning, aching, and pricking qualities (67). Interestingly, the activation of some cold fibers by noxious heat may be the basis for the paradoxical cold sensation felt by stimulating cold spots with noxious heat stimuli (67).

Transduction of noxious mechanical stimuli
Whereas heat- and chemical-induced nociceptor responses correlate with pain perception in humans (9, 24), mechanical stimulation of C-MH (24) and rapidly adapting A-HTM (18) fibers may not (24) (Tables 1 and 2). The perception of pinprick pain intensity, however, is related to activity in capsaicin-insensitive A-fiber nociceptors (e.g., A-M and A-MH type I) (78). Transduction channels mediating mammalian noxious (and innocuous) mechanical stimuli have been elusive (5, 91, 92). Transduction in soma membranes on a submillisecond time scale suggests direct gating by pressure efficacies of stimulation protocols applied in behavioral and ex vivo spots with noxious heat stimuli (67).

C-fibers in the skin and exacerbates nociceptor excitability and pain (see below). It is at the spinal level that nonnociceptive neurons are recruited by strong nociceptor activation through functional modulation of local circuits (105).

Conduction
Nociceptors express a wide variety of voltage-gated channels (e.g., Na, Ca, K) that transduce the receptor potential into an action potential or, more commonly, a set of action potentials that encode the intensity of a noxious stimulus applied within their receptive fields. There are 9 known Na, 10 Ca, and 40 K genes in mammals (http://www.iuphar-db.org/DATABASE/), many of which have multiple splice variants with different functional characteristics (99). Cell excitability and firing behavior (e.g., threshold for action potential generation, action potential and undershoot amplitude and duration, and maximal firing frequency) depend on the complement of these channels as well as those contributing to frequency modulation (e.g., hyperpolarization-activated cyclic nucleotide-gated cation channel [HCN] and A-type K,4.3 and K,3.4 channels) (54). For instance, nociceptors responsive to noxious cold require the expression of the tetrodotoxin-resistant (TTX-resistant) Na,1.8 channel at the peripheral terminal (100), and mice lacking Na,1.8 and Na,1.7 display deficits in mechanosensation (95, 101). Peripheral CGRP release by inflammatory mediators is unaltered by TTX, suggesting an important role of TTX-resistant Na, in regulated pain thresholds, consistent with their robust modulation by bradykinin (BK) and PGE (102) (see below). Since enhanced excitability of primary sensory neurons in inflammatory and pathologic pain states is a major contributor to the perception of pain, specific pharmacological agents that specifically dampen aberrant activity are desirable in the design of pain therapeutics. To this end, an understanding of species-specific differences is critical, as exemplified by the dramatically different phenotypes in mice and humans lacking Na,1.7: although mice lacking Na,1.7 show a mechanosensitive (pinch) and formalin-induced (5%) pain phenotype (103), humans lacking Na,1.7 are insensitive to pain altogether (104).

Central modulation
Nociceptors release a variety of substances from their central terminals that have the potential of exciting second-order neurons through multiple mechanisms. Fast and slow synaptic transmission are mediated in large part by glutamate and peptides (e.g., substance P, CGRP), respectively (7, 38). Of particular importance to pain perception is the plasticity in synaptic strength (i.e., the ability to enhance homosynaptic as well as heterosynaptic connections) between primary afferents and the relay and interneurons they drive, presynaptic and postsynaptic modulation by descending facilitatory and inhibitory pathways in the spinal cord, and the effector aspects of nociceptor function activated by strong GABAergic/glycinergic depolarization of presynaptic terminals leading to the dorsal root reflex (37, 105). Anterograde transmission of action potentials from the spinal cord to the periphery results in release of peptides and other inflammatory mediators in the skin and exacerbates nociceptor excitability and pain (see below). It is at the spinal level that nonnociceptive neurons are recruited by strong nociceptor activation through functional modulation of local circuits (105).

Adaptive and maladaptive shifts in pain threshold
Injury to the skin induces protective physiological responses aimed at decreasing the likelihood of exacerbating the injury. After an injury induced by pungent chemicals (e.g., capsaicin, mustard oil) and burn, stimulation of the injured area produces enhanced pain to noxious stimuli (primary mechanical and thermal hyperalgesia) dependent on C-fiber activity that manifests as a decrease in threshold to activate C-MH fibers and to perceive pain (9, 19, 106). Immediately surrounding the injured area, a zone of flare (reddening) develops and stimulation of even a larger secondary zone produces pain in response to normally innocuous stimuli (e.g., brush stroke) (secondary mechanical allodynia) as well as enhanced responsiveness to noxious mechanical (secondary mechanical hyperalgesia) and thermal (heat) hyperalgesia if spatial summation is invoked (secondary thermal hyperalgesia) (21, 105, 107). Here, noxious punctate stimulation of C-nociceptors induces secondary mechanical hyperalgesia mediated by A-nociceptors (7) and innocuous dynamic mechanical stimuli (gentle stroking) provokes nonnociceptor A-fiber–mediated pain...
(108). Cellular mechanisms underlying this complicated response involve both peripheral and central processes (14, 38, 105, 107) and require nociceptor input, particularly A-MH and C-MH fibers (19, 91, 105). After a burn, A-MH fibers (most likely type I) mediate primary heat hyperalgesia in glabrous skin (9).

What are the cellular mechanisms mediating hyperalgesia? Electrical stimulation of the majority of C-polymodal fibers produced plasma extravasation in their peripheral receptor field (109). Central propagating impulses can antidromically invade peripheral arborizations innervating other areas in the afferent’s receptive field (axon reflex), causing the release of peptides (e.g., substance P, CGRP, somatostatin) and/or other bioactive substances from the terminal (e.g., cytokines) into the interstitial tissue (17). The released substances produce a myriad of autocrine or paracrine effects on endothelial, epithelial, and resident immune cells (Langerhans), which lead to arteriolar vasodilatation (“flare,” via effects on endothelial, epithelial, and resident immune cell pathways) exists at multiple levels including stimulus transduction (“hyperalgesic priming”) evoked by cytokine- and neurotrophin-induced recruitment of Glu/PKCε signaling in nociceptors can produce prolonged sensitization and mechanical hyperalgesia and may contribute to chronic pain (114). Prolonged pain perception observed in inflammatory pain models is generally believed to be produced by ongoing nociceptor activity (15); for example, produces nocifensive behaviors through its activation of TRPA1 (115, 116). Secondary hyperalgesia to punctate pinprick stimuli is mediated at least in part by capsaicin-insensitive A-fiber nociceptors (e.g., A-MH type I and A-M; Table 2) by central sensitization processes (78, 108).

Do labeled lines transmit noxious stimulus information? Determining the extent to which pain qualities are dependent on the activation of subpopulations of neurons and intensity coding poses a considerable challenge and is an active area of research (13). Psychophysical studies on spinal cord injury patients suffering from partial or complete loss of thermal sensitivity support a model in which both pain-specific pathways and nonnociceptive pathways are integrated (117). Significant crosstalk between these pathways exists at multiple levels including stimulus transduction (118), peripheral terminals during neurogenic inflammation, and central connections during central sensitization and may underlie paradoxic temperature sensation. To address the extent by which particular nociceptive signaling pathways encode particular modalities, a number of approaches have been taken. Genetically encoded tracers have enabled visualization of specific subpopulations of sensory neurons (e.g., Mrgpr family, ref. 30; TRPM8, refs. 76 and 77) and determination of their innervation patterns as well as cellular function. Pharmacologic and hereditary genetic ablations have defined the role of nociceptors in pain but until recently have included multiple or entire nociceptor populations (e.g., capsaicin-induced ablation of TRPV1-expressing neurons; refs. 42 and 119) or sensory and autonomic populations (2). Genetically mediated ablation using toxins (e.g., diphtheria A) transcribed by promoters of genes expressed in specific neuronal subtypes (e.g., Na,1.8-expressing neurons, ref. 120) provides a means to address the contributions of particular cells to acute nociception and hyperalgesia and allodynia after injury.

Mice expressing diphtheria toxin under the Na,1.8 promoter reveal significant loss of sensory neurons (85% and 13% of unmyelinated and myelinated neurons, respectively, consistent with Na,1.8 expression in most nociceptors as well as some myelinated sensory neurons). These mice are unresponsive to acute noxious mechanical (pressure-induced, not punctate) and cold stimuli and defective in the development of inflammatory pain, but are normal with regard to acute responsiveness to heat stimuli and the ability to develop neuropathic pain (120). These data are remarkably consistent with gene-deletion studies (103). The sensitivity of C-MH fibers innervating hairy skin to cold, heat, and mechanical stimuli is reduced in mice constitutively lacking MrgprD-expressing cells reported to be TRPV1 and peptide negative (121). However, when genetic ablation of this population of cells is done in adulthood, behavioral deficits were observed to mechanical but not thermal stimuli (122). An additive loss of both mechanical- and heat-induced nocifensive behaviors was achieved after further pharmacologic ablation of central TRPV1⁺ terminals, suggesting a separation of mechanical and thermal modalities at all levels of sensory processing in the pain pathways subserved by MrgprD-and TRPV1-expressing cells (122). The extent to which this separation is maintained for other murine nociceptor populations is a subject of active research.

**Future challenges**

Despite significant progress in understanding the complexities of mammalian nociception and pain perception in the last half century, our knowledge is far from complete with regard to the identity of the full complement of sensors of noxious stimuli (particularly with regard to mechanotransduction), the role of nociceptor heterogeneity in physiological and pathological pain, the coding of the quality of the stimulus, and the modulation of pain pathways by peripheral and central mechanisms. A focus on mechanisms underlying thermal nociception and hyperalgesia is in large part due to the identification of the TRP family of channels. The future identification of elusive mechanotransducers in somatosensory neurons will likewise thrust the direction of research toward a cellular/molecular understanding of mechanical hyperalgesia and allodynia. The application of genetic technologies and pharmacological approaches to understanding the contributions of molecules, signaling pathways, and cell populations to nocifensive behaviors to particular stimulus modalities in normal...
and pathophysiological states in rodents will inspire hypotheses that ultimately must be tested in humans.

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