Perspectives on: Information and coding in mammalian sensory physiology

Probing mammalian touch transduction

Diana M. Bautista[1] and Ellen A. Lumpkin[2,3]

[1]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720
[2]Department of Dermatology and [3]Department of Physiology and Cellular Biophysics, Columbia University College
 of Physicians and Surgeons, New York, NY 10032

Humans rely on our sense of touch for a broad range of essential behaviors, such as feeding, successful child rearing, and avoiding bodily harm. Although widely regarded as one of the five basic senses, touch is a complex sense that encompasses numerous modalities, including stretch, pressure, and vibration. Touch-sensitive neurons display a corresponding diversity of force sensitivities, physiological outputs, and cellular morphologies. Although forward genetic screens have identified several essential molecules in invertebrate mechanosensory neurons, we are only now beginning to uncover molecular players that govern the unique functions of discrete populations of touch receptors in mammals. Recent progress has resulted from the convergence of mouse genetics, genomics, developmental neurobiology, in vitro approaches, and neurophysiological techniques. With this tool kit, we are now poised to answer long-standing questions: Do distinct molecules transduce force in light-touch and pain receptors? What cell types and circuits subserve different perceptual qualities in tactile discrimination? This Perspective describes the most recent advances in our knowledge of molecules, cells, and circuits that encode tactile stimuli, which will help uncover the mechanisms governing touch transduction in mammals.

Our somatic senses of touch and pain enable numerous behaviors fundamental to human existence, allowing us to eat, communicate, and survive. Acute pain is a warning signal that alerts us to noxious mechanical, chemical, and thermal stimuli, which are potentially tissue damaging. During inflammation or injury, we experience a heightened sensitivity to touch that encourages us to protect the injured site. Despite this essential protective function, pain can outlast its usefulness and become chronic. Numerous pathophysiological conditions result in the chronic dysregulation of mechanosensory signaling, leading to pain triggered by light touch (allodynia), as well as enhanced sensitivity to noxious mechanical stimuli (hyperalgesia) (Gilron, 2006).

Light-touch receptors, which mediate discriminative touch, enable fine tactile acuity that allows us to manipulate objects with high precision. As humans, we depend on this skill for everyday tasks that range from eating with utensils to texting. Discriminative touch is also central to social interactions, such as mating, maternal bonding, and successful child rearing (Tessier et al., 1998; Feldman et al., 2010). Indeed, proper brain development requires input from peripheral touch receptors (Fox, 2002). Depriving infants of mechanosensory stimulation leads to striking developmental and cognitive deficits (Kaffman and Meaney, 2007). For example, premature human infants housed in incubators display delayed neurological development and growth, which can be improved by only 45 min of touch a day (Ardiel and Rankin, 2010). In touch-deprived rodents, attentional and behavioral deficits persist through adulthood, underscoring the importance of mechanosensory inputs during development (Ardiel and Rankin, 2010).

To understand the senses of touch and pain, we must unravel peripheral mechanisms that encode tactile stimuli and discover how the brain interprets these signals to dictate behavior. The transduction of a physical force on the skin into an electrical signal is the first step in the encoding of tactile stimuli. In this Perspective, we focus on the most current developments in our understanding of the cells and molecules that mediate touch transduction in the periphery. We refer the reader to recent reviews that more comprehensively cover principles of mechanotransduction and somatosensory signaling (Kung, 2005; Basbaum et al., 2009; Chalfie, 2009; Lumpkin et al., 2010).

Mammalian touch receptors are diverse

The skin is innervated by a variety of somatosensory neurons with distinct morphological end-organs and physiological properties (Fig. 1). This array of cutaneous

Correspondence to Diana M. Bautista: dbautista@berkeley.edu; or Ellen A. Lumpkin: eal2166@columbia.edu

Abbreviations used in this paper: DRG, dorsal root ganglia; IA, intermediate adapting; NT, neurotrophin; RA, rapidly adapting; SA, slowly adapting; TRPV, transient receptor potential vanilloid.

neuronal subtypes is thought to represent different tactile qualities, such as shape, texture, and vibration (Johnson, 2001), as well as a wide range of noxious stimuli (Basbaum et al., 2009). With few exceptions, the correspondence between an end-organ and its physiological response is only correlative, and class-specific molecular markers are just now beginning to emerge (Loewenstein and Rathkamp, 1958; Woodbury and Koerber, 2007; Bourane et al., 2009; Luo et al., 2009).

Each somatosensory neuron has a soma located in the trigeminal ganglia or dorsal root ganglia (DRG) and a branching sensory afferent that sends signals from the periphery to the spinal cord and/or hindbrain. The peripheral branches of touch-receptive afferents innervate the skin, where they transduce mechanical stimuli into action potentials. These cutaneous sensory neurons can be physiologically classified based on conduction velocity (set by degree of myelination), mechanical threshold, adaptation properties, and modality, defined as the type of stimulus to which they best respond.

In general, light-touch receptors are thickly myelinated Aβ or thinly myelinated Aδ afferents. These somatosensory neurons tend to have large somatal diameters and express neurofilament 200, an intermediate filament protein. Within this broad category, rapidly adapting (RA) and slowly adapting (SA) receptors can be distinguished.

RA afferents, which fire action potentials selectively at the onset and offset of a touch stimulus, innervate several different cutaneous structures (Fig. 1). In the hairy skin covering most of our body, lanceolate endings and circumferential afferents surround hair follicles, where they are thought to signal hair movements. Notably, down-hair afferents are Aδ fibers that are among the

most sensitive light-touch receptors in mammalian skin. In the glabrous skin of our palms, RA afferents innervate Pacinian corpuscles and Meissner's corpuscles, which are vibration receptors that encode texture. The lamellae of these corpuscles serve as mechanical filters to set the adaptation profiles of the Aβ afferents they envelope (Loewenstein and Mendelson, 1965).

SA afferents fire action potentials throughout a sustained touch stimulus (Fig. 1). SAI afferents, which have the highest spatial acuity of mammalian touch receptors, are proposed to represent object features such as edges and curvature (Johnson, 2001). These Aβ afferents innervate Merkel cells (Woodbury and Koerber, 2007), which are keratinocyte-derived epidermal cells that are required for SAI responses (Maricich et al., 2009; Morrison et al., 2009; Van Keymeulen et al., 2009). SAII afferents, which are sensitive to directional skin stretch, are thought to contribute to hand grip and awareness of finger position (Johnson, 2001; Zimmermann et al., 2009). These Aβ afferents are proposed to terminate in Ruffini endings, although the presence of this end-organ in different species and skin areas is debated. Along with Aβ afferents, the hairy skin is innervated by a rare subset of unmyelinated C-afferents that are activated by innocuous touch stimuli and are marked by selective expression of vesicular glutamate transporter 3 (Seal et al., 2009).

Nociceptors, which initiate pain perception, are thought to be free nerve endings that fall into C-afferent or Aδ-afferent categories. A large variety of biochemically and physiologically distinct C-afferent subtypes respond to an array of mechanical and thermal stimuli, as well as endogenous and exogenous chemicals (Basbaum et al., 2009). In many cases, nociceptors are polymodal, responding robustly to multiple sensory stimuli. Although most
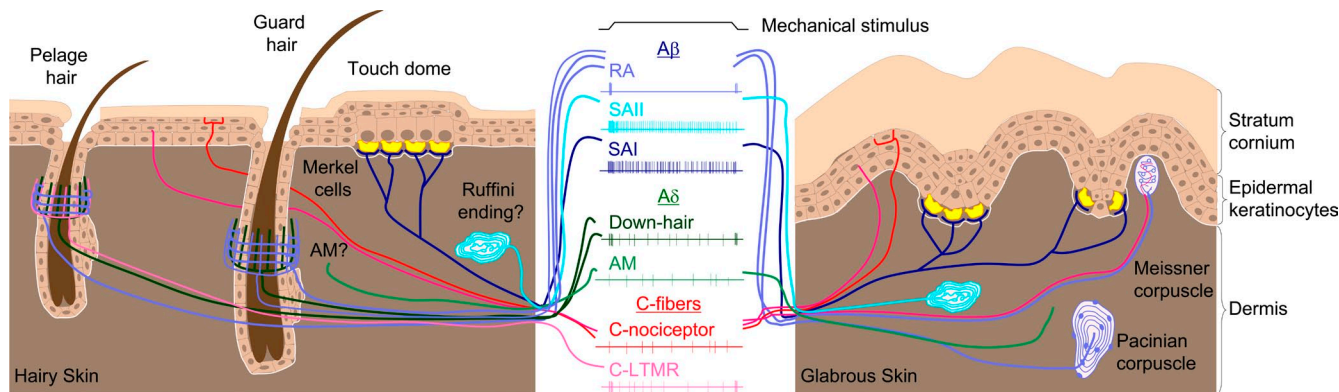

Figure 1. Cutaneous touch receptors. Mechanosensory afferents innervating mammalian skin display morphological and functional diversity. Cartoons depict end-organs in hairy skin (left) and glabrous skin (right), although innervation density is not meant to be representative. For physiologically defined afferent classes, typical action potential trains evoked by touch stimuli are schematized (center). Thickly myelinated Aβ afferents (blue shades) are touch receptors that display RA or SA responses to mechanical stimuli. RA afferents innervate hair follicles, Pacinian corpuscles, and Meissner's corpuscles. SAI afferents innervate epidermal Merkel cells (yellow), and SAII afferents are thought to innervate Ruffini endings. Thinly myelinated Aδ afferents (green shades) include down-hair afferents and A-mechanonociceptors. C-afferents (red and magenta), which surround hair follicles (Park et al., 2003) and abundantly innervate the epidermis, include peptidergic nociceptors, nonpeptidergic nociceptors, and C low-threshold mechanoreceptors.

C-afferents have traditionally been classified as nociceptors, based on their high mechanical thresholds and projection patterns to the spinal cord (Smith and Lewin, 2009), recent studies have implicated C-afferents in other cutaneous senses, such as warm and cool (Peier et al., 2002; Dhaka et al., 2008). High-threshold A-afferent mechanonociceptors are also observed electrophysiologically, although the cutaneous end-organs of these afferents are not known (Zimmermann et al., 2009).

C-afferents richly innervate the epidermis of hairy and glabrous skin (Fig. 1). Peptidergic afferents, which express neuropeptides such as Substance P or calcitonin gene-related peptide, innervate mid-layers of the epidermis. In contrast, nonpeptidergic afferents, most of which express the Mas-related G protein–coupled receptor MrgD, selectively innervate the outermost living skin layer (stratum granulosum) (Zylka et al., 2005). Interestingly, under normal and inflammatory conditions, mice lacking MrgD-positive afferents display decreased responsiveness to noxious mechanical stimuli but normal sensitivity to heat and cold (Cavanaugh et al., 2009). Thus, these afferents may play a selective role in acute mechanical pain and tactile hypersensitivity.

An intriguing open question is whether cutaneous afferents themselves mediate transduction in all mechanosensory modalities or whether epidermal cells also play a role in sensory signaling (Lumpkin and Caterina, 2007). It is clear that nociceptors express some sensory transduction channels, such as the capsaicin receptor transient receptor potential vanilloid (TRPV)1 (Caterina et al., 1997); however, keratinocytes also express putative sensory transduction channels, including TRPV3 and TRPV4 (Lumpkin and Caterina, 2007). Moreover, keratinocytes, Merkel cells, and Pacinian corpuscles express neurotransmitters (Lumpkin et al., 2010), receptors for which are expressed in somatosensory afferents. For example, keratinocytes release ATP in response to sensory stimuli in vitro, and MrgD-positive epidermal sensory neurons express the ATP-gated ion channel P2X3 (Dussor et al., 2008). Although these findings are suggestive, the roles of epidermal cells in touch transduction have not been defined.

Molecular specification of somatosensory cell types

Developmental studies, particularly in genetically modified mouse models, have begun to illuminate mechanisms underpinning the variety of mammalian touch receptors (Luo et al., 2007). Almost all nociceptors require nerve growth factor and its receptor TrkA for specification. At late embryonic stages, nonpeptidergic C-afferents begin to express the transcription factor Runx1 and Ret, a receptor for glial-derived neurotrophic factor ligands (Kramer et al., 2006; Luo et al., 2007). Postnatally, these nonpeptidergic nociceptors turn off TrkA expression, whereas peptidergic C-afferents maintain TrkA expression and require nerve growth factor for survival.

Touch receptors are also specified by neurotrophic factors and developmental transcription factors. RA afferents depend on early embryonic Ret expression and the transcription factor MafA for proper development (Bourane et al., 2009; Luo et al., 2009). Down-hair lanceolate endings are distinguished by their developmental dependence on neurotrophin (NT)-4 (Stucky et al., 1998); Merkel cell–neurite complexes generally require NT-3 and its receptor TrkC for postnatal survival (Airaksinen et al., 1996). In whisker follicles, Merkel cell innervation depends on the transcription factor Runx3 (Senzaki et al., 2010). Proprioceptive neurons, which represent another NT-3–dependent mechanosensory population, are also lost in Runx3 mutants (Levanon et al., 2002; Kramer et al., 2006). Like mechanosensory hair cells of the inner ear, epidermal Merkel cells are vertebrate-specific cells whose development depends on the transcription factor Atonal 1 (Maricich et al., 2009; Morrison et al., 2009; Van Keymeulen et al., 2009).

Based largely on their distinct developmental pathways, some types of touch receptors can now be identified with genetically encoded markers (Lumpkin et al., 2010). These markers are essential tools for identifying molecules that govern the distinct responses of touch-receptor subtypes.

Molecular mechanisms of mammalian touch transduction

In mechanosensory cells, ion channels underlie the transduction of mechanical stimuli into electrical signals. There are two models of how such ion channels are activated. The first model postulates that force-sensitive ion channels are directly activated by changes in membrane tension or distortion. This is the case for the osmosensitive bacterial channels MscS and MscL (Kung, 2005) and members of the two-pore potassium channel family, KCNK (Kung et al., 2010). The second model posits that gating requires tethering molecules that link the transduction channel to the cytoskeleton or extracellular matrix. This model stems from studies in mechanosensory hair cells, where cadherin family proteins and myosins are required for mechanotransduction (Schwander et al., 2010); however, the molecular basis of mechanotransduction in mammalian somatosensory neurons remains enigmatic.

Members of the TRP channel, acid-sensing ion channel, and KCNK channel families have been proposed to function as transduction channels in somatosensory neurons. Because genetic deletion of candidates only subtly alters cellular and/or behavioral mechanosensitivity, the importance of these channels in mammalian mechanotransduction remains controversial. These issues have been extensively discussed in several reviews and will not be covered in detail here (Lewin and Moshourab, 2004; Christensen and Corey, 2007; Lumpkin and Caterina, 2007; Basbaum et al., 2009). More recently, two members of the FAM38 gene family,

FAM38A and FAM38B, have been implicated in somatosensory mechanotransduction.

A role for FAM38A and FAM38B in mechanotransduction stems from an unbiased screen to identify genes required for mechanosensitivity in the Neuro2A mouse neuroblastoma cell line (Coste et al., 2010). Each gene is a complex locus predicted to produce more than a dozen isoforms through alternative promoters and splicing (Thierry-Mieg and Thierry-Mieg, 2006). The proteins encoded by these genes, Piezo1 and Piezo2, are large membrane proteins with up to 30 and 34 predicted transmembrane domains, respectively (Fig. 2 A); however, no putative pore domains or channel-like repetitive domains have been identified. Piezo1 is broadly expressed, including in mechanosensitive tissues such as bladder, lung, and skin (Thierry-Mieg and Thierry-Mieg, 2006; Coste et al., 2010). Piezo1 is also expressed in senile plaque–associated astrocytes (Satoh et al., 2006). Piezo2 transcripts are also detected in several tissues but appear to be most abundant in DRG, bladder, and lung (Thierry-Mieg and Thierry-Mieg, 2006; Coste et al., 2010).

Notably, Piezo1 was also identified in a functional screen for transcripts that regulate integrins, which are mechanosensitive cell adhesion molecules (McHugh et al., 2010). Integrins are transmembrane receptors that serve as a mechanical link between the extracellular matrix and the cytoskeleton. They serve as signaling hubs that, in response to mechanical load, initiate numerous intracellular signaling cascades that govern gene transcription, cell motility, and differentiation (Legate et al., 2009). Integrins mediate mechanotransduction in a variety of physiological contexts, including cell rigidity, migration, organogenesis, and development. FAM38A was

shown to activate integrin signaling by recruiting the R-Ras GTPase to the ER. Whether Piezo1 and Piezo2 are functional ion channels, accessory subunits of mechanosensitive channels, or signaling molecules within a mechanosensitive pathway (e.g., integrin signaling) remains unanswered. In favor of a channel hypothesis, however, expression of Piezo1 or Piezo2 confers displacement- and suction-evoked currents in heterologous cells, such as HEK293 cells (Fig. 2 B).

The diversity of candidate transduction channels raises an important question: what criteria must be satisfied by a bona fide mechanotransduction channel in mammalian somatosensory neurons? Christensen and Corey (2007) previously outlined a set of functional criteria for assessing whether a candidate ion channel is directly activated by mechanical stimuli. Here, we extend this set of criteria to assess whether a candidate mediates mammalian somatosensory mechanotransduction, using Piezo1 and Piezo2 as examples. Most studies of previous transduction candidates used different stimuli and criteria to assess mechanosensitivity, thus making it difficult to compare between studies.

Is the candidate in the right place? It is possible that distinct molecules transduce mechanical stimuli in the different classes of touch receptors schematized in Fig. 1. Thus, at a minimum, a candidate transduction molecule must be expressed in the skin or sensory ganglia and localize to at least one sensory cell type. Because transduction occurs in cutaneous end-organs, bone fide transduction channels should also localize to the plasma membranes of peripheral endings. It is worth noting that a candidate need not be highly expressed to function as a transduction channel, especially if it
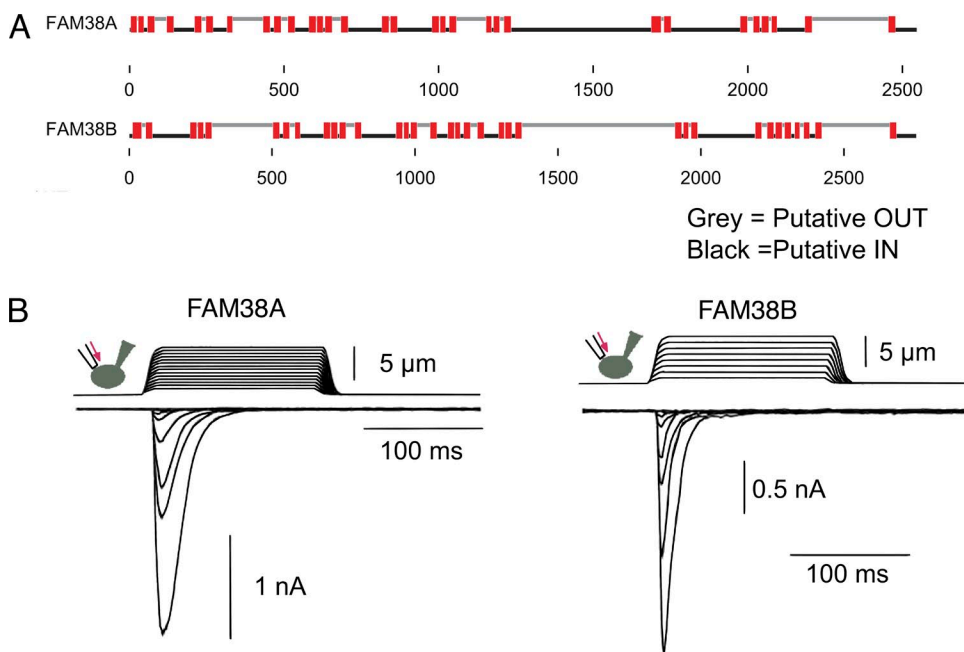

Figure 2. Piezo1 and Piezo2 are candidate mechanotransduction molecules. (A) Predicted hydropathy plots for Piezo1 and Piezo2 proteins. The plot displays putative transmembrane (red), intracellular (black), and extracellular (gray) domains, as predicted by the TMHMM 2.0 server. (B) Mechanically activated currents in HEK293T cells expressing Piezo1 (FAM38A; left) or Piezo2 (FAM38B; right). Representative inward currents in response to a series of 1-µm mechanical steps applied via a glass probe. Whole cell recordings performed at −80 mV. B is modified with permission from Coste et al. (2010).

mediates transduction in only a small population of touch-sensitive neurons.

How well do the Piezos meet these expression criteria? Quantitative PCR analysis shows preferential expression of Piezo2 in somatosensory ganglia and Piezo1 enrichment in the skin. In situ hybridization shows Piezo2 localization in ~20% of DRG neurons. Most of these are likely to represent nociceptors, as they coexpress nociceptive markers such as peripherin or TRPV1. Other Piezo2-positive DRG neurons express the myelination marker NF200; these Aβ or Aδ neurons might include light-touch receptors (Coste et al., 2010). Antibody staining of heterologously expressed Piezo2 shows high intracellular levels and, to a lesser extent, plasma membrane expression (Coste et al., 2010; McHugh et al., 2010). Similarly, a GFP-tagged Piezo1 localizes to the ER in HeLa cells (McHugh et al., 2010). The subcellular distribution of endogenously expressed Piezo1 or Piezo2 in the skin or DRG neurons has not yet been reported. Thus, the tissue distribution is consistent with a role for Piezos in mechanotransduction, but key information about subcellular localization is still lacking. Moreover, because Piezo2 is expressed in only a subset of DRG neurons, additional candidates must be identified in other somatosensory cell types.

Is the candidate intrinsically mechanosensitive? If an ion channel is directly gated by force, a candidate's mechanical properties can be directly compared with endogenous transduction mechanisms. One caveat is that heterologous expression will not produce mechanosensitive currents if accessory proteins or specific cellular contexts are required for force gating. Indeed, the Deg/ENaC isoforms that transduce gentle touch in *Caenorhabditis elegans* do not appear to be mechanically gated when heterologously expressed (Lumpkin et al., 2010). This stumbling block has made it difficult to assess mammalian Deg/ENaC mechanotransduction candidates, such as the acid-sensing ion channels, that do not confer mechanosensitivity in heterologous cells. For such ion channels, we must rely on other physiological properties, such as selectivity or pharmacological profiles, for comparison with endogenous currents.

Like the mechanosensitive KCNK channels (Kung et al., 2010), either Piezo1 or Piezo2 expression alone is sufficient to confer mechanically evoked currents in heterologous cell types (Coste et al., 2010). This finding is promising because the mechanosensitivity, pharmacology, and biophysical characteristics of Piezo-dependent currents can now be directly compared with those of endogenous mechanically activated currents in sensory neurons.

Does the candidate display characteristics of endogenous transduction channels in sensory neurons? Somatosensory neurons retain mechanosensitivity when dissociated and placed in culture. Because it is not clear which in vitro mechanical stimuli best represents tactile stimulation in vivo, a variety of mechanical stimulus paradigms have been tested on dissociated sensory neurons. Several of these paradigms reliably produce mechanosensitive responses in sensory neurons; however, their relation to physiological forces in tissues remains unclear. Nonetheless, in vitro recordings are, at present, the most direct way to assess mechanically evoked responses at the cellular level.

Hypo-osmotic solutions induce cell swelling that leads to calcium influx and neuronal excitation in a subset of sensory neurons (Fig. 3 A; Viana et al., 2001). Osmotic responses require extracellular calcium but are not significantly blocked by voltage-activated calcium channel antagonists, suggesting that swelling triggers calcium influx through an unknown conductance. A second stimulus paradigm is radial stretch of neurons cultured on elastic membranes. Like osmotic stimuli, radial stretch triggers calcium increases in a subset of sensory neurons that require extracellular calcium and are not inhibited by voltage-activated calcium channel blockers (Fig. 3 B; Bhattacharya et al., 2008). Third, like many mammalian cell types, cultured sensory neurons have stretch-activated channels that are gated by suction or pressure applied through a recording pipette (see, for example, Cho et al., 2006). The fourth and most commonly used technique for probing cellular mechanosensitivity is focal displacement applied to the soma or neurite (Fig. 3 C). Such stimulation triggers calcium influx and several currents with distinct properties.

Several groups have reported displacement-evoked mechanosensitive currents; however, stimulation protocols, recording conditions, parameters measured, and model organism vary between these reports. As such, basic properties of mechanically evoked currents differ somewhat between studies, making it difficult to define benchmarks for comparison with candidate mechanotransduction channels. Touch-evoked currents can be elicited by 2–16-µm displacements of cell somata (McCarter et al., 1999; Drew et al., 2002, 2007; Drew and Wood, 2007; Hao and Delmas, 2010; Rugiero et al., 2010) or <1-µm displacement of neurites (Hu and Lewin, 2006). One commonality is the existence of three types of displacement-evoked currents: RA, intermediate adapting (IA), and SA (Table I and Fig. 3 C, bottom).

RA currents display fast kinetics of activation and desensitization (0.5 and 1 ms, respectively) and block by several pharmacological agents. In rat neurons, nonselective RA cation currents are blocked by: Ca^{2+}; Gd^{3+}, which inhibits many types of stretch-activated ion channels; the conotoxin NMB1; the styryl dye FM1-43; the TRP channel inhibitor ruthenium red; and cytochalasin B, which inhibits actin polymerization (McCarter et al., 1999; Drew et al., 2002, 2007; Drew and Wood, 2007; Hao and Delmas, 2010; Rugiero et al., 2010).

Mouse neurons display different characteristics in different studies. In one study, a 16-µm displacement of cell somata elicited ruthenium red–sensitive nonselective cation currents (Drew et al., 2004). In other studies, ≤1-µm displacements elicited sodium-selective currents blocked by Gd^{3+} and NMDG (Hu and Lewin, 2006; Lechner et al., 2009).

At least two populations of RA neurons can be distinguished by their action potential shapes and the developmental stage at which they appear (Lechner et al., 2009). At mouse E13.5, a subset of large DRG neurons, which express TrkB or TrkC, display mechanosensitive RA currents that are Na^+ selective. These neurons are likely to correspond to low-threshold Aβ afferents, such as light-touch receptors and proprioceptors. At E15.5, a second population of RA neurons appears that have small somatal diameters and broad action potentials characteristic of nociceptors.

Other classes of putative nociceptors display IA and SA mechanically evoked currents in culture (Hu and Lewin, 2006). IA currents are nonselective, inactivate in tens of milliseconds, are blocked by NMB1 (rat) and Gd^{3+} (mouse), and are relatively rare in embryonic neurons (Lechner et al., 2009). SA currents, which emerge postnatally in dissociated sensory neurons (Lechner et al., 2009), are nonselective, inactivate over hundreds of milliseconds, and are blocked by Gd^{3+}, ruthenium red in mouse and rat neurons, cytochalasin B, Ca^{2+}, FM1-43 (rat neurons) (McCarter et al., 1999; Drew et al., 2002,

2007; Drew and Wood, 2007; Hao and Delmas, 2010; Rugiero et al., 2010), and the TRPA1 antagonist HC030031 in mouse neurons (Vilceanu and Stucky, 2010).

In cultured rat sensory neurons, mechanically evoked RA and SA currents display calcium-independent and voltage-dependent desensitization during sustained mechanical stimuli (Hao and Delmas, 2010; Rugiero et al., 2010). This is markedly different from the adaptation properties of mechanosensitive currents measured in inner-ear hair cells, indicating that distinct mechanisms regulate transduction in these mechanosensory cell types.

Mechanically evoked currents in Piezo1- or Piezo2-expressing HEK293T cells share several features with displacement-evoked currents in sensory neurons. For example, similar to currents in DRG neurons, Piezo-dependent currents are activated by micrometer focal displacement of somata. Activation kinetics appear to be in the low millisecond range. Piezo1 inactivation occurs with a time constant of 15 ms, most similar to IA currents in sensory neurons. Piezo1 is blocked by Ca^{2+}, Gd^{3+}, and ruthenium red, whereas Piezo2 is blocked by NMDG, Gd^{3+}, and ruthenium red. The spider toxin GsMTx-4, which inhibits stretch-activated channels in a variety of cell types (Bowman et al., 2007), also blocks Piezo1-depedent currents (Bae et al., 2011).

Piezo-dependent currents are also strikingly similar to suction-activated currents observed in a subset of small-diameter cultured sensory neurons (Cho et al., 2006). For example, the single-channel conductance of
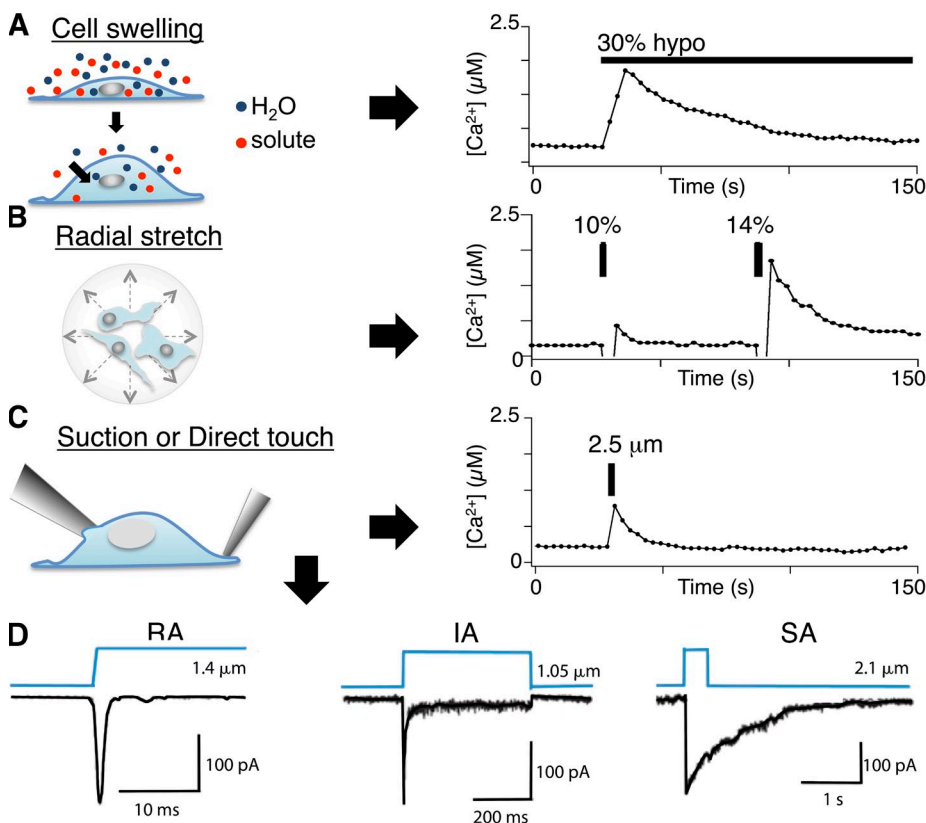

Figure 3. Cell-based assays to probe mechanotransduction. (A) Application of hypo-osmotic solutions causes stretch-evoked calcium signals in DRG neurons. (B) Radial stretch of DRG neurons grown on silastic membranes elicits dose-dependent calcium influx. (C) Membrane suction activates stretch-activated channels while focal pressure applied to the DRG soma triggers calcium influx in cultured DRG neurons. (D) Focal pressure applied to the neurites of sensory neurons elicits RA, IA, and SA currents. D is modified with permission from Lechner et al. (2009. *EMBO J.* 28:1479–1491).

Piezo1-dependent channels in N2A cells matches that of one class of endogenous stretch-activated channels (23 pS). Like Piezo-dependent currents, these stretch-activated channels are Gd^{3+}-sensitive nonselective cation channels.

Collectively, these physiological features support a parsimonious model in which Piezo proteins form mechanosensitive channels; however, many questions remain unanswered. At a biophysical level, stimulus–response relations, adaptation properties, and activation/inactivation kinetics need to be more thoroughly defined.

Is the candidate required for mechanotransduction in cultured sensory neurons? A bone fide transduction channel must be required for native mechanotransduction currents. Thus, gene disruption, RNA interference, or selective antagonists that target a candidate should alter stimulus–response properties, adaptation, ionic selectivity, or conductance of endogenous currents in sensory neurons. Functional disruption of a mechanotransduction channel is also expected to be modality specific, altering only mechanical sensitivity and not responsiveness to thermal or chemical stimuli. Promisingly, treatment of

cultured DRG neurons with Piezo2 short-interfering RNA decreased the proportion of sensory neurons with RA currents and showed a trend toward increased incidence of mechanically insensitive neurons (Coste et al., 2010). In contrast, the proportion of neurons exhibiting IA or SA currents was comparable in control and Piezo2-targeted cultures. Collectively, these findings suggest that Piezo2 is specifically required for mechanically evoked RA currents in cultured sensory neurons.

Is the candidate required for touch-evoked responses in vivo? An essential step in validating candidate transduction molecules is to use in vivo approaches to determine the functional importance of the candidate in somatosensory signaling. This is typically achieved by disrupting a candidate gene in mice and then assaying somatosensory signaling with intact electrophysiological recordings and behavioral assays.

Intact recordings provide key information by demonstrating whether a candidate is required for touch sensitivity in specific subsets of touch receptors and whether it functions in the periphery (Fig. 1). In rodents, mechanically evoked action potentials can be recorded

TABLE I

Properties of mechanically activated currents in cultured sensory neurons and HEK293 cells expressing Piezo1 or Piezo2

Cell type	Ionic selectivity	Stimuli	Activation tau	Inactivation tau	Adaptation mechanism	Block
		μm	ms	ms		
Rat DRG RA[a,b,c,d,e,f]	Nonselective cation	2–12[a]	ND	3	Voltage-dep., Ca^{2+}-indep.	Ca^{2+}, Gd^{3+}, NMB1, ruthenium red, FM1-43, cytochalasin B
Rat DRG IA[d,e]	Nonselective cation	2–12	ND	21	ND	NMB1
Rat DRG SA[a,b,c,d,e,f]	Nonselective cation	2–12	ND	296 (τ_1) 1,140 (τ_2)	Voltage-dep., Ca^{2+}-indep.	Gd^{3+}, ruthenium red, cytochalasin B, Ca^{2+}, FM1-43
Mouse DRG RA[g,h]	Sodium[g]	0.75–1 (neurite) 4–6 (soma)	0.8–1.0	1.05–1.92	ND	Gd^{3+}, NMDG
	Nonselective cation[h]	16	3	47–57		Ruthenium red
Mouse DRG IA[g]	ND	0.75–1 (neurite) 4–6 (soma)	0.5–0.7	17–26	ND	Gd^{3+}
Mouse DRG SA[g,i]	Nonselective cation	0.75–1 (neurite) 4–6 (soma)	0.4–1.3	>230	ND	Gd^{3+}, ruthenium red, HC030031[i]
HEK293 Piezo1[j]	Nonselective cation[j]	3	ND	17	ND	Ruthenium red Gd^{3+}, Ca^{2+}
HEK293 Piezo2[j]	Nonselective cation[j]	ND	ND	7	ND	Ruthenium red Gd^{3+}, NMDG

[a]McCarter et al. (1999).
[b]Drew et al. (2002).
[c]Drew and Wood (2007).
[d]Drew et al. (2007).
[e]Hao and Delmas (2010).
[f]Rugiero et al. (2010).
[g]Hu and Lewin (2006).
[h]Drew et al. (2004).
[i]Vilceanu and Stucky (2010).
[j]Coste et al. (2010).

extracellularly from teased peripheral afferents in an ex vivo skin-saphenous nerve preparation (for protocols see Zimmermann et al., 2009). This recording configuration has been widely used to evaluate the importance of developmental pathways, transduction candidates, and voltage-activated ion channels in peripheral sensory signaling. For example, when compared with wild-type controls, mice lacking stomatin domain protein SLP3 show a high proportion of mechanically insensitive cutaneous afferents in skin nerve recordings (Wetzel et al., 2007). Responses can also be measured intracellularly from DRG somata in vivo (Ma et al., 2010) or in an ex vivo skin–DRG preparation, which allows a neuron's peripheral end-organs and central projections to be visualized with neuronal tracers (Woodbury and Koerber, 2007; Seal et al., 2009). This is an important advantage because it can be used to identify the morphology of touch receptors that are classified by their physiological properties (Woodbury and Koerber, 2007).

Two standard behavioral assays are used to measure touch sensitivity in rodents. First, calibrated von Frey filaments are used to apply force to the plantar surface of a mouse hind paw, and either the force required to elicit paw withdrawal or the number of withdrawal responses to a given force is recorded (Chaplan et al., 1994). Second, the Randall–Selitto test uses a clamping device that applies progressively higher pressure on the tail (or hind paw) until the rodent withdraws, at which point force magnitude is measured (Randall and Selitto, 1957). Although these tests are robust for probing function of pain-sensing nociceptors, they are not designed to analyze the wide array of sensory neurons that mediate discriminative touch.

New behavioral assays are needed to assess sensitivity to light touch, texture, and vibration. One such assay is a two-choice preference-based tactile acuity test, whereby mice prefer exploring textured floor gratings over smooth surfaces (Wetzel et al., 2007). Mice lacking SLP3 display altered texture preferences, suggesting that SLP3 is required for normal responses to light-touch stimuli (Wetzel et al., 2007). The use of mice that lack specific subsets of sensory afferent types will greatly facilitate the design of new behavioral assays that are fine-tuned to a specific class of mechanoreceptor (Bourane et al., 2009; Luo et al., 2009; Maricich et al., 2009).

Does the candidate gene encode a pore-forming transduction channel? When a transduction channel candidate is shown to be necessary and/or sufficient for mechanically evoked currents, a key remaining validation step is to determine whether the gene encodes a pore-forming ion channel. This is critical for defining mechanosensory mechanisms because, rather than functioning as a transduction channel, a candidate might be required for proper expression, trafficking, or gating of transduction channels. For example, Piezo1 might play a role in

regulating the store-operated calcium channel Orai (Wu et al., 2007). FAM38A knockdown in HeLa cells decreases calcium release from intracellular stores and attenuates calcium influx through Orai channels (McHugh et al., 2010). Although the authors speculate that Piezo1 might function as an ER calcium release channel, it is equally plausible that it modulates Orai function, which is responsible for calcium influx and the refilling of calcium stores; any decrease in Orai activity would lead to smaller stores and diminished influx, as observed. Thus, future experiments are needed to determine whether Piezos are pore-forming channels or channel modulators, as the approaches outlined above cannot distinguish between these possibilities.

Protein engineering offers ingenious methods for demonstrating that a gene encodes a pore-forming transduction channel. Point mutations can be engineered to confer pharmacological sensitivity or to cause signature alterations in specific biophysical properties, such as ionic selectivity or conductance (O'Hagan et al., 2005). These mutant isoforms can then be used to test for functional rescue of candidate gene disruption in mechanosensory neurons. If the gene encodes a bona fide transduction channel, endogenous mechanically evoked currents should display the pharmacological or biophysical signature of the point mutant after rescue. This approach has been used to successfully validate Deg/ENaC subunits and TRP-4 as mechanotransduction channels in *C. elegans* and myosins as adaptation motors in mechanosensory hair cells (Holt et al., 2002; O'Hagan et al., 2005; Kang et al., 2010).

Because Piezo proteins lack sequence similarity to all known ion channels, implementing this strategy is likely to require extensive structure–function analysis to identify putative pore regions, to define signature point mutations, and to confirm that these mutations do not alter protein trafficking or subcellular localization. The discovery that Piezo genes induce robust mechanosensitive currents in many cell types makes this powerful approach possible.

Conclusions

Among sensory systems, the molecular mechanisms underlying touch remain most enigmatic. Based on studies in cultured sensory neurons and heterologous systems, Piezos are promising new candidates for mediating mechanotransduction; however, key studies are needed to understand the nature of these molecules and the roles they play in somatosensation and other mechanosensitive cell types. As described above, the critical experiments needed to demonstrate a requirement for Piezo proteins in cutaneous somatosensory transduction include showing an altered tactile phenotype in Piezo-deficient mice and proving that Piezo isoforms contribute to a pore-forming channel in vivo. In addition, as new tools for probing touch in vitro and in vivo

become available, other candidate molecules must also be revisited and new candidates remain to be discovered. Only by defining the biophysical and pharmacological signatures for each subtype of sensory neuron, and matching behavioral output to each subtype, can we understand the complex mechanisms underlying our sense of touch.

This Perspectives series includes articles by Farley and Sampath, Schwartz and Rieke, Reisert and Zhao, and Zhang et al.

REFERENCES

Airaksinen, M.S., M. Koltzenburg, G.R. Lewin, Y. Masu, C. Helbig, E. Wolf, G. Brem, K.V. Toyka, H. Thoenen, and M. Meyer. 1996. Specific subtypes of cutaneous mechanoreceptors require neurotrophin-3 following peripheral target innervation. *Neuron.* 16:287–295. doi:10.1016/S0896-6273(00)80047-1

Ardiel, E.L., and C.H. Rankin. 2010. The importance of touch in development. *Paediatr. Child Health (Oxford).* 15:153–156.

Bae, C., F. Sachs, and P.A. Gottlieb. 2011. The mechanosensitive ion channel Piezo1 is inhibited by the peptide GsMTx4. *Biochemistry.* 50:6295–6300. doi:10.1021/bi200770q

Basbaum, A.I., D.M. Bautista, G. Scherrer, and D. Julius. 2009. Cellular and molecular mechanisms of pain. *Cell.* 139:267–284. doi:10.1016/j.cell.2009.09.028

Bhattacharya, M.R., D.M. Bautista, K. Wu, H. Haeberle, E.A. Lumpkin, and D. Julius. 2008. Radial stretch reveals distinct populations of mechanosensitive mammalian somatosensory neurons. *Proc. Natl. Acad. Sci. USA.* 105:20015–20020. doi:10.1073/pnas.0810801105

Bourane, S., A. Garces, S. Venteo, A. Pattyn, T. Hubert, A. Fichard, S. Puech, H. Boukhaddaoui, C. Baudet, S. Takahashi, et al. 2009. Low-threshold mechanoreceptor subtypes selectively express MafA and are specified by Ret signaling. *Neuron.* 64:857–870. doi:10.1016/j.neuron.2009.12.004

Bowman, C.L., P.A. Gottlieb, T.M. Suchyna, Y.K. Murphy, and F. Sachs. 2007. Mechanosensitive ion channels and the peptide inhibitor GsMTx-4: history, properties, mechanisms and pharmacology. *Toxicon.* 49:249–270. doi:10.1016/j.toxicon.2006.09.030

Caterina, M.J., M.A. Schumacher, M. Tominaga, T.A. Rosen, J.D. Levine, and D. Julius. 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature.* 389:816–824. doi:10.1038/39807

Cavanaugh, D.J., H. Lee, L. Lo, S.D. Shields, M.J. Zylka, A.I. Basbaum, and D.J. Anderson. 2009. Distinct subsets of unmyelinated primary sensory fibers mediate behavioral responses to noxious thermal and mechanical stimuli. *Proc. Natl. Acad. Sci. USA.* 106:9075–9080. doi:10.1073/pnas.0901507106

Chalfie, M. 2009. Neurosensory mechanotransduction. *Nat. Rev. Mol. Cell Biol.* 10:44–52. doi:10.1038/nrm2595

Chaplan, S.R., F.W. Bach, J.W. Pogrel, J.M. Chung, and T.L. Yaksh. 1994. Quantitative assessment of tactile allodynia in the rat paw. *J. Neurosci. Methods.* 53:55–63. doi:10.1016/0165-0270(94)90144-9

Cho, H., J.Y. Koo, S. Kim, S.P. Park, Y. Yang, and U. Oh. 2006. A novel mechanosensitive channel identified in sensory neurons. *Eur. J. Neurosci.* 23:2543–2550. doi:10.1111/j.1460-9568.2006.04802.x

Christensen, A.P., and D.P. Corey. 2007. TRP channels in mechanosensation: direct or indirect activation? *Nat. Rev. Neurosci.* 8:510–521. doi:10.1038/nrn2149

Coste, B., J. Mathur, M. Schmidt, T.J. Earley, S. Ranade, M.J. Petrus, A.E. Dubin, and A. Patapoutian. 2010. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science.* 330:55–60. doi:10.1126/science.1193270

Dhaka, A., T.J. Earley, J. Watson, and A. Patapoutian. 2008. Visualizing cold spots: TRPM8-expressing sensory neurons and their projections. *J. Neurosci.* 28:566–575. doi:10.1523/JNEUROSCI.3976-07.2008

Drew, L.J., and J.N. Wood. 2007. FM1-43 is a permeant blocker of mechanosensitive ion channels in sensory neurons and inhibits behavioural responses to mechanical stimuli. *Mol. Pain.* 3:1. doi:10.1186/1744-8069-3-1

Drew, L.J., J.N. Wood, and P. Cesare. 2002. Distinct mechanosensitive properties of capsaicin-sensitive and -insensitive sensory neurons. *J. Neurosci.* 22:RC228.

Drew, L.J., D.K. Rohrer, M.P. Price, K.E. Blaver, D.A. Cockayne, P. Cesare, and J.N. Wood. 2004. Acid-sensing ion channels ASIC2 and ASIC3 do not contribute to mechanically activated currents in mammalian sensory neurones. *J. Physiol.* 556:691–710. doi:10.1113/jphysiol.2003.058693

Drew, L.J., F. Rugiero, P. Cesare, J.E. Gale, B. Abrahamsen, S. Bowden, S. Heinzmann, M. Robinson, A. Brust, B. Colless, et al. 2007. High-threshold mechanosensitive ion channels blocked by a novel conopeptide mediate pressure-evoked pain. *PLoS ONE.* 2:e515. doi:10.1371/journal.pone.0000515

Dussor, G., M.J. Zylka, D.J. Anderson, and E.W. McCleskey. 2008. Cutaneous sensory neurons expressing the Mrgprd receptor sense extracellular ATP and are putative nociceptors. *J. Neurophysiol.* 99:1581–1589. doi:10.1152/jn.01396.2007

Feldman, R., I. Gordon, I. Schneiderman, O. Weisman, and O. Zagoory-Sharon. 2010. Natural variations in maternal and paternal care are associated with systematic changes in oxytocin following parent-infant contact. *Psychoneuroendocrinology.* 35:1133–1141. doi:10.1016/j.psyneuen.2010.01.013

Fox, K. 2002. Anatomical pathways and molecular mechanisms for plasticity in the barrel cortex. *Neuroscience.* 111:799–814. doi:10.1016/S0306-4522(02)00027-1

Gilron, I. 2006. Review article: the role of anticonvulsant drugs in postoperative pain management: a bench-to-bedside perspective. *Can. J. Anaesth.* 53:562–571. doi:10.1007/BF03021846

Hao, J., and P. Delmas. 2010. Multiple desensitization mechanisms of mechanotransducer channels shape firing of mechanosensory neurons. *J. Neurosci.* 30:13384–13395. doi:10.1523/JNEUROSCI.2926-10.2010

Holt, J.R., S.K. Gillespie, D.W. Provance, K. Shah, K.M. Shokat, D.P. Corey, J.A. Mercer, and P.G. Gillespie. 2002. A chemical-genetic strategy implicates myosin-1c in adaptation by hair cells. *Cell.* 108:371–381. doi:10.1016/S0092-8674(02)00629-3

Hu, J., and G.R. Lewin. 2006. Mechanosensitive currents in the neurites of cultured mouse sensory neurones. *J. Physiol.* 577:815–828. doi:10.1113/jphysiol.2006.117648

Johnson, K.O. 2001. The roles and functions of cutaneous mechanoreceptors. *Curr. Opin. Neurobiol.* 11:455–461. doi:10.1016/S0959-4388(00)00234-8

Kaffman, A., and M.J. Meaney. 2007. Neurodevelopmental sequelae of postnatal maternal care in rodents: clinical and research implications of molecular insights. *J. Child Psychol. Psychiatry.* 48:224–244. doi:10.1111/j.1469-7610.2007.01730.x

Kang, L., J. Gao, W.R. Schafer, Z. Xie, and X.Z. Xu. 2010. C. elegans TRP family protein TRP-4 is a pore-forming subunit of a

native mechanotransduction channel. *Neuron.* 67:381–391. doi:10.1016/j.neuron.2010.06.032

Kramer, I., M. Sigrist, J.C. de Nooij, I. Taniuchi, T.M. Jessell, and S. Arber. 2006. A role for Runx transcription factor signaling in dorsal root ganglion sensory neuron diversification. *Neuron.* 49:379–393. doi:10.1016/j.neuron.2006.01.008

Kung, C. 2005. A possible unifying principle for mechanosensation. *Nature.* 436:647–654. doi:10.1038/nature03896

Kung, C., B. Martinac, and S. Sukharev. 2010. Mechanosensitive channels in microbes. *Annu. Rev. Microbiol.* 64:313–329. doi:10.1146/annurev.micro.112408.134106

Lechner, S.G., H. Frenzel, R. Wang, and G.R. Lewin. 2009. Developmental waves of mechanosensitivity acquisition in sensory neuron subtypes during embryonic development. *EMBO J.* 28:1479–1491. doi:10.1038/emboj.2009.73

Legate, K.R., S.A. Wickström, and R. Fässler. 2009. Genetic and cell biological analysis of integrin outside-in signaling. *Genes Dev.* 23:397–418. doi:10.1101/gad.1758709

Levanon, D., D. Bettoun, C. Harris-Cerruti, E. Woolf, V. Negreanu, R. Eilam, Y. Bernstein, D. Goldenberg, C. Xiao, M. Fliegauf, et al. 2002. The Runx3 transcription factor regulates development and survival of TrkC dorsal root ganglia neurons. *EMBO J.* 21:3454–3463. doi:10.1093/emboj/cdf370

Lewin, G.R., and R. Moshourab. 2004. Mechanosensation and pain. *J. Neurobiol.* 61:30–44. doi:10.1002/neu.20078

Loewenstein, W.R., and M. Mendelson. 1965. Components of receptor adaptation in a Pacinian corpuscle. *J. Physiol.* 177:377–397.

Loewenstein, W.R., and R. Rathkamp. 1958. The sites for mechano-electric conversion in a Pacinian corpuscle. *J. Gen. Physiol.* 41:1245–1265. doi:10.1085/jgp.41.6.1245

Lumpkin, E.A., and M.J. Caterina. 2007. Mechanisms of sensory transduction in the skin. *Nature.* 445:858–865. doi:10.1038/nature05662

Lumpkin, E.A., K.L. Marshall, and A.M. Nelson. 2010. The cell biology of touch. *J. Cell Biol.* 191:237–248. doi:10.1083/jcb.201006074

Luo, W., S.R. Wickramasinghe, J.M. Savitt, J.W. Griffin, T.M. Dawson, and D.D. Ginty. 2007. A hierarchical NGF signaling cascade controls Ret-dependent and Ret-independent events during development of nonpeptidergic DRG neurons. *Neuron.* 54:739–754. doi:10.1016/j.neuron.2007.04.027

Luo, W., H. Enomoto, F.L. Rice, J. Milbrandt, and D.D. Ginty. 2009. Molecular identification of rapidly adapting mechanoreceptors and their developmental dependence on ret signaling. *Neuron.* 64:841–856. doi:10.1016/j.neuron.2009.11.003

Ma, C., D.F. Donnelly, and R.H. LaMotte. 2010. In vivo visualization and functional characterization of primary somatic neurons. *J. Neurosci. Methods.* 191:60–65. doi:10.1016/j.jneumeth.2010.06.010

Maricich, S.M., S.A. Wellnitz, A.M. Nelson, D.R. Lesniak, G.J. Gerling, E.A. Lumpkin, and H.Y. Zoghbi. 2009. Merkel cells are essential for light-touch responses. *Science.* 324:1580–1582. doi:10.1126/science.1172890

McCarter, G.C., D.B. Reichling, and J.D. Levine. 1999. Mechanical transduction by rat dorsal root ganglion neurons in vitro. *Neurosci. Lett.* 273:179–182. doi:10.1016/S0304-3940(99)00665-5

McHugh, B.J., R. Buttery, Y. Lad, S. Banks, C. Haslett, and T. Sethi. 2010. Integrin activation by Fam38A uses a novel mechanism of R-Ras targeting to the endoplasmic reticulum. *J. Cell Sci.* 123:51–61. doi:10.1242/jcs.056424

Morrison, K.M., G.R. Miesegaes, E.A. Lumpkin, and S.M. Maricich. 2009. Mammalian Merkel cells are descended from the epidermal lineage. *Dev. Biol.* 336:76–83. doi:10.1016/j.ydbio.2009.09.032

O'Hagan, R., M. Chalfie, and M.B. Goodman. 2005. The MEC-4 DEG/ENaC channel of Caenorhabditis elegans touch receptor neurons transduces mechanical signals. *Nat. Neurosci.* 8:43–50. doi:10.1038/nn1362

Park, T.J., C. Comer, A. Carol, Y. Lu, H.S. Hong, and F.L. Rice. 2003. Somatosensory organization and behavior in naked mole-rats: II. Peripheral structures, innervation, and selective lack of neuropeptides associated with thermoregulation and pain. *J. Comp. Neurol.* 465:104–120. doi:10.1002/cne.10824

Peier, A.M., A.J. Reeve, D.A. Andersson, A. Moqrich, T.J. Earley, A.C. Hergarden, G.M. Story, S. Colley, J.B. Hogenesch, P. McIntyre, et al. 2002. A heat-sensitive TRP channel expressed in keratinocytes. *Science.* 296:2046–2049. doi:10.1126/science.1073140

Randall, L.O., and J.J. Selitto. 1957. A method for measurement of analgesic activity on inflamed tissue. *Arch. Int. Pharmacodyn. Ther.* 111:409–419.

Rugiero, F., L.J. Drew, and J.N. Wood. 2010. Kinetic properties of mechanically activated currents in spinal sensory neurons. *J. Physiol.* 588:301–314. doi:10.1113/jphysiol.2009.182360

Satoh, K., M. Hata, S. Takahara, H. Tsuzaki, H. Yokota, H. Akatsu, T. Yamamoto, K. Kosaka, and T. Yamada. 2006. A novel membrane protein, encoded by the gene covering KIAA0233, is transcriptionally induced in senile plaque-associated astrocytes. *Brain Res.* 1108:19–27. doi:10.1016/j.brainres.2006.06.050

Schwander, M., B. Kachar, and U. Müller. 2010. Review series: the cell biology of hearing. *J. Cell Biol.* 190:9–20. doi:10.1083/jcb.201001138

Seal, R.P., X. Wang, Y. Guan, S.N. Raja, C.J. Woodbury, A.I. Basbaum, and R.H. Edwards. 2009. Injury-induced mechanical hypersensitivity requires C-low threshold mechanoreceptors. *Nature.* 462:651–655. doi:10.1038/nature08505

Senzaki, K., S. Ozaki, M. Yoshikawa, Y. Ito, and T. Shiga. 2010. Runx3 is required for the specification of TrkC-expressing mechanoreceptive trigeminal ganglion neurons. *Mol. Cell. Neurosci.* 43:296–307. doi:10.1016/j.mcn.2009.12.003

Smith, E.S., and G.R. Lewin. 2009. Nociceptors: a phylogenetic view. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 195:1089–1106. doi:10.1007/s00359-009-0482-z

Stucky, C.L., T. DeChiara, R.M. Lindsay, G.D. Yancopoulos, and M. Koltzenburg. 1998. Neurotrophin 4 is required for the survival of a subclass of hair follicle receptors. *J. Neurosci.* 18:7040–7046.

Tessier, R., M. Cristo, S. Velez, M. Giron, Z.F. de Calume, J.G. Ruiz-Palaez, Y. Charpak, and N. Charpak. 1998. Kangaroo mother care and the bonding hypothesis. *Pediatrics.* 102:e17. doi:10.1542/peds.102.2.e17

Thierry-Mieg, D., and J. Thierry-Mieg. 2006. AceView: a comprehensive cDNA-supported gene and transcripts annotation. *Genome Biol.* 7:S12. doi:10.1186/gb-2006-7-s1-s12

Van Keymeulen, A., G. Mascre, K.K. Youseff, I. Harel, C. Michaux, N. De Geest, C. Szpalski, Y. Achouri, W. Bloch, B.A. Hassan, and C. Blanpain. 2009. Epidermal progenitors give rise to Merkel cells during embryonic development and adult homeostasis. *J. Cell Biol.* 187:91–100. doi:10.1083/jcb.200907080

Viana, F., E. de la Peña, B. Pecson, R.F. Schmidt, and C. Belmonte. 2001. Swelling-activated calcium signalling in cultured mouse primary sensory neurons. *Eur. J. Neurosci.* 13:722–734. doi:10.1046/j.0953-816x.2000.01441.x

Vilceanu, D., and C.L. Stucky. 2010. TRPA1 mediates mechanical currents in the plasma membrane of mouse sensory neurons. *PLoS ONE.* 5:e12177. doi:10.1371/journal.pone.0012177

Wetzel, C., J. Hu, D. Riethmacher, A. Benckendorff, L. Harder, A. Eilers, R. Moshourab, A. Kozlenkov, D. Labuz, O. Caspani, et al. 2007. A stomatin-domain protein essential for touch sensation in the mouse. *Nature.* 445:206–209. doi:10.1038/nature05394

Woodbury, C.J., and H.R. Koerber. 2007. Central and peripheral anatomy of slowly adapting type I low-threshold mechanoreceptors innervating trunk skin of neonatal mice. *J. Comp. Neurol.* 505:547–561. doi:10.1002/cne.21517

Wu, M.M., R.M. Luik, and R.S. Lewis. 2007. Some assembly required: constructing the elementary units of store-operated Ca2+ entry. *Cell Calcium.* 42:163–172. doi:10.1016/j.ceca.2007.03.003

Zimmermann, K., A. Hein, U. Hager, J.S. Kaczmarek, B.P. Turnquist, D.E. Clapham, and P.W. Reeh. 2009. Phenotyping sensory nerve endings in vitro in the mouse. *Nat. Protoc.* 4:174–196. doi:10.1038/nprot.2008.223

Zylka, M.J., F.L. Rice, and D.J. Anderson. 2005. Topographically distinct epidermal nociceptive circuits revealed by axonal tracers targeted to Mrgprd. *Neuron.* 45:17–25. doi:10.1016/j.neuron.2004.12.015