Invertebrate Nociception: Behaviors, Neurons and Molecules

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ABSTRACT: Genetic analysis of nociceptive behaviors in the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* has led to the discovery of conserved sensory transduction channels and signaling molecules. These are embedded in neurons and circuits that generate responses to noxious signals. This article reviews the neurons and molecular mechanisms that underlie invertebrate nociception. We begin with the neurobiology of invertebrate nociception, and then focus on molecules with conserved functions in vertebrate nociception and sensory biology. © 2004 Wiley Periodicals, Inc. J Neurobiol 61: 161–174, 2004

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SEVERAL CLASSES OF NEURONS MEDIATE MECHANICAL NOCICEPTION IN *C. ELEGANS*

Nociception in *Caenorhabditis elegans* is defined based on characteristic behavioral responses. In response to aversive cues, the animal ceases forward locomotion, moves backward briefly, and reorients to face away from the direction of the stimulus. Aversive cues include touch, certain odorants, high osmotic strength, acidic pH, heavy metals, and other molecules that are toxic to the animals (Ward, 1973; Chalfie and Sulston, 1981; Bargmann et al., 1993; Kaplan and Horvitz, 1993; Sambongi et al., 1999, 2000; Hilliard et al., 2002). Not all harmful compounds generate an avoidance response, indicating that aversion is generated by sensory perception and not by general tissue damage.

Mechanosensation was the first sensory modality to be explored in *C. elegans* in detail. The mechanosensory neurons were identified by testing behaviors after killing candidate cells with a laser microbeam, a technique that has proven generally useful in assigning neuronal functions. The *C. elegans* nervous system consists of 302 neurons, each of which is unique and identifiable in all animals (White et al., 1986). Among these 302 neurons are roughly similar numbers of sensory neurons (recognized by the presence of specialized sensory processes or cilia), motor neurons (recognized by the presence of neuromuscular junctions), and interneurons. Multiple classes of mechanosensory neurons are dedicated to specific body regions and mechanical intensities.

Both harsh and light touch to the body elicit avoidance behaviors that differ depending on the location of the stimulus (Chalfie and Sulston, 1981). Animals reverse and change direction if the mechanical stimulus is delivered to the anterior body, and accelerate forward movement if the stimulus is delivered to the posterior body. These distinct behaviors are generated by mechosensory neurons with spatially segregated receptive fields (Fig. 1). Five "touch cells"—two ALM neurons and one AVM neuron with sensory

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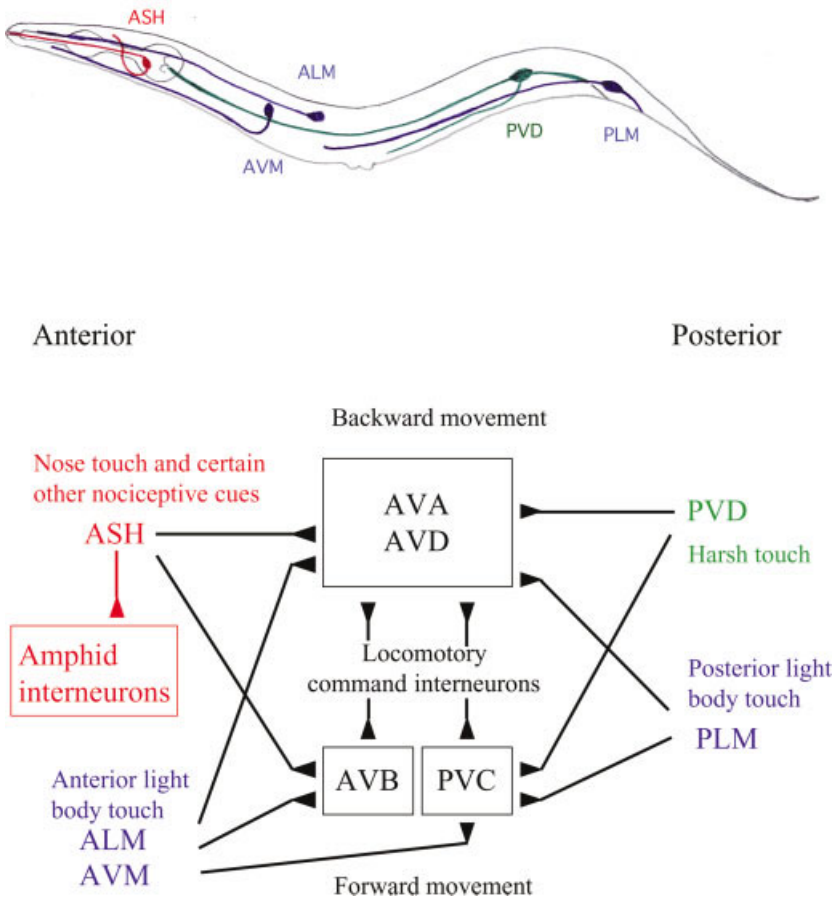

Figure 1 Morphology and synaptic connectivity of nociceptive neurons in *C. elegans.* Upper diagram, neuronal morphologies: anterior is at left and dorsal is up. There are two ALM (blue), PLM (blue), ASH (red), and PVD (green) neurons, but only one of each bilaterally symmetric pair is shown. For all neurons, the sensory process is anterior of the cell body. The position of the pharynx is shown (gray). At bottom, functions and connections of sensory neurons, modified from Hilliard et al., 2002. Black lines denote synapses to the command interneurons; red lines denote synapses to amphid interneurons, a different set of targets. AVB and PVC command interneurons predominantly promote forward movement, whereas AVA and AVD command interneurons predominantly promote backward movement.

processes that extend along the anterior half of the animal and two PLM neurons with sensory processes in the posterior half— mediate avoidance of gentle body touch such as stroking with a hair (Chalfie et al., 1985). Calcium imaging from intact touch cells shows that they are preferentially activated by anterior (ALM) or posterior (PLM) mechanical stimuli (Suzuki et al., 2003). The touch cell processes are attached to the epidermis under the animal's cuticle. They are characterized by unusual 15-protofilament microtubules and long sensory processes surrounded by an extracellular matrix called the mantle.

The neuronal circuit for touch avoidance has been traced from the sensory neurons to the motor neurons (Chalfie et al., 1985). The touch cells form gap junctions and chemical synapses onto five pairs of interneurons called command interneurons, which control locomotion (Fig. 1). The AVA, AVD, and AVE interneurons primarily control backward movement, while AVB and PVC control forward movement (Chalfie et al., 1985). Gap junctions between the touch cells and the forward and backward command interneurons lead to rapid transmission of mechanosensory information; gap junctions and synapses between command interneurons and motor neurons drive reversals and accelerations. Although it is a strong escape response, the touch response is plastic, and can be modulated by nonassociative learning (touch habituation) and associative learning (context-dependent touch habituation) (Rankin et al., 1990;

Rankin, 2000). Long-term habituation of the touch response requires glutamatergic synapses and the AMPA receptor GLR-1, probably at the chemical synapses between the touch cells and the command interneurons (Wicks and Rankin, 1997; Rankin and Wicks, 2000; Rose et al., 2003).

The touch cells act together with other sensory neurons that sense higher intensity noxious signals, or mechanosensory stimuli to other body regions. Either the touch cells or the PVD sensory neurons can detect harsh prodding of the body with a platinum wire (Way and Chalfie, 1988, 1989; Suzuki et al., 2003). Additional classes of neurons detect local mechanical stimuli in the head and the tip of the tail. These areas are rich in sensory nerve endings that are either free, associated with support cells, or attached to the cuticle. Many of these neurons are tipped with sensory cilia that act as the site of transduction. Light touch to the nose is sensed by the two ASH neurons, which have cilia at the tip of the nose (Kaplan and Horvitz, 1993), with a minor contribution from the four OLQ neurons and two FLP neurons. Harsher mechanical stimulation of the nose, the pharyngeal region, and the tail also lead to avoidance behavior, but the sensory neurons that detect these stimuli are unknown.

Not all touch is noxious. Animals slow their rate of locomotion upon encountering a bacterial lawn, a mechanosensory response that is generated by eight ciliated dopaminergic neurons called CEP, ADE, and PDE (Sawin et al., 2000). The light mechanical stimulus that triggers this slowing response can be simulated by textured stimuli like Sephadex beads.

Polymodal Nociceptive Neurons Detect Chemical, Mechanical, and Physical Repellents

The two ASH neurons detect aversive nose touch and many other aversive cues including high osmotic strength (e.g., brackish water), the odors 2-octanone, octanol, and benzaldehyde, acidic pH, quinine, and other bitter compounds, SDS, and heavy metals (Bargmann et al., 1990; Kaplan and Horvitz, 1993; Troemel et al., 1995; Sambongi et al., 1999, 2000; Hilliard et al., 2002). Their broad sensory specificity and particularly their ability to detect both chemical and mechanical repellents is reminiscent of classical polymodal nociceptors such as those found in the dorsal root ganglion (Bargmann and Kaplan, 1998).

The ASH neurons are associated with a pair of sensory organs called the amphids, which each contain 12 classes of sensory neurons and two classes of support cells. Two similar but simpler sensory organs called phasmids are present in the tail. Amphids and phasmids end in small pores through which external chemicals contact the cilia of most amphid neurons. The ASH cilia are directly exposed to the environment at the amphid pores, enabling their detection of chemical and osmotic repellents (Fig. 1) (Perkins et al., 1986).

Many of the behavioral responses mediated by ASH include a minor component from other neurons of the amphids or phasmids. The amphid ADL neurons contribute to avoidance of high osmolarity, octanol, copper, and cadmium (Bargmann et al., 1990; Troemel et al., 1995; Sambongi et al., 1999). The amphid ASK and ASE neurons contribute to avoidance of some water-soluble repellents (Sambongi et al., 1999, 2000; Hilliard et al., 2002).

Like mechanosensation, chemical nociception can incorporate information about the physical location of the stimulus. The amphid neurons that drive backward movement in response to SDS are antagonized by the phasmid sensory neurons, whose sensory endings are at the tip of the tail (Hilliard et al., 2002). Apparently, an anterior–posterior sensory comparison provides a rapid mechanism for assessing the location of the potential toxin and generating an appropriate behavior. More complex spatial responses are generated by another amphid neuron, AWB, which detects aversive volatile odors. Unlike ASH, AWB does not generate an efficient rapid escape response of reversal and turning. Instead, AWB drives long-range chemotaxis away from a source of aversive odor (Troemel et al., 1997).

The neural circuit for avoidance directed by the polymodal nociceptor ASH involves glutamatergic synapses from ASH to the forward and backward command interneurons (Hart et al., 1995; Maricq et al., 1995; Lee et al., 1999; Bellocchio et al., 2000; Mellem et al., 2002; Takamori et al., 2002). ASH senses a diverse array of nociceptive cues, but there is evidence that these cues remain segregated to some degree (Hart et al., 1995; Maricq et al., 1995). This downstream distinction is thought to be generated by interneurons that "decode" the amount or temporal pattern of ASH neurotransmitter release. Support for this model is provided by analysis of the glutamate receptor subunits *glr-1, glr-2,* and *nmr-1* that are present on command interneurons. Mutations in *glr-1* and *glr-2* eliminate the nose touch response and cause partial defects in osmotic avoidance behaviors (Hilliard et al., 2002; Mellem et al., 2002). *nmr-1* mutations do not compromise nose touch responses, but *nmr-1*; *glr-1 glr-2* mutants have a more severe osmotic avoidance defect than either the *nmr-1* mutant or the *glr-1 glr-2* double mutant (Mellem et al., 2002). These results are consistent with a model in

which nose touch and hyperosmotic solutions evoke different levels or patterns of glutamate release from ASH.

ASH expresses the FMRFamide-related neuropeptides FLP-18 and FLP-21 as well as other neuropeptides (Li et al., 1999a, 1999b; Nathoo et al., 2001; Rogers et al., 2003). This expression is an interesting parallel to the expression of neuropeptides like Substance P and CGRP by vertebrate nociceptors. Genetic and physiological evidence suggests that neuropeptides downregulate activity at the ASH-interneuron synapse. Mutations in the proprotein convertase EGL-3, which promotes neuropeptide maturation, suppress both the nose touch and osmotic avoidance defects of *glr-1* mutations, and apparently lead to glutamate release sufficient to activate the NMDA receptor NMR-1 (Kass et al., 2001; Mellem et al., 2002).

Animals expressing the heterologous rat capsaicin-sensitive channel VR1 in ASH avoid the ligand capsaicin, which is not aversive to wild-type animals (Tobin et al., 2002). The creation of this "artificial behavior" indicates that ASH activation is sufficient, as well as necessary, for escape behaviors.

Nociception Regulates More Complex Behaviors in *C. elegans*

A classical view of nociception is that it serves both to generate an immediate emergency response from the organism—the reflexive withdrawal of a hand in response to a hot surface or a worm's sudden reversal upon encountering a toxic chemical—but that it also intersects with higher order processes associated with the perception of pain.

Although most neuroscientists would not argue that a worm feels pain, nociception does feed into at least one complex behavior in *C. elegans*, a social foraging behavior present in many wild strains of *C. elegans*. In the wild, feeding behavior is determined by a polymorphism in the neuropeptide receptor *npr-1*. Less active alleles of *npr-1* result in foraging behavior in which animals feed in clusters, while the highly active *npr-1* allele of the wild reference strain N2 results in solitary feeding behavior (de Bono and Bargmann, 1998).

Ablation of the ASH and ADL nociceptive neurons suppresses the aggregation behavior of *npr-1* social feeders, as do mutations that eliminate ASH and ADL sensory functions (de Bono et al., 2002). These results suggest that nociceptive cues induce social feeding behavior. Adverse conditions can induce aggregation behavior in a number of invertebrate and vertebrate species (Pitcher and Parrish, 1993; Choe and Crespi, 1997).

C. elegans mec Genes: The First Molecular Model for Mechanosensation

The dissection of body touch mechanosensation has been complemented by an exhaustive genetic analysis of the molecular components of touch sensation. Focused, precise screens for mutants that fail to respond to light touch to the body have reached genetic saturation, perhaps the only behavioral screens for which this can be said (Chalfie and Au, 1989). From these screens, 18 mechanosensory (*mec*) genes have been identified. This array of *mec* mutants has revealed an elaborate molecular framework for mechanosensation.

The MEC Channel: The Multisubunit DEG/ENaC Channels and Mechanosensation

A great success of the Mec screen was the identification of a novel ion channel family, the DEG/ENaC (degenerin/epithelial Na+ channel) family (Table 1). MEC-4 and MEC-10 are founding members of this set of sodium channels, which are extensively reviewed elsewhere (Mano and Driscoll, 1999; Goodman and Schwarz, 2003). DEG/ENaC channels are widespread in the animal kingdom; they have extensive extracellular domains and assemble from several subunits with two transmembrane domains per subunit. Dominant mutations that constitutively activate these channels lead to necrotic death of the neurons in which they are expressed—hence, the name degenerin (Driscoll and Chalfie, 1991), and the pioneering mammalian member was the epithelial sodium channel, or ENaC. There are approximately 50 family members in *C. elegans* alone (Goodman and Schwarz, 2003). However, the two genes *mec-4* and *mec-10* play integral and specific roles in mechanosensation. *mec-4* and *mec-10* are preferentially expressed in the touch cell neurons, and loss of function mutations in either *mec-4* or *mec-10* result in loss of the behavioral response to gentle touch (Driscoll and Chalfie, 1991; Huang and Chalfie, 1994; Goodman et al., 2002). In addition, *mec-4* mutants fail to generate calcium transients in the touch cells in response to gentle touch (Suzuki et al., 2003). This defect is probably not due to generally compromised neuronal function or physiology; in a *mec-4* mutant background, the neurons maintain resting currents and respond to harsh touch. These observations all support the hypothesis that

Table 1 Ion Channels Implicated in Invertebrate Nociception and Mechanosensation, and Other Functions of Related Ion Channels

	Caenorhabditis elegans			*Drosophila melanogaster*		
Channel Family	Molecule	Expression	Putative Functions	Molecule	Expression	Putative Functions
DEG/ENaC	MEC-4 MEC-10	Touch cell neurons	Mechanosensation (proposed components of mechanosensitive channels)			
				PPK1	Dendrites of embryonic/larval peripheral mechanosensory neurons	Locomotion (Proprioception?)
	UNC-8	Motor neurons	Stretch sensation?			
	UNC-105	Muscles	Stretch sensation?			
	FLR-1 (others)	Intestine	Stretch sensation?			
				PPK11	Taste bristles	Salt sensation
				PPK19	Taste bristles	Salt sensation
TRPA				Painless	md-da Type II neurons	Response to noxious temperature, touch
				dANKTM1	Not described	Responds to mildly elevated temperatures when heterologously expressed
TRPN	CeNompC	Dopaminergic ciliated mechanosensory neurons	Mechanosensation?	NompC	Ciliated mechanosensory neurons	Mechanosensation and proprioception
TRPV	OSM-9	Multiple ciliated sensory neurons, including nociceptors and mechanosensory neurons	Chemosensation, Osmosensation, Nociception, Nose mechanosensation			
	OCR-1 OCR-2 OCR-3 OCR-4	Subsets of OSM-9-expressing cells	OCR-2= Chemosensation, Osmosensation, Nociception, Nose mechanosensation	Nanchung	Mechanosensory ciliated chordotonal neurons	Mechanosensation (hearing)

MEC-4 and MEC-10 form core subunits of a mechanosensitive channel.

When expressed in *Xenopus* oocytes, MEC-4 and MEC-10 generate a channel whose activity is dramatically increased in the presence of the dominant "degeneration" mutations in MEC-4 and MEC-10 (Goodman et al., 2002). Genetic interactions based on these dominant phenotypes as well as functional expression

indicate that the channel function of *mec-4* and *mec-10* depends on two additional mechanosensory genes, *mec-2* and *mec-6*. Like *mec-4, mec-2* and *mec-6* are required for calcium transients in the touch cells in response to light touch (Suzuki et al., 2003). *mec-6* encodes a single-pass membrane-spanning protein with limited homology to paraoxanases that is required for *mec-4(d)* induced degeneration, and *mec-2* encodes a membrane-associated stomatin-like protein (Chalfie and Wolinsky, 1990; Chelur et al., 2002). Expression of either MEC-6 or MEC-2 dramatically increases MEC-4(D)/MEC-10(D)–dependent Na+ currents in *Xenopus* oocutes, and expression of MEC-2 and MEC-6 leads to a further synergistic enhancement of the current (Goodman et al., 2002). All four of these proteins colocalize, and MEC-6 physically interacts with the other three proteins of the putative channel complex (Chelur et al., 2002).

DEG/ENaC channel subunits function in vertebrate mechanosensitive and nociceptive neurons. The brain sodium channel BNC1/ASIC2 is expressed in lanceolate nerve endings that lie adjacent to and surround hair follicles (Price et al., 2000). In mice lacking functional BNC1, rapidly adapting mechanosensory neurons fire only about half the number of action potentials as wild-type mice in response to a 20-μm stimulus. Another vertebrate family member, DRASIC/ASIC3, acts in several classes of mechanoreceptors. A mouse DRASIC mutation reduced the responsiveness of mechanoreceptors to noxious pinch, and of nociceptors to acid and noxious heat (Price et al., 2001). DRASIC can be activated heterologously by protons, and nerve preparations from mice lacking this channel showed changes in acid-evoked responses in both mechanoreceptors and nociceptive neurons. The electrophysiological properties of dorsal root ganglion (DRG) sensory neurons in mouse knockouts suggest that BNC1/ASIC2, ASIC/ASIC1 (Waldmann et al., 1997), and DRASIC/ASIC3 form subunits of acid-gated heteromultimeric channels (Bassilana et al., 1997; Lingueglia et al., 1997; Babinski et al., 2000; Benson et al., 2002). Amiloride-sensitive ENaC channels are implicated in a distinct set of nonnoxious sensory functions in mammalian salt and acid taste (Mano and Driscoll, 1999).

The Touch Cell Skeleton

A model of mechanosensation in vertebrate hair cells proposes that tethering of a channel to an intracellular skeleton and rigid extracellular structures provides the force that leads to channel opening (Hudspeth, 1989). Molecules that emerged from the *mec* screen are candidates to provide internal and external links for force generation.

The touch cells have unusual 15-protofilament large-diameter microtubules that are important for coupling mechanical stimuli to neuronal activation (Chalfie and Thomson, 1979, 1982). These atypical 15-protofilament microtubules are encoded by *mec-7*, a touch cell-specific β-tubulin, and *mec-12*, an α-tubulin (Savage et al., 1989; Fukushige et al., 1999). On the basis of genetic interactions, the proposed MEC-2 channel subunit has been proposed to link the mechanosensory channel with the microtubule network (Huang et al., 1995).

A specialized extracellular matrix called the mantle is associated with touch cells (Chalfie and Thomson, 1979, 1982). In this matrix are MEC-5, a collagen, and MEC-9, a secreted protein with EGF-like and Kunitz domain-like repeats (Du et al., 1996). *mec-1* mutants lack most of the extracellular matrix and have defects in the attachment of touch cell processes near the cuticle (Chalfie and Sulston, 1981). By analogy to hair cells (Hudspeth, 1989), *mec-1, mec-5,* and *mec-9* are thought to encode the extracellular structure that tethers the mechanosensitive channel.

The MEC genes present a remarkably detailed picture of the components of one nociceptive mechanotransduction system. This system has also provided essential insights into mammalian sensory processing through its identification of the DEG/ENaC channel family. Further exploration of DEG/ENaC channel components and regulation has great potential: there are many related genes with unknown functions in *C. elegans* as well as mammals.

OSM-9 and OCR-2: TRPV Proteins for Polymodal Nociception

All ASH-mediated sensory behaviors require the TRPV channels OSM-9 and OCR-2, the *C. elegans* relatives of a vertebrate channel family also intimately involved in nociception (Caterina et al., 1997; Colbert et al., 1997; Tobin et al., 2002). The TRP superfamily of ion channels are a diverse group of proteins with six membrane-spanning domains (Montell et al., 2002a). Most TRP channels contain a series of N-terminal ankyrin repeats. Based on sequence and structural features, the TRP superfamily has been parsed into seven families (Montell et al., 2002b): TRPC, which includes the canonical *Drosophila* TRP channels, TRPV, TRPN, TRPM, TRPP, TRPA, and TRPML (Table 1).

Within the vertebrate TRPV subfamily, the best-characterized members are TRPV1 and TRPV4. TRPV1, originally called VR1, is responsive to nox-

ious temperatures, the chili pepper irritant capsaicin, acidic pH, anandamide, and a number of other stimuli (Caterina et al., 1997; Tominaga et al., 1998; Zygmunt et al., 1999). Expressed in small-diameter polymodal nociceptive neurons, TRPV1 plays important roles in nociception (Caterina et al., 2000). TRPV4 is a nonspecific cation channel gated by osmotic stimuli and temperature, expressed in osmosensitive and mechanosensitive neurons and nonneuronal osmosensitive cells, and implicated in several forms of osmosensation *in vivo* (Halpern et al., 1995; Liedtke et al., 2000; Strotmann et al., 2000; Delany et al., 2001; Watanabe et al., 2002; Alessandri-Haber et al., 2003; Liedtke and Friedman, 2003; Mizuno et al., 2003).

The *C. elegans* TRPV channel OSM-9, which cofounded the family with TRPV1, is required for osmosensory, mechanosensory, and some olfactory responses (Colbert et al., 1997). *osm-9* is essential for all forms of ASH nociception, and is expressed in ASH and several other nociceptive neurons (ADL and OLQ). In addition, *osm-9* acts downstream of G protein-coupled receptors in the detection of attractive odorants by the AWA olfactory neurons (Colbert et al., 1997; Roayaie et al., 1998). Another group of sensory neurons, including the AWC olfactory neurons, use a cGMP-gated transduction channel encoded by *tax-2* and *tax-4* for primary odor sensation, and use *osm-9* for sensory adaptation (Coburn and Bargmann, 1996; Komatsu et al., 1996, 1999; Colbert and Bargmann, 1995).

A recent report identified a protein called HrTRPV from the Ascidian *Halocynthia roretzi* that is closely related to OSM-9. Like TRPV4, when heterologously expressed HrTRPV could mediate calcium influx in response to hypoosmolar conditions (Kondoh et al., 2003).

C. elegans has five TRPV genes: *osm-9* and four *osm-9*/capsaicin receptor-related (*ocr*) genes with sequence homology to *osm-9* and each other (Caterina et al., 1997; Colbert et al., 1997; Bargmann, 1998; Harteneck et al., 2000). OCR proteins are expressed in a number of sensory neurons and one nonneuronal cell type, but are always coexpressed with OSM-9. Mutations in *ocr-2* result in nociceptive defects similar to those of *osm-9* mutants—defects in osmotic avoidance, nose touch, and response to noxious chemicals sensed by ASH. Like *osm-9* mutants, *ocr-2* mutants are also compromised for chemotaxis to attractive volatile odorants sensed by AWA. OSM-9 and OCR-2 have been proposed to form a heteromeric channel whose localization to cilia is specified by OCR-2 (Tobin et al., 2002).

Although they have not been directly reconstituted in heterologous systems, the OSM-9/OCR channels may be polymodal channels. Other TRPV channels respond to both physical stimuli and G-protein–coupled receptor signaling; similarly, OSM-9/OCR-2 respond to osmolarity, mechanical signals, and G-protein signaling. Rescue of *C. elegans osm-9* mutants with vertebrate channels supports this model. Expression of vertebrate TRPV4 in ASH rescued the osmotic avoidance and nose touch defects of *osm-9,* although it failed to rescue the ASH volatile avoidance defects (Liedtke et al., 2003). Remarkably, the vertebrate channel interacted with all of the known *C. elegans* genetic pathways for nose touch and osmotic avoidance, including the *C. elegans* G-proteins and the modality-specific OSM-10 protein required for osmosensation. These interactions suggest that the homology between OSM-9 and TRPV4 is functional, and that rescue did not represent a mere bypass of endogenous pathways. By contrast, expression of vertebrate TRPV1 in the ASH neurons of *osm-9* mutants does not rescue typical *C. elegans* nociception, although it does create an artificial capsaicin avoidance behavior. This artificial behavior is independent of the endogenous signaling proteins in ASH such as OSM-10. Although certainly not proof that OSM-9 can directly sense osmotic stimuli, the fact that a known osmosensitive channel can specifically restore these behaviors in *osm-9* mutants suggests that OSM-9 and TRPV4 may share common regulatory mechanisms.

The detailed mechanisms of nociception upstream of OSM-9/OCR-2 are unknown. The novel protein OSM-10 is involved in osmosensation, but not other ASH nociceptive modalities (Hart et al., 1999). G-protein signaling is believed to initiate sensation of many noxious chemicals in ASH (Roayaie et al., 1998).

Other Nociceptive Signaling Mechanisms?

Noxious temperature is a common nociceptive stimulus that has been studied extensively in vertebrates and invertebrates. *C. elegans* has an acute thermal avoidance response, but the cellular locus of this action is unclear (Wittenburg and Baumeister, 1999). *osm-9* (TRPV) mutants are not compromised for this behavior, suggesting that alternative molecular mechanisms may be involved.

Some chemosensory neurons that sense repulsive cues do not express either *osm-9* or *ocr* genes. Signaling in the aversive odorant-sensing neuron AWB involves G protein coupled receptor signaling through the cyclic nucleotide gated channel TAX-2/TAX-4 (Troemel et al., 1995, 1997). Detection of aversive cues by the amphid neuron ASK may also proceed

through a *tax-4*–dependent pathway (Coburn and Bargmann, 1996; Komatsu et al., 1996; Hilliard et al., 2002). Other pathways for touch, specifically the pathways for detection of very light touch by dopaminergic neurons, and detection of harsh touch, remain to be described.

DROSOPHILA NOCICEPTION

Type I and Type II Neurons in the Peripheral Nervous System

Two major classes of sensory neurons, type I and type II, populate the peripheral nervous system of *Drosophila*. External sensory neurons that terminate in a single ciliated dendrite are referred to as type I neurons. Type I neurons cover the fly, and underlie mechanosensory responses mediated by sensory bristles as well as the detection of other mechanical and thermal stimuli. Morphologically, type I neurons resemble the ciliated amphid neurons in *C. elegans* (Jarman, 2002). The activity of type I neurons can be recorded in the sensory bristle, whose deflection results in rapid depolarization (Corfas and Dudai, 1989). Type I sensory dendrites are surrounded by a high K^+, low Ca^{2+} endolymph that is secreted by supporting cells (Kernan and Zuker, 1995). The brief 200-μs latency of mechanoreceptor currents after bristle deflection argues against second-messenger–dependent mechanisms of neuronal activation (Walker et al., 2000), so bristle deflection most likely directly gates a mechanosensitive channel.

Type I neurons can also be organized into more complex sensory organs in structured arrays. Type I neurons form the internal chordotonal organs that are thought to serve as insect proprioceptors, and type I chordotonal neurons form Johnston's organ, a sound-detecting organ located in the second antennal segment. Interestingly, as described below, there appear to be fundamental differences in the mechanosensory mechanisms in different type I structures.

Type I neurons also appear to detect temperature. Calcium imaging of type I terminal sensory organ neurons in the head revealed increases in activity in response to even subtle drops in temperature (Liu et al., 2003b). Blocking synaptic transmission in these neurons by expression of the tetanus toxin light chain (Sweeney et al., 1995) resulted in an almost complete elimination of a cold temperature avoidance behavior in a choice assay (Liu et al., 2003b).

In contrast to type I neurons, type II neurons extend multiple dendrites and lack sensory cilia. The dendritic arborization (md-da) neurons, one of the three subtypes of type II neurons, spread dendrites along the epidermis and have terminals embedded in the cuticle (Bodmer and Jan, 1987). Morphologically, the naked dendrites resemble vertebrate nociceptors. Blockage of synaptic transmission in the md-da neurons through the expression of the tetanus toxin light chain eliminated the response of larvae to noxious heat and strong mechanical stimulation, suggesting an important nociceptive role for these neurons (Tracey et al., 2003).

Genetic Screens for Mechanosensation Defects in Type I Neurons: the NompC TRPN Protein

An early genetic approach to identifying components of mechanosensory transduction in type I neurons was based on a grooming behavior that is triggered by the sensation of debris on the body (Svebenyi, 1969; Dawkins and Dawkins, 1976; Phillis et al., 1993). Coating flies with dust triggers extensive grooming and dust removal. Different classes of mutants were isolated based on defective dust removal, and it was hypothesized that mutations could affect the initial sensation of the particles by the sensory bristles, transmission of this information to downstream neurons, or the function of downstream motor programs. The difficulty of identifying the mutations in mechanoreception rather than downstream circuits was overcome in a second screen, in which an initial screen for uncoordinated mutants was followed by extracellular recordings of sensory bristles in the mutants (Kernan et al., 1994). Of special interest were mutations in the *nomp* genes (no mechanoreceptor potential), which resulted in the absence of mechanoreceptor potentials (Kernan and Zuker, 1995).

Null *nompC* mutants have morphologically normal mechanoreceptive cells but no observable mechanoreceptor potential upon stimulation of sensory bristles; milder mutants have altered mechanoreceptor potentials, suggesting a close relationship between this gene and sensory detection (Kernan et al., 1994; Walker et al., 2000). *nompC* mutants are defective in coordinated movement, potentially due to defective mechanosensation. The *nompC* gene was cloned, and encodes a potential mechanosensitive channel, the founding member of the TRPN subfamily (Walker et al., 2000). A striking feature of the TRPN family is a large number of N-terminal ankyrin repeats, and NompC is predicted to have 29 ankyrin repeats at the N-terminus. A *C. elegans* ortholog of *nompC* is expressed in the cilia of dopaminergic neurons that sense very light touch (Walker et al., 2000). A zebrafish ortholog of *nompC* is expressed on zebrafish

sensory hair cells, and is required for hair cell mechanotransduction (Sidi et al., 2003). Thus, *nompC*-related genes are associated with mechanotransduction in both invertebrates and vertebrates.

Mutations in a second gene, *nompA,* disrupt contacts between sensory endings and cuticular structures (Chung et al., 2001). *nompA* encodes a protein expressed in support cells of the peripheral sense organs that sheathe the neuronal sensory process. NompA might be an extracellular anchor protein for sensory channels, as proposed for the (unrelated) extracellular mantle proteins in *C. elegans* such as MEC-9.

NompA and NompC are expressed only in the peripheral nervous system. Thus, there are probably other mechanosensitive signaling systems in *Drosophila*, as noted below.

Nociceptive Transduction in Larval Type II Neurons Relies on the Painless TRPA (ANKTM1-like) Channel

Although *Drosophila* larvae generally respond to light touch by halting their forward movement and slightly reversing, touch with a hot probe results in a distinct behavior in which the larvae roll laterally (Kernan et al., 1994; Tracey et al., 2003). This robust response can be induced at temperatures as low as 39°C, and is dependent on the md-da type II sensory neurons. A genetic screen for mutants that are defective in the response to noxious heat identified 49 mutants that failed to respond quickly to noxious temperatures (Tracey et al., 2003). Mutations in the *painless* gene resulted in defects in this response at temperatures up to 52°C, as well as defects in nociceptive mechanosensation. When stimulated with a 45 mN von Frey filament, only 13% of *painless* larvae responded with the rolling behavior of wild-type animals. The response to light touch (10 mN) was not affected in the *painless* mutants, suggesting that *painless*-dependent transduction is specific to nociceptive sensory cues.

Painless encodes a protein with eight ankyrin repeats that defines the TRPA subfamily of ion channels (Corey, 2003). The closest vertebrate relative of *painless* is ANKTM1, a vertebrate TRPA family member that is expressed in nociceptive neurons and activated by cold (Story et al., 2003). anti-Painless antibodies localize the protein to punctate regions within dendritic arbors, suggesting that it detects peripheral temperature changes. Although *painless* has not been reconstituted in heterologous systems, recordings from abdominal peripheral nerves confirmed that neurons from *painless* mutants, in contrast to those from wild-type animals, failed to increase spiking frequency after raising temperature to 42°C (Tracey et al., 2003). *painless* is central to larval nociception, and based on its sequence, it is a strong candidate to respond directly to noxious temperature and touch.

Although vertebrate ANKTM1 is related to *painless*, it is even more closely related to the *Drosophila* gene now called dANKTM1. In contrast with the vertebrate channel, heterologous expression of the dANKTM1 cDNA generated transient currents in response to heating rather than cooling (Viswanath et al., 2003). The temperature threshold for these currents was 24–29°C, suggesting that dANKTM1 channel activation could occur at physiologically relevant temperatures. The expression pattern and phenotype of mutations in this channel have not yet been described. Two other uncharacterized *Drosophila* relatives of *painless* have been noted that are more closely related to *painless* than to dANKTM1 (Viswanath et al., 2003). It will be interesting to characterize these proteins both in heterologous systems and *in vivo.* By analogy to vertebrate thermosensitive channels that are tuned to different temperatures, these proteins may contribute to the recognition of alternative noxious temperatures.

Evolutionary Connections in Invertebrate Sensation: Orthologues of *C. elegans* Nociceptive Molecules Have Sensory Roles in *Drosophila*

Nanchung (Nan) is the closest *Drosophila* relative to the *C. elegans ocr* TRPV genes. It is expressed exclusively in the mechanosensory cilia of the chordotonal organs, suggesting the possibility that it may comprise part of the mechanotransduction channel in *Drosophila* proprioception or hearing. Indeed, mutations in *nan* completely eliminate antennal sound-evoked potentials (Kim et al., 2003).

Touch and hearing appear to share many common elements in *Drosophila,* as most mutants identified as defective in mechanosensation also show hearing defects (Eberl et al., 2000). However *nompC* mutations have only a small effect on the sound-evoked response in Johnston's organ, in contrast to the dramatic defects they produce in bristle receptor transduction (Kernan et al., 1994). Thus, the Nanchung TRPV channel subunit may be the primary mechanosensory channel in fly hearing, with NompC carrying out a different role in the cells. Alternatively, NompC may contribute to hearing at certain frequencies or intensities.

Might a sound-sensitive channel be structured similarly to the *C. elegans* OSM-9/OCR-2 channel? There is a single *osm-9* ortholog in *Drosophila*, but its

expression pattern has not been described. It will be interesting to see if this gene is coexpressed with Nan and, if so, how it may modify the properties of Nan channels.

Like TRPV4, Nanchung is activated by hypoosmotic solutions when expressed heterologously (Kim et al., 2003). This sensitivity may reflect changes in membrane properties that mimic mechanical stimuli, or a separate physiological response to osmotic stimuli, akin to the multiple modes of activation postulated for OSM-9/OCR-2.

DEG/ENaC Family Members in *Drosophila* are Expressed in Mechanosensory Neurons

Drosophila relatives of the DEG/ENaC family members exist, and one of these relatives, Pickpocket1 (PPK), is expressed in the dendrites of embryonic and larval peripheral mechanosensory neurons (Adams et al., 1998; Darboux et al., 1998). A mutation in the *ppk1* gene did not result in overt altered external touch sensation in larvae (Ainsley et al., 2003), but the mutant larvae did exhibit changes in crawling behavior, with increased locomotor speed and fewer turns than wild-type animals. One interpretation of this result is that the channel may be important for proprioceptive signals that influence locomotion. Mutations affecting development or structural components of the chordotonal organs result in an opposite phenotype to *ppk1* mutants, with increased turning frequency and reduced duration of linear motion (Caldwell et al., 2003). Disruption of the chordotonal organs, unlike *ppk1* mutations, also reduced sensitivity to touch. These behavioral results present the beginnings of a mechanosensory behavioral circuit for larval *Drosophila,* and the possibility of understanding how different mechanosensory cues are integrated as the larva navigates through its environment.

The *Drosophila* Pickpocket11 and Pickpocket19 DEG/ENaC family members are expressed in the larval taste-sensing terminal organ and the adult taste bristles. These channels are implicated in taste, and have been shown to be important in larval sensation of Na^+ and K^+ ions and the adult response to high salt concentrations (Liu et al., 2003a). Other *Drosophila* DEG/ENaC family members remain to be characterized.

CONCLUSION

What makes a neuron nociceptive? The *C. elegans* polymodal ASH neurons are similar to vertebrate polymodal nociceptive neurons; their functions are unambiguously nociceptive, and depend on the TRPV channel family that is prominent in vertebrate nociception. Initial studies in vertebrates emphasized the idea of molecules, particularly ion channels, specific to nociception (McCleskey and Gold, 1999). However, the most striking result from functional comparisons within and between species is the versatility of DEG/ENaC, TRPV, and TRPN channel families, which may all be involved in nociception, nonnociceptive mechanosensation, and other sensory modalities. For example, in *Drosophila*, the TRPV channel Nanchung plays a role in hearing nonnoxious courtship song. The neuronal circuits, rather than the specific channel molecule, define the nociceptive nature of the stimulus.

One common theme in both invertebrate and vertebrate nociception is the ability to quickly detect physical stimuli and translate them into neuronal signaling—a necessity for noxious stimuli that present a danger to an organism. These observations leave interesting open questions about the evolution of these systems. Were there primitive DEG/ENaC, TRPV, and TRPN nociceptive systems that now appear in modified forms across different species? Or were rapid sensory transduction mechanisms opportunistically adapted to the nociceptive needs of each species after the vertebrate/invertebrate split?

Another common theme is the cellular and molecular complexity of nociception. Spatial maps and intensity coding of touch stimuli are remarkably primitive and conserved features of animal mechanosensory systems. The importance of the invertebrate systems has been their power to identify the proteins, conserved with vertebrates, that rapidly translate physical cues into neuronal signaling and behavior. Further studies of the molecular and cellular architecture of nociception should provide insights into the basic neurobiology underlying sensory integration and perception.

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