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Research Focus

Sensation is *painless*

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Emily Dickinson declared: 'After great pain, a formal feeling comes'. This formal feeling begins when sensory neurons are activated by noxious stimuli, such as stepping on a tack. Recently, Seymour Benzer's group identified sensory neurons in *Drosophila* larvae that mediate aversive responses to noxious heat and mechanical stimuli. Thresholds for behavioral and nerve responses are elevated by mutations in the *painless* gene, which encodes a TRP ion channel protein. Painless thus joins an elite group of TRPs implicated in sensory transduction in insects, nematodes, mammals and fish.

Sensation continuously guides and modifies behavior, assuring that animals are able to find food and mates, to move toward favorable environments, and to avoid predators. Information about the external world is carried by light, odors, sounds, touches, temperature and even electric fields. Sensory cells specialized to detect each kind of stimulus respond to these cues and provide animals with qualitative, quantitative and spatial information about the world. Many animals also have sensory neurons activated preferentially by stimuli with the potential to cause tissue damage. In humans and other mammals, such sensory neurons are called nociceptors and generate signals interpreted by the CNS as pain.

A crucial step in sensation is the activation of 'transducer' channels, which open in proportion to the strength of the stimulus. Such channels can be activated indirectly, as in visual transduction, or directly, as hypothesized for hearing [1]. Only now are we beginning to uncover potential transducer channels needed for hearing, touch, temperature and pain sensation. Progress in this arena has accelerated through the use of genetic screens in the nematode *Caenorhabditis elegans*, *Drosophila* fruit flies, and the zebrafish *Danio rerio* [2]. Two classes of ion channel proteins have emerged from these studies as potential mechanotransducer channels: DEG/ENaCs and TRPs. (The DEG/ENaC superfamily is named after the first two sub-families identified: degenerins, *C. elegans* genes that when mutated can cause cellular degeneration, and ENaCs, subunits of vertebrate epithelial Na^+ channels. TRP channels are named after the product of the *transient receptor potential* gene in *Drosophila*). This article highlights Painless, a newly characterized member of the TRPN subfamily of TRP channels required for sensation of noxious thermal and mechanical stimuli [3], and will discuss its sisters, no-mechanoreceptor-potential C (NompC) [4,5] and the ankyrin-like ANKTM1 [6,7].

Naked neurons and nociceptors

The epidermis of *Drosophila* larvae is densely innervated by sensory dendrites from type II or multidendritic (md) sensory neurons [8]. These cells, which are not clearly associated with accessory structures, display morphological similarities to vertebrate sensory neurons that also terminate in free nerve endings embedded in the skin. The precise function of md sensory neurons was unknown until recently. The elegant work of Tracey *et al.* [3] showed that signaling by md-da neurons is required for normal responses to noxious thermal ($T > 41\ °C$) and mechanical ($F > 45$ mN) stimuli in *Drosophila* larvae (Figure 1a). Thus md-da neurons, or a subset of them, are likely to be nociceptors. Consistent with this notion, most if not all md-da neurons are thermosensitive [9]. It should be noted, however, that responses to both thermal and mechanical stimuli have yet to be measured in individual md-da neurons, leaving open the question of whether thermal and mechanical stimuli are detected by distinct md-da neurons or whether individual md-da neurons are polymodal sensory neurons that can detect both kinds of stimuli. Additional mechanosensory neurons are present

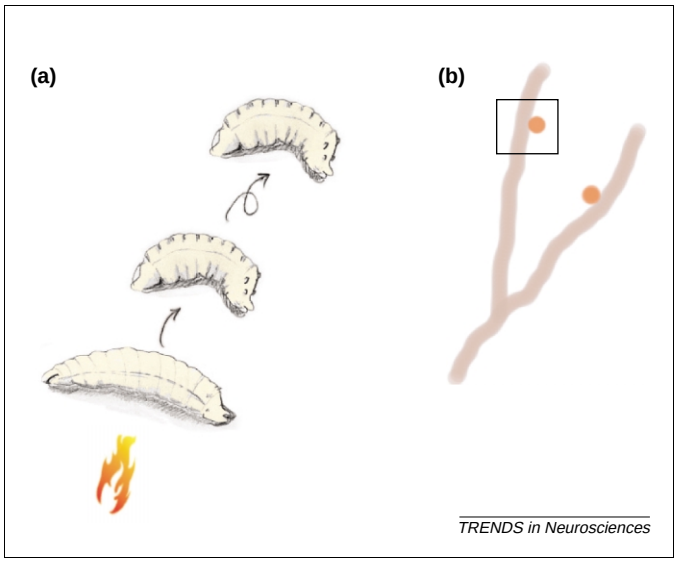

Figure 1. Responses to noxious stimuli in *Drosophila* larvae depend on intact multidendritic md-da neurons and wild-type *painless*. **(a)** The response to noxious thermal ($T > 41\ °C$) and mechanical ($F > 45$ mN) stimuli. Wild-type animals rapidly (latency < 1 s) bend and roll away from the site of stimulation. Genetic screens for animals that fail to respond to noxious heat have uncovered several genes needed for this robust, aversive behavior. The *painless* gene, which is predicted to encode a transient receptor potential (TRP) ion channel, is the first of these genes to be cloned. This behavior also requires signaling from md-da neurons because responses to heat are eliminated by targeted expression of tetanus toxin. **(b)** Painless protein is expressed in the sensory dendrites of md-da neurons and concentrated in puncta. Taken together, these results support the idea that Painless contributes to a sensory transduction channel; Painless puncta might represent sites of sensory transduction.

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in *Drosophila* larvae, because the md-da neurons are not required for responses to non-noxious mechanical stimuli. It will be interesting to learn how sensory thresholds vary among md-da neurons and whether such functional variation correlates with recently described anatomical subtypes [10].

Polymodal Painless?

A nociceptor is a sensory neuron that responds to diverse stimuli that have the potential to cause tissue damage. This operational definition, originally proposed by Sherrington [11], implies that nociceptors are polymodal – that is, they have the ability to detect noxious thermal, mechanical and/or chemical stimuli. To accomplish this feat, polymodal sensory neurons could express a team of specialists, detectors that respond to only one kind of stimulus. Alternatively, one or a few generalists – detectors activated by multiple kinds of stimuli – could operate in polymodal sensory neurons. Painless could be one such generalist detector, a potential transducer of both thermal and mechanical stimuli. In the absence of the *painless* gene, larvae fail to roll away from thermal and mechanical stimuli that reliably elicit such responses in wild-type animals. In *painless* mutants, heating to >41 °C fails to increase the firing rate in one class of temperature-sensitive units in the abdominal nerve (which contains the axons of md-da neurons). Moreover, Painless protein is localized to md-da dendrites, where it is concentrated in puncta (Figure 1b). An exciting possibility is that Painless puncta represent specialized sites of sensory transduction in md-da dendrites.

Other TRPNs, more sensations…

The generalist Painless is closely related at the molecular level to two additional TRP channels that appear to be more specialized. These two channels, ANKTM1 in mice and flies and NompC in flies and zebrafish, were identified using molecular and genetic approaches and are proposed to transduce thermal and mechanical stimuli, respectively.

The first of these channels, mouse ANKTM1, is activated by noxious cold (threshold $T < 17$ °C) and by the cooling agent, icilin [6]. It is coexpressed with another TRP channel, TRPV1, in a subset of sensory neurons in mammalian dorsal root ganglia. TRPV1 is a generalist detector, activated by noxious heat, pH, vanilloids and endocannabinoids [12]. The subset of dorsal root ganglion neurons that expresses both ANKTM1 and TRPV1 is proposed to comprise polymodal nociceptors [6], although this awaits confirmation in functional studies. Paradoxically, *Drosophila* ANKTM1 (34% identical to mouse ANKTM1) is activated by heating (threshold $T > 24$ °C) and not by cooling [7]. The function of *Drosophila* ANKTM1 and its expression pattern are unknown. Nonetheless, it is easy to imagine exploring the molecular basis for temperature detection by constructing chimeras of these two proteins.

A second TRPN channel related to Painless, NompC, is needed to respond to mechanical stimuli applied to sensory bristles covering the body of adult *Drosophila* fruit flies [4]. Bristles are innervated by ciliated or type I sensory neurons, which are believed to express NompC. Such sensory neurons display striking similarities to vertebrate hair cells, specialized cells that detect sound and vibration in vertebrate inner ears. For instance, the apical surfaces of both types of sensory cells are bathed in an unusual extracellular solution that is high in K^+ [13,14]. Although much is known about transducer currents in vertebrate hair cells [15,16], the molecular identity of transducer channels has remained obscure. A recent breakthrough is the identification of an ortholog of NompC in zebrafish [5]. The zebrafish NompC gene is expressed by hair cells in the inner ear and lateral line sensory organs that sense water flow across the body surface. Consistent with a role for NompC in sensory mechanotransduction by hair cells, inactivating NompC eliminated acoustic startle responses in zebrafish larvae and reduced sensory microphonic potentials recorded in lateral line organs. With this report, the similarity between fly bristle mechanoreceptors and vertebrate hair cells extends to the molecular level.

Future directions

It is assumed, but not proven, that ion channels are direct transducers of thermal and mechanical stimuli. A first test of this idea is to determine the effect of loss-of-function mutations on sensory currents measured *in vivo*. This test has yet to be conducted for Painless or ANKTM1. Extracellular measurements in *Drosophila* bristles [4] and zebrafish lateral-line organs [5] are consistent with the idea that NompC is crucial for sensory mechanotransduction. A residual mechanoreceptor current is present in *Drosophila* bristles lacking NompC, however, suggesting that it is not the only transducer operating in sensory bristles. A second test is to show that each channel responds directly to thermal and/or mechanical stimuli in heterologous cells. This test has been conducted for thermal sensitivity of mouse and fly ANKTM1 [6,7]. Analogous tests for mechanical sensitivity are problematic, however, because specialized cellular structures might be essential for gating. Osmotic swelling has been used as a surrogate mechanical stimulus for TRPV channels implicated in sensory mechanotransduction in mammals [17] and flies [18]. These data show that these channels can respond to mechanical stimuli but it is unclear how activation by osmotic stimulation is related to how channels are activated *in vivo*.

Exactly how transducer channels respond to thermal and/or mechanical stimuli remains an enticing mystery. All TRPN channels contain a tandem array of ankyrin repeats in their N terminals. These domains could anchor TRPN channels to specialized elements in the cytoskeleton, as proposed for NompC [4]. Anchors in sensory cells could regulate whether or not a given TRPN channel is activated by mechanical stimuli. Such channels would have the potential to transduce mechanical energy but would do so only when expressed in a cell that provides a suitable anchor. In this scenario, a generalist such as Painless might be sensitive to both thermal and mechanical stimuli in some neurons and detect only thermal stimuli in others. The importance of cellular context could be tested, for example, by expressing Painless in NompC-expressing cells (and vice versa). Sensitivity to diverse stimuli could also arise from combinatorial expression of

multiple TRPs, as suggested for TRPVs in *C. elegans* [19]. The discovery that md-da neurons and Painless are essential for nociception in *Drosophila* larvae introduces the idea that sensory thermotransduction and mechanotransduction could operate via a single transducer channel.

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