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Cells and Circuits for Thermosensation in Mammals

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Abstract

How is temperature detected and how is the resulting sensory information synthesized to produce appropriate thermosensory responses? Research in the last few years has gone a long way to answering the first part of this question. Excitingly, recent research has uncovered some of the ways sensory inputs are processed spinally, as well as identifying supra-spinal centers involved in processing responses to thermal stimuli. In this review, we explore the new areas of research that have contributed to our comprehension of the way the peripheral sensory neurons are tuned in addition to the receptors used to differentiate thermal stimuli. We also describe recent work which begins to illuminate the processing of primary sensory signals by the spinal cord and regions of the brain.

Introduction

Environmental temperature is a highly sentient stimulus initiating both conscious and unconscious reactions. These reactions to thermal stimuli are sensed by nerve fibers in the skin and other organs. The sensory neurons innervating the skin are pseudo-unipolar with cell-bodies clustered together just outside the CNS, in dorsal root ganglia (DRG) for most of the body, and for the face, oral cavity, and head, in the trigeminal ganglion. Sensory neurons also send axonal projections to the spinal cord and spinal trigeminal nucleus where they transmit signals to higher centers. Thermal challenges elicit varied responses including reflex responses (e.g. paw withdrawal) homeostatic control (maintenance of body temperature), and produce an awareness of environmental temperature (e.g. place preference and learned behavior). These multiple responses are the result of engagement of specific neural circuits present in the spinal cord and the brain.

Peripheral thermosensory system

How are cold and heat distinguished from each other? Early experiments using electrophysiological recordings of peripheral sensory neurons indicated that there are distinct classes of neurons that either respond to cold, or to heat¹. The classes of cold and heat responding neurons could be further grouped dependent on their reaction to other

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stimuli and their level of myelination. Although these results offer a simple explanation for how hot and cold stimuli can be distinguished, they lack a direct relationship with behavioral reactions and additionally, paradoxical responses of the same neuron to both, heat and cold stimuli have been reported². To better understand the basic mechanism involved in temperature detection and establish the link between electrical signals and behavioral response, screens to identify thermo-receptive proteins were initiated about 20 years ago. This line of inquiry led to the discovery of receptors for the hot-temperature mimetic capsaicin and the cold mimetic menthol^{3–5}. *In vitro*, the capsaicin receptor, transient receptor potential cation channel subfamily V member 1 (TRPV1), is activated by temperatures above 42 °C, and the menthol receptor, transient receptor potential cation channel subfamily M member 8 (TRPM8), by temperatures below 25 °C. Importantly, the elimination of the TRPM8 ion-channel in mice profoundly reduced behaviors to cold^{6–8}. In contrast, loss of TRPV1 results in major effects on injury-induced allodynia and hyperalgesia to heat stimuli but does not have dramatic effects on acute behavioral responses to heat^{9–11}. The latter result suggests that there is either receptor redundancy, or that there are other receptors for heat. Recently, it was suggested that a trio of TRP ion-channels, TRPV1, TRPA1, and TRPM3, collectively are the receptors for acute withdrawal responses to heat stimuli¹². Although this is an attractive explanation, it is also possible that, while these TRP proteins are required for responses to heat, similar to TRPM5 being needed for sweet and bitter taste¹³, they may be critical signaling components downstream of a sensor protein. In addition to TRPV1 and TRPM8, *in vitro*, other TRP channels respond to thermal stimulation with various thresholds, covering the whole physiological relevant temperature range, but disappointingly, data from the studies of mice where these genes are knocked out, does not support them being required *in vivo*^{14–16}.

Another way to understand more about how different temperatures are distinguished is to determine the specificity of different classes of sensory neurons. This strategy was used to dissect the contribution of TRPV1- and TRPM8-expressing neurons. TRPV1 and TRPM8 are largely found in separate neuronal populations, suggesting that this is a way hot and cold can be distinguished from each other¹⁷. Initially, lesions using pharmacological approaches were employed to ablate PV1-neurons^{18–20}. Results from these studies showed that even though TRPV1 itself is not required for behavioral responses to heat, TRPV1-neurons are. In addition, using molecular genetics, a more directed and reproducible ablation of TRPV1-neurons also demonstrated that TRPV1-neurons are the principle neurons required for behavioral responses to heat and that these neurons are not necessary for cold responses¹¹. Furthermore, also using molecular genetics, it was established that TRPM8-neurons are required for responses to cold, but not heat^{11, 21}. Interestingly, the elimination of TRPM8-neurons had a greater effect on responses to cold than loss of the TRPM8 ion-channel^{6–8} suggesting that there may be other cold-sensors in these cells. Together, these results provide a simple explanation for how cold and heat are distinguished from each other, cold is selectively encoded by TRPM8-neurons and heat by TRPV1-neurons.

To investigate the dynamic responses of sensory neurons to temperature stimulation on the single-cell level, a recent study utilized calcium imaging to analyze responses of large numbers of cells in the trigeminal ganglion with receptive fields in the oral cavity²². This study confirmed that cold, hot, and warm responses are represented by distinct classes of

cells. It also showed that these neurons could be further subdivided based on rates of adaptation and stimulus threshold (Figure 1A). In particular, the study reported that there are at least four classes of cold-responsive neurons, all of which express TRPM8 (one class is silent in normal conditions and is activated after injury). Intriguingly, recent single-cell sequencing data demonstrates multiple, molecularly distinct classes of TRPM8-cells in the trigeminal ganglion²³, suggesting that these cells provide subtly different information about the quality of cold. Ethologically, for warm blooded animals the detection of cold is probably more important than the detection of heat because of the need to defend, from cold, their body temperature. Calcium imaging also revealed a small population of neurons which respond to warm stimuli similar to those reported previously^{24,25}. Further, it was reported that the responses of these warm neurons are dependent on the TRPV1 ion-channel²² but a different report postulated that another TRP channel, TRPM2 is important for warmth detection²⁶. Together, results from knock-out mice, directed cell-ablation and *in vivo* imaging suggest that cold and heat thermal stimuli are encoded by selectively tuned primary afferents and that inputs from these neurons activate largely separate sensory circuits, at least in regard of behavioral reflex withdrawal and preference tests.

Control of body temperature

Warm blooded mammals keep tight control of their body temperature maintaining it in a very narrow range^{27,28}. Even small fluctuation in core body temperature can have severe consequences and mammals utilize several active compensatory mechanisms when challenged. For appropriate responses to heat or cold, connections between thermosensory detection system(s) and organs that either dissipate heat or are involved in thermogenesis must exist²⁹. In animals lacking the TRPV1-lineage of sensory neurons, which includes both TRPV1- and TRPM8-neurons, the ability to respond appropriately to both external and internal thermal challenge are greatly diminished³⁰. Of note, the afore mentioned trio of TRP channels, TRPV1, TRPA1 and TRPM3, seemed to be dispensable for heat seeking and noxious temperature avoidance behavior¹², indicating that potentially distinct transduction mechanisms feed into homeostatic versus reflex responses to temperature challenge. In addition, TRPM8 and TRPM2-neurons have been shown to be important in long-term control of body temperature³¹. However, thermosensory centers in the CNS are also critical for the control of core body temperature^{27,28}, suggesting that input from TRPV1- and TRPM8-neurons is only part of the means by which core body temperature is managed. The preoptic area (POA) of the hypothalamus is a key thermosensory homeostasis center. It was shown that the TRPM2 ion-channel is one but likely not the only molecule required for heat responses in the POA and excitingly, chemogenetic manipulation of TRPM2-neurons or BDNF/PACAP-neurons is sufficient to evoke large changes in core body temperature^{32,33}. These studies define the neurons which encode control of core body temperature and start to shed light on how changes in core body temperature are detected in the POA. Studies of the role of the POA are still ongoing and in the future, it will be important to delineate the circuits connecting peripheral neurons with the POA and to precisely define the input(s) used to control responses to cold challenge³⁴.

Spinal and supraspinal representation of temperature

Thermosensory afferents target predominantly lamina I in the superficial dorsal horn of the spinal cord³⁵; however, the molecular identity of the post-synaptic neurons is not clear. Unlike the selectivity of the primary sensory neurons, there is cross-talk between inputs within the spinal cord which are likely critical for shaping thermosensory responses^{36–38}. In particular, capsaicin afferents are thought to inhibit spinal cold neurons, through inhibitory interneurons, a circuit that has been suggested to enhance cold sensitivity^{37, 38}. To overcome the low throughput of electrophysiological recordings, more recently calcium imaging was used to get a more complete picture, albeit still without cell-type definition, of the responses of superficial spinal cord neurons to temperature stimulation³⁹. Neurons could be broadly classified based on their responses to cold, heat, or both cold and heat (Figure 1B). For the majority of cold responsive neurons, response magnitudes and duration were indifferent to the absolute temperature of a stimulus, but responses were highly sensitive to the rate of cooling and rapidly adapted. In contrast, heat responsive spinal neurons encode absolute temperature, their responses were indifferent to the rate of heating and did not adapt. There were also neurons which were stimulated by both cold and heat. The numbers of these broadly tuned neurons increased with stimulus intensity and unlike cold sensitive neurons they adapted slowly. The ablation of TRPV1- or TRPM8-neurons substantially affected most but not all heat and cold responsive spinal responses, respectively. Interestingly, TRPV1-afferents also appear to drive responses to extreme cold. There are still open questions about how thermal information is computed and processed by the spinal cord. For example, when responses to warmth were studied behaviorally, in animals lacking either TRPM8-cells, TRPV1-neurons, or both TRPM8- and TRPV1-neurons¹¹, the loss of afferent input from TRPM8-neurons distorts responses to preferred temperatures, suggesting that the attraction to warm reflects input from both TRPM8- and TRPV1-neurons. Indeed, mice that lack TRPM8- and TRPV1-cells display neither aversion or attraction between 10 °C and 50 °C¹¹. It remains to be seen whether this “push-pull” mechanism for generation of a temperature preference occurs in the spinal cord. More generally, the molecular identity of cold, heat, and broadly tuned spinal cord neurons needs to be determined, and the interactions between these neurons needs to be investigated. It will also be important to demonstrate which neurons transmit thermosensory signals from the spinal cord to the brain.

Some brain regions involved in processing thermal stimuli have been broadly defined (Figure 1C), including areas of the thalamus, parabrachial nucleus, the already mentioned POA, and cingulate cortex^{34, 40, 41}. Additionally, most somatosensory information is also represented in the somatosensory cortex, S1. To uncover details about the coding of cold, intrinsic imaging was performed of the forepaw S1-cortex in mice, as were whole-cell recordings in awake and anaesthetized animals⁴². The S1-cortex was demonstrated to be sensitive to cold stimulation and the chemical silencing of the forepaw S1 prevented appropriate responses in mice trained in a cold operant task, demonstrating that the S1 is not only activated by cold but is required for its percept being linked to a trained operation. S1 cold responses were not distinguishable from responses elicited by a vibrotactile stimulus, a result which is concordant with both cold and touch being used to code for the physical

localization of objects⁴³. Therefore, S1 provides a spatial representation of both, cold and touch.

Future directions

Thermosensation provides animals with a limited repertoire of information about environmental temperature, cold, warm and heat. We now know that sensory neurons are selective sensors of thermal stimuli, that the spinal cord is a processing center for sensory signals, and we also now know much about the major brain regions involved in producing responses to temperature cues. Although we have taken dramatic steps in understanding of many aspects of thermosensation in the past few decades, there is still a large amount of work necessary to fully understand this sense. Key advances needed include the delineation of afferent thermosensory CNS pathways with cellular resolution and the characterization of circuits responsible for integration of these stimuli with internal state as well as sensory information from other senses. Another area that we don't understand well are the adaptive properties of the thermosensory system. For instance, when one is cold, a 40 °C stimulus is attractive but, on a hot summer day, the same stimulus is aversive. Future advances in these areas will also help paving the way to intervene with maladaptive responses of the thermosensory system.

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Highlights;

- TRPV1- and TRPM8-neurons are required for responses to hot and cold, respectively.
- Different classes of trigeminal TRPM8-cells respond differently to cold stimuli.
- Spinal cord thermosensory neurons transform sensory signals from afferent inputs.
- The POA senses internal temperature and controls thermal homeostasis.
- S1 somatosensory cortex is required for operant tasks for cold cues.

Figure 1;

Pathways for thermosensory detection and processing.

Schematic representation of the major pathways for sensory detection (A), and processing (B, C) of thermal stimuli. A, peripheral sensory tissues are innervated by two largely non-overlapping populations of neurons, those expressing Trpv1 (red) and those expressing Trpm8 (blue). Note that a small number of Trpv1⁺/Trpm8⁺ neurons exists (purple) but their response characteristics are not known. Schematic represents the cell-types (center) and their responses to different temperatures. Each population is drawn in correspondence to their

approximate relative abundance when considering innervation of the oral cavity from the TG²². B, neurons in the spinal cord can be broadly divided into three classes based on their responses to temperature, heat responders (red), cold responders (blue), and hot and cold responders (yellow). Some of the notable features of these classes of neurons are listed. C, some of the CNS regions defined to have roles in thermosensation are schematized. The functions of these areas are listed, and example actions are noted.