Cold emerging from the fog

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Proposed mechanisms for the sensation of cold have focused on single proteins. A paper in this issue reports that cold transduction depends on a complex interplay among ion channels.

Cold is detected by a small subpopulation of peripheral sensory nerves that enter the central nervous system in the superficial dorsal horn of the spinal cord and respond to cold with bursts of action potentials[1]. Despite extensive research into the mechanism of cold transduction, there is no agreement on the specifics. Mostly, investigators have been searching for a single-channel mechanism analogous to that for transduction of noxious heat: a strong stimulus (>43°C) activates vanilloid receptor-1 (VR1), a recently identified protein in the transient receptor potential (TRP) family that provides one of the major mechanisms for noxious heat transduction by peripheral nerves[2]. VR1 channels open, allowing depolarizing current to flow into the heat-sensitive nerves and evoke action potential firing. However, it may be that with cold, the mechanism is not so simple. In this issue, Viana *et al.*[3] suggest a new way of thinking about transduction of a cold stimulus into an excitatory signal. Using trigeminal ganglion neurons from newborn mice, they show that cold transduction is not simply due to activation of a single channel but rather is an emergent property dependent on the expression, density and activation of several different channels expressed in cold-sensitive neurons.

The peripheral fibers of some sensory neurons respond to gentle cooling, and others respond to noxious cold. These fibers fire single or multiple bursts of action potentials in a pattern that reflects the intensity or rapidity of cooling[4]. Cold-sensitive nerves are generally considered to be a small subset of the small-diameter fibers, although with extreme cold temperatures, a much larger proportion of sensory nerves may be cold sensitive[5]. To identify cold-sensitive trigeminal neurons, Viana *et al.*[3] put dissociated neurons into short-term cell culture, then used a cold-evoked change in intracellular calcium concentration to rapidly screen for the small subpopulation (9%) that were sensitive to cold. Each responsive cell was studied using electrophysiological techniques to probe the mechanism generating cold sensitivity. Some cold-sensitive cells depolarized transiently to drive a burst of action potentials, and others showed oscillations in membrane potential that evoked repeated bursts of action potentials similar to the activity of cold fibers recorded in the skin. The current driving the depolarization, referred to as I_{cold}, was proposed to reflect activation and de-activation of multiple channels with different time, temperature and voltage dependencies, which produce a net decrease in conductance that is usually transient.

Viana *et al.*[3] found that as the temperature drops, the leak channels that normally conduct potassium ions (K^+) close, causing membrane depolarization (**Fig. 1a**). As this occurs, the depolarizing influence of the inwardly rectifying I_h current mediated by HCN channels (hyperpolarization-activated cyclic nucleotide–gated K^+ channel[6]) begins to diminish as a result of the decrease in membrane potential and the cold stimulus itself. While I_{cold} is on, the net depolarization causes action potential firing and associated calcium entry through voltage-gated calcium channels. This complex interplay of channels that all contribute to the cold response is in contrast to previously proposed mechanisms that have focused on cold modulation of single channels or pumps. These hypotheses include a simple decrease in leak conductance, such as might be mediated by the two-pore potassium channel TREK-1 (ref. 7), modulation of Na^+–K^+ ATPase activity or modulation of channels in the ENac\DEG family[8]. It was recently proposed that cold-induced current is due to activation of a relatively non-selective cation current, activated with a lower threshold in the presence of menthol[9]. A cold- and menthol-sensitive receptor (CMR1) with the same characteristics has very recently been cloned by McKemy *et al.*[10], adding weight to the hypothesis that this cation channel is an important element in cold transduction (**Fig. 1a**). Similar to VR1, CMR1 is in the TRP family of proteins, reinforcing the idea that these TRP proteins are important in transduction of temperature.

According to Viana *et al.*[3], the cold response is determined not only by the prominent expression of certain channels in cold-sensitive neurons, but also by the prominent expression of another K^+ current, termed IK_D (**Fig. 1a**), in cold-insensitive neurons. In these neurons, the strong hyperpolarizing influence provided by IK_D prevents the depolarizing action of cold from eliciting action potentials (**Fig. 1b**). In cold-sensitive neurons, however, the initial depolarization is not prevented by IK_D (**Fig. 1a**) because these channels are expressed at low levels. The importance of IK_D in preventing cold response in cold-insensitive neurons was tested by adding the IK_D blocker 4-aminopyridine (4-AP) to the bath and re-testing for sensitivity to cold stimulus. Under these conditions, some previously cold-insensitive neurons become responsive to cold (**Fig. 1c**), indicating that a potassium-channel brake prevents these neurons from responding. This, in turn, raises the interesting possibility that small changes in the balance of channel expression or properties in cold-insensitive neurons could transform cold-insensitive fibers into cold-sensitive fibers *in vivo*.

The study by Viana *et al.*[3] brings us a step closer to understanding the complex mechanism of cold transduction, but much remains to be clarified. Peripheral somatosensory nerves are responsible for reporting the occurrence and location of touch, temperature, pain and position. Detection of the stimuli for these sensations occurs in the peripheral terminals of the sensory nerves. It is important to bear in mind that Viana *et al.* performed all of their experiments at the level of the soma, which may not reflect the density and distribution of channels found in the terminals of the same cells. Nevertheless, by identifying mechanistic elements of cold transduction, Viana *et al.* make it possible to hypothesize new mechanisms contributing to the setting of cold threshold. Many of the channels contributing to the cold response are modulated either directly or indirectly by changes in intracellular second messengers, particularly cyclic nucleotides, making them good candidates for dynamic regulation of cold threshold. For example, the activation curve of HCN channels shifts to the right as cyclic nucleotides bind to the intracellular binding site[11]. Under these conditions, HCN channels will provide a more powerful depolarizing influence on membrane potential and contribute to a

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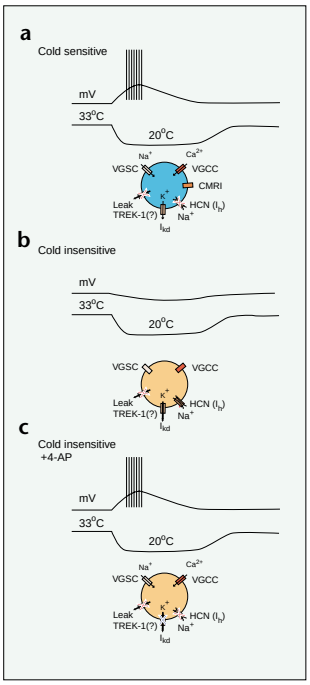

Fig. 1. Multiple ion channels contribute to cold-induced firing of action potentials in cold-sensitive neurons and pharmacologically modified cold-insensitive neurons. (**a**) A representation of how a combination of channels might contribute to the cold response. Closing of leak channels (possibly TREK-1) and opening of CMR1 channels followed by closing of HCN channels causes cold-sensitive neurons to fire a transient burst of action potentials during a cold stimulus. (**b**) Activation of IK_D exerts a brake on any depolarizing influences, inhibiting the firing of a cold-insensitive neuron. (**c**) In the presence of 4-AP, a blocker of IK_D, a cold-insensitive neuron fires action potentials in response to a cold stimulus. VGSC, voltage-gated sodium channels; VGCC, voltage-gated calcium channels.

stronger cold response. Another way that cold sensitivity of sensory neurons may be regulated is by changing relative channel density through transcriptional regulation of channel expression.

Menthol, a cyclic terpene alcohol found in mints, induces the sensation of cooling in the mouth and on the skin. Thus it is intriguing that it turns out to be a useful tool for studying cold sensitivity in individual sensory neurons. Menthol has been shown to enhance cold-induced influx of calcium[9] and to activate menthol receptors that are highly calcium permeable[10,12]. All the cold-sensitive neurons in the Viana *et al.* study[3] either were activated directly by menthol or responded to less cool temperatures in the presence of menthol. In contrast, none of the cold-insensitive neurons was sensitive to menthol. Thus it is likely that the recently cloned menthol receptor, CMR1, is a critical molecular mediator of cold and menthol transduction. Other studies, however, including the one by Viana *et al.*[3], indicate that unlike noxious heat sensation, in which there is a clear threshold for cellular excitation (around 43°C), the threshold for responses to innocuous cold (15–28°C) or noxious cold (<15°C) are broadly distributed. This indicates a more complex cellular response than is most probably mediated by CMR1 alone.

The effect of menthol is analogous to the action of capsaicin, the spicy component of hot chili peppers and an agonist for the noxious heat receptor, VR1. Interestingly, in both the Viana *et al.*[3] and McKemy *et al.*[10] studies, about half of the cold- and menthol-sensitive neurons were also sensitive to capsaicin. This suggests that some cold-sensitive neurons are also responsive to noxious heat, consistent with studies of the properties of cold fibers[4]. This observation raises an interesting question of how cold signals are distinguished from those of noxious heat in the same sensory fiber that is responsive to both signals. The answer might lie in the differential firing pattern encoded by each stimulus. For example, Viana *et al.* reported that some cold-sensitive neurons display a rhythmic bursting firing pattern in response to cold stimuli. If this type of cold-sensitive neuron is also responsive to noxious heat, it would be interesting to test its response pattern to a noxious heat stimulus.

Although Viana *et al.*[3] and Reid and others[9,10] come to different conclusions about the nature of I_{cold}, it may be that they are in fact looking at different components of the same complex response. The novelty of the Viana study is that cold responsiveness is a complex interplay among multiple channels, and that these basic elements are present in many sensory neurons, even those that are not normally sensitive to cold.

1. Christensen, B. N. & Perl, E. R. *J. Neurophysiol.* **33**, 293–307 (1970).
2. Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D. & Julius, D. *Nature* **389**, 816–824 (1997).
3. Viana, F., de la Pena, E. & Belmonte, C. *Nature Neurosci.* **5**, 254–260 (2002).
4. Kenshalo, D. R. & Duclaux, R. *J. Neurophysiol.* **40**, 319–332 (1977).
5. Simone, D. A. & Kajander, K. C. *J. Neurophysiol.* **77**, 2049–2060 (1997).
6. Clapham, D. E. *Neuron* **21**, 5–7 (1998).
7. Maingret, F. *et al. EMBO J.* **19**, 2483–2491 (2000).
8. Askwith, C. C., Benson, C. J., Welsh, M. J. & Snyder, P. M. *Proc. Natl Acad. Sci. USA* **98**, 6459–6463 (2001).
9. Reid, G. & Flonta, M. L. *Nature* **413**, 480 (2001).
10. McKemy, D. D., Neuhausser, W. M. & Julius, D. *Nature* (in press).
11. Ludwig, A., Zong, X., Jeglitsch, M., Hofmann, F. & Biel, M. *Nature* **393**, 587–591 (1998).
12. Okazawa, M., Terauchi, T., Shiraki, T., Matsumura, K. & Kobayashi, S. *Neuroreport* **11**, 2151–2155 (2000).

The frontal cortex: does size matter?

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The human frontal cortex has been reported to be proportionally larger than in other primates. Magnetic resonance scans of humans, apes and monkeys now cast doubt on this idea.

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Historically there has been a long search for specializations of the human brain that might account for our intellectual preeminence. It has often been claimed that our frontal lobes, and in particular the prefrontal cortex, are especially enlarged relative to other animals. The evidence that is most frequently cited comes from the classic work of Brodmann[1], who measured the size of the prefrontal cortex and neocortex in man and non-human primates. One can use these values to plot the size of the human prefrontal cortex against that of the entire neocortex, and then perform a regression analysis to ask what value for prefrontal cortex one would expect for a neocortex as large as it is in the human brain. From Brodmann's data, the prefrontal cortex is roughly two times as large as expected. The possible functional rele-