level of wave frequency must occur in order for binocular inputs onto single LGN neurons to be coactive and stabilized. Lastly, their results underscore the notion that activity patterns continue to reinforce the precise structure of neural circuits after they form.

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Numbing the Senses: Role of TRPA1 in Mechanical and Cold Sensation

In this issue of *Neuron*, Kwan et al. demonstrate that TRPA1 is critical for the transduction of noxious cold and mechanical stimuli, as well as in mediating the activation of nociceptors by endogenous and natural irritants. Differences between the present report and a previous study indicate that further study is needed to reach a consensus on the role of TRPA1 in the transduction of mechanical and noxious cold stimuli.

The continuously intensive investigation of the Transient Receptor Potential (TRP) ion channel family has revealed their striking contribution to sensory biology. Across a diversity of vertebrate and invertebrate species, they are the molecular machinery required for the transduction of stimuli encompassing the five classical senses. Thermal stimuli within the perceptible range (from painfully hot to cold) activate a subset of TRP channels (dubbed thermoTRPs) from three diverse subfamilies (Patapoutian et al., 2003). In addition to their distinct thermal thresholds, all of the thermoTRPs are chemosensitive; they are activated specifically by endogenous, synthetic, and plant-derived molecules, most of which evoke cutaneous thermal and pain sensations.

A number of recent studies demonstrate unequivocally that similar to the noxious heat and capsaicin receptor TRPV1, cold-activated TRPA1 comprises a molecular site of integration of multiple pain producing stimuli, including pungent components derived from mustard oil, cinnamon oil and garlic, and the endogenous pro-algesic bradykinin (Bandell et al., 2004; Jordt et al., 2004). The issue of whether TRPA1 is activated by noxious cold, however, is not without controversy for two main reasons. First, calcium imaging and electrophysiogical studies of heterologously expressed TRPA1 failed to reproduce the finding that TRPA1 is activated by noxious cold temperatures, as first observed by Story et al. Second, the use of newly identified nonthermal TRPA1 agonists and variation in thermal/pharmacological response profiles of cultured sensory neurons began to reveal disparate observations among laboratories (Bandell et al., 2004; Jordt et al., 2004). Many of these observations were contrary to the initial hypothesis that the dual cold and heat sensitivity of a small subset of neurons could be explained by the expression of TRPA1 in a subset of TRPV1-positive neurons (Story et al., 2003).

While the field awaited knockout mice to resolve the "cold controversy," Corey et al. showed through a series of in vitro knockdown studies that TRPA1 was a promising candidate for the mechanosensitive transduction channel of hair cells in the inner ear (Corey et al., 2004). New and exciting questions arose as to whether mice deficient for TRPA1 would be impaired in their ability to hear as well as to sense cold and noxious chemical agents. Our anticipation is answered now with not one, but two independently generated lines of TRPA1 knockout (TRPA1$^{-/-}$) mice reported recently by Bautista et al. in *Cell* and by Kwan et al. (2006) in this issue of *Neuron*. However TRPA1 remains somewhat silent about its mysteries, not for lack of hearing in these mice, but rather stubborn inconsistencies regarding the role(s) of TRPA1 in somatosensation.

The widely accepted model for auditory mechanotransduction proposes that an ion channel gated by mechanical force is located at the tips of hair cell stereocilia (Gillespie et al., 2005). Mechanical forces are thought to be transmitted to the channel via an elastic structure (the "gating spring"). The very features of TRPA1, an ion channel with a predicted 16 N-terminal ankyrin repeats (a built in gating spring), made it an attractive candidtate as a potential mechanosensor. To this end, Corey et al. showed in late 2004 that TRPA1 mRNA is first expressed in the inner ear at the initiation of hair cell mechanotransduction in utero and that the protein is localized within stereocilia postnatally. They then utilized RNA interference (siRNA and morpholino) to knockdown mouse

and zebrafish TRPA1. Knockdown of TRPA1 diminished hair cell transduction currents, providing the most compelling evidence of the study (Corey et al., 2004). Taken together, these data branded TRPA1 a strong candidate for the mechanosensitive transduction channel in vertebrate hair cells. However, similar to the report by Bautista et al., Kwan et al. report in this issue of *Neuron* that they observe no hearing deficits in TRPA1$^{-/-}$ mice. Measures of behavioral responses to sound and vestibular challenge, styryl dye uptake in ex vivo preparations, and electrophysiological recordings from auditory brain stem and hair cells show that all properties of the native transduction channel appear intact in TRPA1$^{-/-}$ mice.

The previous study by Corey and colleagues was compelling and siRNA is a powerful tool for targeted gene knockdown, but unexpected effects such as hybridization to nontarget sequences are difficult to rule out. Perhaps previous siRNA experiments silenced a gene related to mechanosensory transduction. The authors postulate here that targeted disruption of TRPA1 during development may lead to compensatory mechanisms via a yet unidentified channel, one that may form heteromeric complexes with TRPA1. Such a channel could be a TRP family member, similar in sequence to TRPA1, and off-target effects could lead to nonspecific silencing of the channel. This certainly warrants further study; clearly a molecular key to our understanding of mechanotransduction is "still out there."

It has been recently hypothesized that TRPA1 also possesses properties reminiscent of peripheral mechanonociceptors and a contribution of TRPA1 to somatic mechanosensation is revealed by this study in *Neuron* (Nagata et al., 2005). Contrary to the findings of Bautista et al., the Corey group reports that mice lacking TRPA1 are deficient in the detection of acute high-threshold mechanical stimuli applied to the extremities. Blunted sensitivity to mechanical stimulation of the hindpaws of mice becomes apparent at forces below the threshold intensities reported by the Julius group, but differences could arise from the manner in which behavioral tests were performed (50% threshold testing versus stimulus-response functions). Although direct mechanical activation of TRPA1 has not yet been demonstrated, these data provide strong evidence suggesting that TRPA1 mediates responses to high-threshold mechanical stimuli in peripheral nociceptors.

Bradykinin (BK), an endogenous pro-algesic agent released during inflammation, sensitizes neurons to thermal and mechanical stimuili resulting in lowered thresholds of mechanical and thermal pain (hyperalgesia). Bandell et al. previously showed that TRPA1 robustly couples to BK signaling via the G protein-coupled BK receptor B2R in vitro, revealing yet another mechanism by which TRPA1 is involved in mediating pain (Bandell et al., 2004). Given this, Kwan et al. go on to confirm an in vivo role of TRPA1 in the development of BK-induced mechanical hyperalgesia. The threshold of mechanically induced pain (indicated by paw withdrawal) after BK-mediated inflammation is significantly higher in TRPA1$^{-/-}$ mice compared to wild-type, suggesting mice lacking TRPA1 are not subject to heightened BK-induced pain sensitivity. Furthermore, a haplo-insufficiency effect of TRPA1 gene disruption is apparent in these assays. In both acute mechanonociception and mechanical hyperalgesia, the phenotype observed in heterozygous mice is intermediate relative to their wild-type and knockout littermates. This indicates an interesting gene-dosage effect not reported for other targeted thermoTRP channels.

A host of pungent, irritating compounds, including allyl isothiocyanate (mustard oil), allicin (derived from fresh garlic), and cinnamaldehyde (cinnamon oil) are potent TRPA1 agonists and burning in sensory quality (Bandell et al., 2004; Jordt et al., 2004; Bautista et al., 2005; Macpherson et al., 2005). A focus of studies led by Bautista and Kwan is whether TRPA1 is necessary for the pain response generated by mustard oil. Mustard oil (present in wasabi and horseradish) induces a multitude of sensory effects encompassing taste, smell, and pain modalities. In terms of pain, mustard oil causes acute nociceptive responses and a neurogenic response (plasma extravasation and release of proinflammatory molecules), which heightens inflammation. Behavioral tests from both studies of TRPA1$^{-/-}$ mice show attenuated acute nocifensive behaviors in response to mustard oil. Kwan et al. show that TRPA1-deficient mice are less sensitive to water spiked with mustard oil, still consuming a quantity of water containing such high concentrations of the chemical that wild-type mice cease drinking entirely. Both studies also investigated whether TRPA1 is required to mediate pain responses in peripheral nociceptors and find TRPA1-deficient mice do not show pain responses similar to wild-type when the chemical is applied to the hindpaw. Again, Kwan et al. observe an intermediate phenotype in heterozygous mice. Although significant attenuation of pain behaviors is observed by both groups, one subtle difference is that Kwan et al. observe a slight residual sensitivity to mustard oil in TRPA1$^{-/-}$ mice in their assays, whereas Bautista et al. report a complete lack of pain response to mustard oil.

Clearly, TRPA1 contributes to sensitivity to mustard oil; however, a discrepancy remains regarding the specificity of this chemical. As expected and identical to the *Cell* study, a population of cultured DRG neurons dually activated by mustard oil and capsaicin (TRPA1 and TRPV1 agonists, respectively) is present in wild-type mice but absent in TRPA1$^{-/-}$ mice. Also identical to the *Cell* study, total capsaicin sensitivity remains completely intact indicating that ablation of TRPA1 does not induce trans-effects on TRPV1 expression. Here, the similarity ends. In contrast to Bautista and coworkers who detect no mustard oil responses in cultures derived from newborn TRPA1 null mice, Kwan et al. identify a subset of adult neurons that exhibit responses to mustard oil but not capsaicin. This result resembles the observations reported by Bandell et al. who previously hypothesized another receptor for mustard oil is present in DRG neurons (Bandell et al., 2004). The data of Kwan et al. suggest this as well because all TRPA1-expressing neurons express TRPV1 and should be activated by both TRPA1 and TRPV1 agonists. These inconsistencies are difficult to resolve, but differences in the two studies could result from experimental approach. It is possible that the capsaicin-insensitive population of neurons activated by mustard oil in adult cultures is not present in cultures derived from newborn animals.

Neither study resolves whether TRPA1 accounts for cold sensitivity in DRG cultures. The experiments of Kwan et al. were not extended to investigate cold sensitivity of adult DRG neurons. Bautista et al. observe two populations of cold-sensing neurons in their cultures, one responding to menthol and neither to mustard oil, suggesting that one population expresses TRPM8 and the other is cold sensitive via an unknown mechanism exclusive of TRPA1 (Bautista et al., 2006).

Compounding the issue of whether TRPA1 plays a role in thermosensation, the data derived from cold-related behavioral assays of the Julius and Corey groups also differ. Both groups performed analyses of cold sensing by utilizing the acetone and cold plate tests. Bautista et al. observe no behavioral differences in response to cold stimuli among wild-type, heterozygous, and knockout mice, but Kwan et al. report here that mice lacking TRPA1 are deficient in their ability to sense cold. Behavioral responses were scored differently between the two groups. Bautista et al. measured the time until mice first showed a response to the cold challenge (latency), while Kwan et al. recorded the number of times mice responded over the course of the assays (Bautista et al., 2006). Which measure is most relevant to the detection of cold pain? Qualitatively cold pain has acute components but also develops over time. Regardless, these differences raise important issues pertaining to the performance and analysis of behavioral assays. Furthermore, cold-related pain behavioral assays are not straightforward or consistent. Often mice show no apparent response to very cold temperatures (unpublished observations). Perhaps thermal preference tests of TRPA1-deficient mice (two-temperature choice and gradient assays) will reveal more subtle roles of this channel. We must also consider the contribution of TRPM8 to cold sensing, and surely these types of issues will arise when performing behavioral analyses of TRMP8 knockout animals.

The study Kwan et al. reveals an additional layer of complexity in cold-related pain. They include mice of both genders in their tests, showing a trend toward diminished sensitivity in male mice that is greatly unmasked in female mice. Gender differences in pain sensitivity are documented in rodent behavioral assays and human psychophysical tests, with females exhibiting a lower pain threshold compared to males. This variable must be considered in phenotypic analysis when conducting pain studies (Mogil and Chanda, 2005).

Another lingering issue arises when one considers the strategies that were used to knock out the TRPA1 gene in the two reports. In both cases, exons including the pore-loop domain of TRPA1, but not the other coding exons, were deleted. Thus, in both knockout lines, a truncated transcript for TRPA1 is potentially generated. Indeed, supplemental data provided by Bautista et al. show robust expression of a truncated transcript in TRPA1$^{-/-}$ mice. In the study by Kwan et al., the authors utilized a creative approach to avoid potential impact of a truncated transcript. These authors include in their targeting vector design an in-frame ER retention signal that would be incorporated into a truncated transcript generated from the targeted allele. Thus, any potential truncated TRPA1 protein that might be generated in the TRPA1$^{-/-}$ mice should be retained in the ER and therefore not expressed on the cell surface. Whether truncated proteins are indeed generated in either TRPA1$^{-/-}$ lines is not known, and thus the potential impact of these truncated proteins should be considered when interpreting these studies. For example, is it possible that a truncated TRPA1 could alter the function of other TRP channels by coassembly? Would such a truncated protein act in a dominant-negative fashion to inhibit TRP channels? Until antibody studies can confirm the absence or presence of truncated TRPA1 proteins in the TRPA1$^{-/-}$ animals, these will remain open questions.

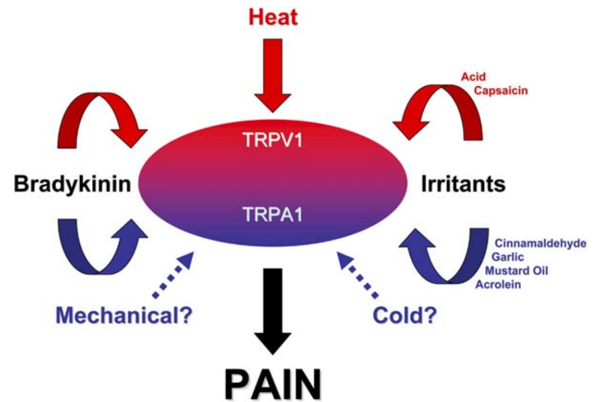

Figure 1. Model of Our Current Understanding of Signaling through TRPA1

It is hypothesized that polymodal nociceptors (represented by the oval) express both TRPA1 and TRPV1 (denoted by blue and red, respectively). TRPV1 is gated by noxious heat as well as modulated by bradykinin and chemical irritants. Consistent with a recent report by Bautista et al., Kwan et al. demonstrate in this issue of *Neuron* that TRPA1 contributes to the transduction of these noxious signals. Mechanical and cold stimuli may also activate TRPA1, but these relationships are represented as dashed lines given the conflicts between the present study and that of Bautista and colleagues.

Although TRPA1 has been successfully targeted and knockout mice analyzed by two independent groups, it seems we cannot summarily conclude on the role of TRPA1 in somatosensation (Figure 1). Further analyses of these mice are clearly necessary to clarify the contribution of TRPA1 to mechano-, chemo- and thermosensation. In terms of cold sensing, perhaps when TRPM8 knockout mice become available, side-by-side analysis of cold-related behavior in both strains and the generation of double knockout will be informative. Despite the differences between this study in *Neuron* and that of Bautista et al. in *Cell*, both demonstrate a significant role of TRPA1 in mediating pain, suggesting it is a potential target in the development of new therapeutics.

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Dendritic Enlightenment: Using Patterned Two-Photon Uncaging to Reveal the Secrets of the Brain's Smallest Dendrites

It has been a longstanding challenge for experimentalists to manipulate precisely the spatial and temporal patterns of synaptic input to the dendritic tree in order to mimic activity occurring in the intact brain and determine their importance for synaptic integration. In this issue of *Neuron*, Losonczy and Magee have used rapid multisite two-photon uncaging of glutamate to define patterns of synaptic input on a submillisecond and micron scale to investigate the rules for summation of synaptic inputs in the fine oblique dendrites of pyramidal neurons.

Most synapses are made onto the dendrites of neurons. This is essential for the wiring up of the brain (Chklovskii, 2004) but also has direct consequences for the way individual synaptic inputs are integrated by the postsynaptic neuron to generate its action potential (AP) output (Häusser and Mel, 2003). The integrative properties of dendrites are governed by their passive cable properties, but dendrites are also known to contain various types of voltage- and calcium-dependent conductances (Johnston et al., 1996). Together they can shape the rules for synaptic integration such that synaptic inputs summate sublinearly or approximately linearly at the soma (Cash and Yuste, 1999; Urban and Barrionuevo, 1998). However, under some conditions, particularly when many synaptic contacts are concurrently active in a small region of the dendritic tree, regenerative activation of dendritic Na^+, Ca^{2+} and NMDA receptor channels may occur, resulting in a supralinear response: a dendritic spike. The exact conditions for the generation of these local spikes, in particular in the thin basal and oblique branches of pyramidal neurons are unclear. How many synaptic inputs need to arrive on a small dendritic branch, and in which time interval, for the branch to exit the approximately linear operating regime and generate a local dendritic spike? Does the threshold for evoking such a spike, or its spatial extent and peak amplitude, depend strongly on the exact spatial pattern of inputs onto a single branch? And once a spike is evoked in a particular dendrite, how does its effect spread to the soma, and how does this affect the AP output of the neuron?

The ability of the thin dendritic branches of hippocampal and neocortical pyramidal neurons to support initiation of local dendritic spikes has been known for some time. Schiller et al. (2000) demonstrated that local dendritic spikes can be evoked in the basal branches of layer 5 pyramidal neurons by focal extracellular stimulation of nearby axons, and by one-photon uncaging of glutamate. Using a combination of pharmacology and modelling they showed that these spikes are carried by Na^+, Ca^{2+}, and predominantly by NMDA receptor conductances. Applying the same techniques to the basal dendrites of hippocampal CA1 pyramidal neurons, Ariav et al. (2003) showed that these, too, support local dendritic spikes, which in this case are dominated by a fast, Na^+-based initial component followed by a slow, NMDA receptor-dependent component. Again in layer 5 pyramidal neurons, Polsky et al. (2004) demonstrated using extracellular stimulation at two locations that nearby inputs on the same branch summated supralinearly as they cooperated in the initiation of a local dendritic spike in that branch, while spatially separated inputs to different branches summated linearly. These experiments provide support for a two-layer "neural network" model of synaptic integration in the dendritic tree of a pyramidal neuron, in which the individual thin dendritic branches correspond to the first layer of thresholding units whose output is then relayed to the second-layer thresholding unit corresponding to the AP initiation site near the soma of the neuron (Häusser and Mel, 2003; Mel, 1993; Poirazi et al., 2003).

In order to understand the "arithmetic" of dendrites in realistic detail, experiments are required which provide more quantitative control over the spatiotemporal organization of synaptic input patterns delivered to individual dendritic trees. Existing methods either do not permit fine control of the spatial pattern of synaptic input, or do not provide a physiological time course or AMPA/NMDA ratio for individual synaptic conductance inputs. For example, experiments using focal extracellular stimulation of nearby axons cannot accurately control which and how many synapses are activated on the postsynaptic neuron in question. Furthermore, dendritic spikes are typically elicited only after two successive extracellular stimulation events (Schiller et al., 2000), as this helps to increase recruitment of NMDA receptor-mediated conductances. Similarly, one-photon uncaging tends to activate glutamate-gated conductances