effect of calcineurin on the extinction of auditory fear conditioning, another form of associative memory that also depends on the amygdala. Demonstrating a link between calcineurin and Zif268, Baumgärtel *et al.*[1] showed that genetic inhibition of calcineurin increased basal Zif268 levels and modified the expression of a subset of proteins in the same direction as in Zif268-overexpressing mice. Thus, although not directly shown by the authors, these data suggest the exciting possibility that calcineurin inhibition during learning gates Zif268-dependent protein expression, thereby supporting memory persistence (**Fig. 1**). It will be interesting to see whether calcineurin overactivation blocks training-induced Zif268 increases and whether accelerated memory extinction can be rescued to control levels by pretraining knockdown of calcineurin in the amygdala.

Notably, the authors found no differences in memory extinction when transgene expression in any of the mouse strains was induced after conditioning. This indicates that calcineurin controls memory persistence through a mechanism that takes place early during the establishment of the memory trace, but not once it is formed. This is in contrast with previous studies where genetic[5] or pharmacological[6] inhibition of calcineurin after fear conditioning prevented memory extinction. Because the former study used the same transgenic mice as Baumgärtel *et al.*[1], the results suggest that extinction operates in different ways depending on the type of memory. In any case, the present data strongly suggest that fluctuations in calcineurin activity are inconsequential to the mechanism of extinction in CTA.

Finally, because calcineurin can indirectly activate PP1, another serine/threonine phosphatase, the authors also examined the role of PP1 in CTA. Memory extinction appeared normal in PP1 mutant mice. In this experiment, however, the authors initiated the extinction trials shortly after conditioning, a regime known to alter the properties of extinction[7,8], thus complicating comparison with the calcineurin data. Also, PP1 activity after CTA was measured in cytoplasmic fractions rather than in the membrane fractions where PP1 targets numerous synaptic proteins. In addition, CTA learning is impaired in mutant mice lacking the PP1-anchoring protein spinophilin[9]. In view of the evidence, it may be premature to rule out a role for PP1 in CTA.

The insightful results of Baumgärtel *et al.*[1] raise a number of questions for future research. What specific synaptic connections in the amygdala undergo calcineurin inhibition after training, and is synaptic strength modified accordingly? What activity-dependent mechanism modulates calcineurin in behaving animals, and what signaling pathway couples calcineurin to Zif268 induction? One possible mechanism for the sustained inhibition of calcineurin is the upregulation of endogenous, membrane-associated calcineurin inhibitors, a number of which are expressed in the brain[10]. It will also be interesting to clarify which of the proteins commonly regulated by calcineurin and Zif268 in transgenic mice are modified during CTA and how they contribute to memory retention and extinction. From a biochemical standpoint, the persistence of aversive memory suggests that the signaling program initiated by calcineurin inhibition during conditioning may be particularly resistant to the molecular mechanisms of extinction. One postulated cellular correlate of memory extinction is synaptic depotentiation[11], the process in which synaptic strength is reversed to prepotentiation levels. It remains to be seen whether synaptic depotentiation is differentially affected in calcineurin mutants and control mice during CTA learning and extinction.

The results reported by Baumgärtel *et al.*[1] contribute substantially to the growing appreciation of the importance that negative regulatory mechanisms are likely to have in the formation and storage of memories. Indeed, although positive regulators (such as kinases) have received most of the attention so far, it is clear that only the conjunction of opposing signals can provide sufficient versatility, balance and meaning for memories to fulfill their biological functions.

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Fire in the hole: pore dilation of the capsaicin receptor TRPV1

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The capsaicin receptor TRPV1 is important in pain sensation. A new study suggests that this nonselective cation channel shows dynamic alterations in ion permeability, which may contribute to mechanisms of pain hypersensitivity.

Ion channels are generally described by bedrock characteristics that include such parameters as ionic selectivity, conductance, voltage sensitivity, sensitization and inactivation, and pharmacological profiles, among others. Although many of these properties are subject to modulation, ion selectivity is generally considered to be an invariant functional fingerprint that does not change in response to different physiological conditions. This dogma was challenged some years ago with the analysis of ATP-gated ion channels of the P2X family, which suggested that prolonged agonist exposure increased the pore size, leading to enhanced permeability of the channel to large cations[1,2]. This phenomenon, known as pore dilation, has been largely relegated to P2X receptors until now. In this issue,

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Chung *et al.*[3] provide evidence to suggest that pore dilation also applies to other ion channels, in this case the thermosensitive ion channel TRPV1.

TRPV1, a member of the extended family of nonselective cationic TRP channels, is important in pain sensation. In addition to being activated by capsaicin, the pungent ingredient in 'hot' chili peppers, TRPV1 is also a thermosensitive channel that contributes to the detection of noxious heat by a subpopulation of primary afferent sensory neurons[4]. Notably, a variety of inflammatory agents can alter the temperature threshold of TRPV1 activation, a process that contributes to pain hypersensitivity in the context of tissue injury. This process includes allosteric modulation of the channel by pro-algesic agents, such as extracellular protons, that directly interact with TRPV1. Other inflammatory mediators, such as bradykinin or nerve growth factor, enhance TRPV1 sensitivity indirectly by activating membrane receptors that couple to phospholipase C signaling pathways[5]. These inflammatory mediators markedly alter TRPV1 activation thresholds and augment the excitatory response. However, less attention has been given to the effects of inflammatory mediators on other biophysical properties, such as ion permeation and selectivity.

Borrowing from the approach used to define pore dilation in P2X channels, Chung *et al.*[3] investigated the effects of sustained agonist exposure on TRPV1 ion permeability using the standard techniques of ion substitution and reversal potential analysis. On the basis of these experiments, they found a time- and concentration-dependent change in ionic permeability following prolonged exposure to chemical (but not thermal) stimuli (**Fig. 1**). For example, the relative permeability to calcium ions (as compared with sodium) increased three-fold. Perhaps most notably, TRPV1 also becomes permeable to large organic cations. Indeed, activation of both native and recombinant TRPV1 promotes uptake of large molecules[6], including dyes such as YO-PRO1 and FM1-43. Chung *et al.*[3] found that uptake is a dynamic feature of TRPV1, occurring only after repetitive or prolonged application of capsaicin or protons. Mutational and chemical modification of residues in the putative pore region alters this stimulus-evoked change in ionic selectivity, suggesting that these changes in cation permeation (operationally defined as pore dilation) reflect direct changes to the pore itself.

Similar mutational studies have been carried out with P2X receptors, but whether the observed changes in ion selectivity reflect true pore dilation remains controversial. A variety of alternative mechanisms have been proposed to account for the observed stimulus-dependent increase in permeability to large cations. One possibility invokes downstream activation of a secondary pore (channel or transporter) that permits uptake of dyes and other cations. For example, $P2X_7$ receptors are proposed to associate with connexin hemi-channels that promote dye uptake in a $P2X_7$-dependent manner[7,8]. Another model suggests that association of activated single channels leads to the formation of channel multimers whose permeation properties change as a function of density[9]. In either case, mutational studies do not distinguish among such possibilities so long as the functional analysis is limited to the measurement of whole-cell currents, which represent a population average of ion permeation properties. In the end, definitive evidence for bona fide pore dilation will require single-channel measurements and the analysis of changes to unitary conduction properties.

Irrespective of the exact biophysical mechanism underlying pore dilation, enhanced cation permeability resulting from TRPV1 activation may have a number of interesting and important physiological ramifications. First, the ability of TRPV1 to accommodate large permeant ions has practical applications with regard to marking or otherwise modifying cells that specifically express this ion channel and respond to capsaicin. An elegant example involves the agonist-dependent delivery of the membrane-impermeant local anesthetic QX-314 to TRPV1-expressing sensory nerve terminals[10]. This provides a potential strategy for generating analgesia and avoiding the typical numbing side effects common to nonspecific anesthetic regimes by sparing effects on other cell types, such as motor neurons. Second, changes in cation permeability are likely to have very proximal effects on TRPV1 itself (through calcium-dependent desensitization), as well as on other neighboring channels that are also modulated by intracellular calcium, such as voltage-dependent channels and the wasabi/irritant receptor TRPA1. This, in turn, will affect a host of downstream processes, including neurotransmitter release from both central and peripheral terminals, thereby modulating the magnitude of both acute pain and neurogenic inflammatory responses. Of particular interest is the finding that protein kinase C–dependent phosphorylation of TRPV1 can enhance the pore dilation effect (**Fig. 1**). This may enhance the abilities of inflammatory mediators, such as bradykinin or nerve growth factor, to potentiate TRPV1 and produce pain hypersensitivity through their actions on phospholipase C signaling pathways.

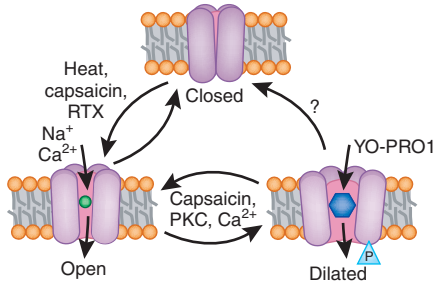

Figure 1 The TRPV1 pore dilation model proposed by Chung *et al.*[3]. The initial open state of TRPV1 shows low permeability to large cations. Prolonged exposure to chemical agonists induces a transition to a dilated state, in which permeability to large cations is increased.

Finally, prolonged exposure to capsaicin selectively injures or kills TRPV1-expressing sensory neurons via an as-yet-undefined mechanism that may include aspects of necrosis and/or apoptosis[11]. The enhanced permeability to calcium and other (large) cations may very well contribute to cell death pathways. This might account for the paradoxical use of capsaicin as an analgesic agent: high doses or prolonged exposure can lead to decreased pain sensation through death or injury of the sensory nerve fiber—quite possibly the source of the macho tolerance displayed by chili aficionados to earth-scorching habaneros.

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