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How I learned to stop worrying and love calcineurin

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Abstract

Many kinases have been implicated in memory formation, but a new study suggests that a phosphatase, calcineurin, is important for the long-lasting nature of emotional memories by making them resistant to extinction.

Ever wondered what is it about emotional or traumatic memories that make them more vivid, recurrent and stable than other memories? In this issue, Baumgärtel *et al.*¹ show that calcineurin (a phosphatase also called PP2B) confers their characteristic dominance and resilience to extinction on emotional memories, even in the face of later events that prove that their maintenance is no longer justified or useful.

To study what makes emotional memories persist, the authors chose conditioned taste aversion (CTA), a protocol in which an animal learns to reject an appetitive novel taste (such as saccharin solution) when it is paired with an illness-inducing chemical (LiCl₂ injection)². A single taste-illness pairing suffices to produce a robust and long-lasting aversive memory for the taste. Like other forms of associative learning, CTA depends on NMDA receptors and changes in gene expression³. Although the neuroanatomical substrates supporting CTA memory are incompletely defined, they include the amygdaloid complex, the insular cortex and the parabrachial nucleus in the brain⁴.

Baumgärtel *et al.*¹ found that CTA training triggered a decrease in calcineurin activity selectively in the amygdala. This finding alone is noteworthy, given the scarcity of reports that have demonstrated an actual change in calcineurin activity associated with learning. More strikingly, the inhibition of calcineurin was observed three days after learning. One is left wondering whether this reflects a sustained decrease from the time of learning or a more complex pattern of calcineurin activity. A kinetic study of calcineurin activity following training would also provide clues as to the molecular mechanism of calcineurin inhibition, given that the protein level was unchanged. Calcineurin is a serine/threonine phosphatase activated by Ca²⁺/calmodulin that sometimes inhibits the proteins that it regulates. Hence, one would expect the decrease in calcineurin activity to stimulate downstream neuronal signaling. In keeping with this, CTA increased the mRNA level of the memory-related transcription factor Zif268, again three days after training. Given that immediate early genes, such as Zif268, typically outlast the duration of the stimulus by only a few hours, this result may point to the existence of a tonic inhibitory mechanism over Zif268 that becomes relieved on calcineurin inhibition.

Baumgärtel *et al.*¹ then used conditional transgenic mice in which calcineurin activity was either decreased or increased in the forebrain to show that it is the level of calcineurin activity at the time of learning, and only then, that determines the robustness and persistence of the aversive memory. Indeed, mice with genetically inhibited calcineurin showed undiminished aversion to saccharin even after several presentations. Similar results were found in transgenic mice overexpressing Zif268. Conversely, memory was extinguished substantially faster in mice with higher calcineurin activity compared with controls. The authors observed a similar

bidirectional 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 genetic⁵ or pharmacological⁶ 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 spinophilin⁹. 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 brain¹⁰. 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 depotentiation¹¹, 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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Figure 1.

The role of calcineurin in conditioned taste aversion. **(a)** Mice learn to reject a new taste when it is paired with an illness-producing toxin. A reduction in brain calcineurin (CaN) activity makes the memory for the taste aversion more resistant to extinction. Conversely, reversal of taste aversion is facilitated when CaN activity is high at the time of learning. **(b)** CTA triggers a decrease in CaN activity in the amygdala that may relieve inhibition of a Zif268-dependent signaling pathway. Altered CaN-dependent protein synthesis may contribute to the persistence and robustness of emotional memory.