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NEUROSCIENCE

In search of the memory molecule

The protein PKM–ζ has been proposed to regulate the maintenance of memory in rodents, but this theory has been questioned. The finding that another isoform of the protein acts as a backup if PKM–ζ is lacking will influence this debate.

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An understanding of memory has long been a goal of neuroscience. One question that has attracted particular attention is whether there is a specific molecule that maintains memories. After almost two decades of careful work, neuroscientist Todd Sacktor and colleagues thought they had the answer. In 2006, the authors reported[1] that an atypical isoform of the enzyme protein kinase C, called PKM-ζ, was involved in maintaining memories in mice, and that an inhibitor of PKM-ζ could erase memories. The results were subsequently questioned[2,3], and controversy ensued. Writing in *eLife*, the same group that performed the 2006 study opens a new chapter in this debate[4], arguing that PKM-ζ should be restored to its pre-eminent status as the memory molecule.

More than half a century ago, the psychologist Donald Hebb proposed that the synaptic connections between two neurons are strengthened when the neurons fire together[5]. He suggested that this form of synaptic strengthening provided the basis for the formation of long-term memories, enabling many neurons to be linked together in cell assemblies that serve as the physical substrates of memory, called engrams. It was later discovered[6] that high-frequency neural stimulation led to persistent increases in synaptic strength, known as long-term potentiation (LTP). Most neuroscientists embraced the idea that understanding LTP was the key to understanding memory[7]. The race was on to identify the molecular machinery involved in LTP.

One molecule in particular emerged from the fray. Although dozens of molecules were involved in initial synaptic strengthening following high-frequency stimulation, only PKM-ζ seemed to be crucial for maintaining these strengthened connections[8]. In PKM-ζ, therefore, the activity of a single molecule was linked to the persistence of memory. Subsequently, several experiments showed that inhibition of PKM-ζ after memory formation (for example, by using a 13-amino-acid

protein fragment called ZIP, which mimics the natural substrate that inactivates PKM-ζ) led to memory erasure[1,9].

However, enthusiasm surrounding PKM-ζ waned dramatically following the discovery that mice in which PKM-ζ had been deleted showed normal LTP and memory[2,3]. More puzzling still, ZIP produced LTP-reversing and memory-erasing effects in mice that lacked PKM-ζ, similar to its effects in normal mice that expressed the enzyme. The amnesiac effects of ZIP, therefore, must be acting through another mechanism.

Do these results indicate that PKM-ζ is not necessary for memory? Much of the initial clamour surrounding the 2013 papers[2,3] focused on this possibility. It seems unlikely, however, because more than one method for inhibiting PKM-ζ erases memories[9]. An alternative possibility is that PKM-ζ has an essential role in LTP maintenance and memory persistence in normal mice, but compensatory processes that are sensitive to ZIP emerge in PKM-ζ-deficient mice.

This brings us to the detective work of the current study. Tsokas *et al.*[4] first confirmed that ZIP reversed LTP in both normal and PKM-ζ-deficient mice, indicating that trivial procedural differences could not resolve the controversy. Next, the authors showed that induction of LTP produced an increase in PKM-ζ in slices taken from the hippocampal region of the brains of normal mice, and that there was a sustained increase in another atypical protein kinase C isoform, PKC-ι/λ, in slices from PKM-ζ-deficient mice. Moreover, injecting either PKC-ι/λ or PKM-ζ directly into hippocampal CA1 pyramidal neurons
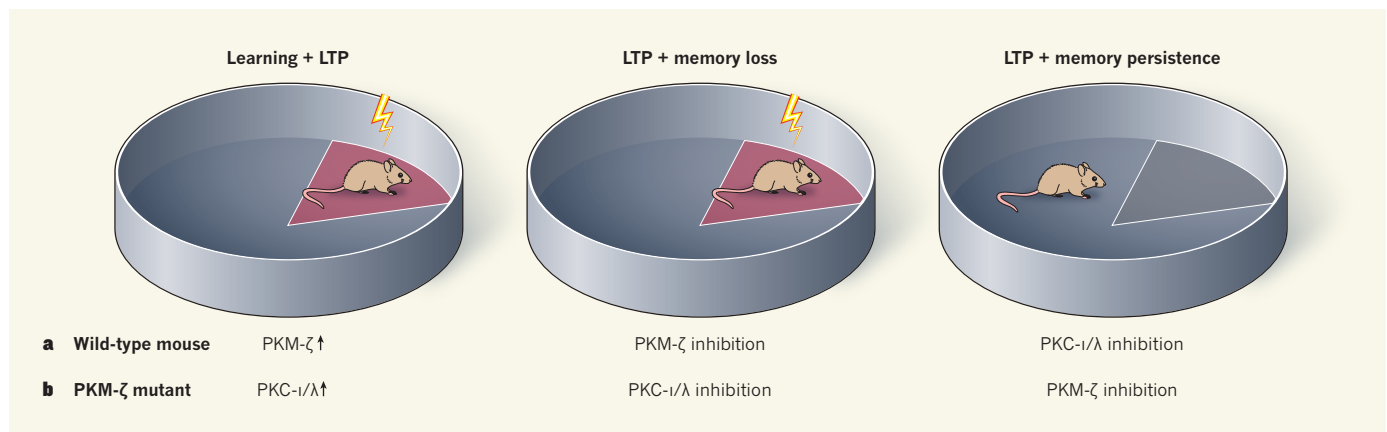

Figure 1 | Memory loss modulated. In place-avoidance tests, mice learn that they will receive a foot shock if they move over a certain part of a rotating test arena. During this learning, the synaptic connections between neurons are strengthened in a process called long-term potentiation (LTP), which is required for memory formation. Tsokas *et al.*[4] investigated how two atypical isoforms of the enzyme protein kinase C — PKM-ζ and PKC-ι/λ — regulate memory maintenance following LTP induction. **a**, In wild-type mice, levels of PKM-ζ rise following learning. Inhibition of PKM-ζ in these mice causes loss of LTP and hence loss of memory, so the mice forget how to avoid a shock. By contrast, inhibition of PKC-ι/λ has no effect on memory of the learned activity. **b**, In mice that lack the gene encoding PKM-ζ, PKC-ι/λ is elevated following LTP induction. Inhibition of PKC-ι/λ causes LTP and memory loss, whereas PKM-ζ inhibition has no effect. Thus PKM-ζ is the main substrate for memory maintenance in normal conditions, but PKC-ι/λ can compensate in its absence.

induced LTP in slices from normal mice. ZIP treatment reversed the effects of either protein injection, hinting that PKC-ι/λ might be the mystery molecule that compensates for loss of PKM-ζ.

To test this idea directly, Tsokas and colleagues inhibited either PKM-ζ or PKC-ι/λ and examined LTP in hippocampal slices (Fig. 1). In slices from control mice, inhibiting PKM-ζ blocked LTP, but PKC-ι/λ inhibition had no effect. By contrast, in PKM-ζ-deficient mice, inhibiting PKC-ι/λ blocked LTP, but PKM-ζ inhibition was ineffective. The same pattern emerged when the authors examined the effects of PKC-ι/λ and PKM-ζ inhibition on memory in control and PKM-ζ-deficient mice.

Do these latest results restore the position of PKM-ζ as the leading memory molecule? The allure of the PKM-ζ theory is the idea that a single molecule is responsible for maintaining LTP and memories. The current findings are not inconsistent with this view. However, in their experiments, Tsokas *et al.* inhibited PKM-ζ in normal mice before (rather than after) LTP and memory induction. This means that they cannot directly evaluate the enzyme's role in the persistence of LTP and memory.

The PKM-ζ saga serves as a cautionary tale about the specificity of the tools that we use to examine brain function and establish causality. The controversy exposed the bluntness of ZIP as a tool for probing PKM-ζ function because it clearly affects other molecules and may even lead to neuronal silencing[10]. Equally, seemingly more specific interventions, such as genetic deletion of PKM-ζ, produced a cascade of unintended compensatory changes, which clouded interpretations and masked predicted outcomes. This limitation is not restricted to genetic mutations, but extends to any intervention that perturbs brain function (such as optogenetic or chemogenetic strategies in which genetically introduced proteins can be activated and inhibited in response to light or drugs).

As the PKM-ζ debate rumbles on, there is a broader mystery to consider. Molecular neuroscientists such as Tsokas and colleagues present a static view of the engram, in which patterns of synaptic changes that are initiated during memory encoding are maintained over the lifetime of the memory. By contrast, systems neuroscientists present a more dynamic picture, emphasizing memory maintenance in the midst of broad changes in the synapses[11]

and even the neurons[12] that correspond to the engram. A full account of memory persistence needs to merge these molecular and systems perspectives, allowing the twain to meet. ∎

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