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PERSPECTIVE

Relocating coincidence detection for associative learning

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To successfully navigate and survive in a changing environment, animals need to learn to associate sensory stimuli with positive or negative experiences. But how do neurons 'know' when two stimuli occur simultaneously? This 'coincidence detection' function has been ascribed in many systems to Ca^{2+}/calmodulin-dependent adenylyl cyclase, which produces cAMP given coincident depolarisation and activation of G-protein coupled receptors, and is required for associative learning across phyla (Hawkins & Byrne, 2015). In this issue of *The Journal of Physiology*, Yamada et al. use an elegant combination of optogenetics, electrophysiology and pharmacology to show that in associative synaptic plasticity in *Drosophila*, a key coincidence detector is actually located downstream of cAMP (Yamada et al., 2024).

Flies learn to associate odours with positive (reward) or negative (punishment) valence, and these memories are stored in Kenyon cells (KCs), the principal neurons of the mushroom body. KCs respond sparsely to odours and synapse onto mushroom body output neurons (MBONs) leading to behaviours like approach or avoidance. KC-MBON synapses are modulated by reward/punishment-encoding dopaminergic neurons (DANs). Reward DANs are paired with avoidance MBONs, and punishment DANs with approach MBONs (Fig. 1*A*), so learning generally occurs through synaptic long-term depression (LTD), weakening the 'incorrect' action (Amin & Lin, 2019).

Based on behavioural genetics results, synaptic depression underlying aversive learning in the mushroom body is thought to occur presynaptically. Here, Yamada et al. provide further evidence of presynaptic plasticity by measuring a commonly used proxy for presynaptic release probability (P_r): the so-called 'paired-pulse ratio', where presynaptic neurons are stimulated twice in quick succession and the postsynaptic responses are measured. When P_r is high, the second postsynaptic response is usually smaller than the first because the first stimulus depleted the vesicle pool. When P_r is low, this short-term depression is less and the second response may even be bigger than the first due to presynaptic Ca^{2+} left over from the first stimulus (Glasgow et al., 2019). Indeed, when Yamada et al. optogenetically stimulated a sparse subset of KCs while applying dopamine (Fig. 1*B*), KC-MBON synapses were depressed and the paired-pulse ratio ($\frac{Amplitude\ 2nd\ response}{Amplitude\ 1st\ response}$) increased (Fig. 1*C*). While the paired-pulse ratio can sometimes change via postsynaptic mechanisms (Glasgow et al., 2019), the most plausible interpretation is that P_r decreased, i.e. the depression occurred presynaptically. By using this approach, the study joins others in expanding the *Drosophila* learning field toward linking insights from behavioural genetics to detailed synaptic physiology.

Yamada et al. next applied their optogenetic stimulation paradigm to test the role of cAMP in KC-MBON plasticity. Olfactory learning requires a Ca^{2+}-dependent adenylyl cyclase in KCs, and co-activation of KCs and DANs has been reported to cause synergistic production of cAMP in KCs (Amin & Lin, 2019), suggesting that cAMP production reports on coincident odour and reward/punishment and leads to presynaptic depression in KCs. Surprisingly, Yamada et al. found that increasing cAMP levels alone (by injecting forskolin to locally activate adenylyl cyclase) is not sufficient to induce LTD at KC-MBON synapses. Rather, LTD requires KC activation in addition to cAMP, indicating that LTD is triggered by another coincidence detector downstream of cAMP (Fig. 1*D*). Furthermore, when imaging cAMP levels while simultaneously applying dopamine and sparsely activating KCs, Yamada et al. did not detect any difference in cAMP levels between activated and non-activated KC axons. They further showed that cGMP has the opposite effect to cAMP, causing presynaptic long-term potentiation (LTP) at

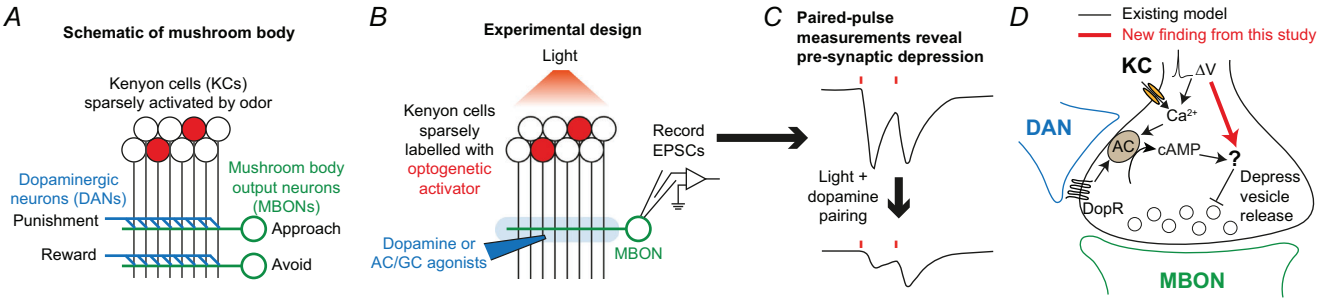

Figure 1. Physiological measurement of presynaptic plasticity at KC-MBON synapses
A, schematic of mushroom body anatomy. *B*, schematic of experimental design in Yamada et al. (2024). *C*, paired-pulse measurements reveal presynaptic depression at KC-MBON synapses. Red ticks represent optogenetic stimulation of KCs. *D*, this study relocates the key coincidence detector for KC-MBON plasticity: a process downstream of cAMP must depend on KC activity (red arrow). AC, adenylyl cyclase; GC, guanylyl cyclase; DopR, dopamine receptor; EPSC, excitatory postsynaptic current.

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KC-MBON synapses, but again, only when paired with KC activation. cGMP-triggered LTP is slower than cAMP-triggered LTD, matching behavioural studies showing that guanylate cyclase triggers delayed 'inverted' memories that promote forgetting by offsetting the 'normal' memory.

How unique are these findings to aversive learning in *Drosophila*? Yamada et al. studied a particular KC-MBON synapse involved in aversive learning; it will be interesting in future studies to measure paired-pulse ratios at other KC-MBON synapses, including those involved in appetitive learning, which also has a postsynaptic component (Pribbenow et al., 2022). Beyond *Drosophila*, could other systems using cAMP for associative plasticity also have additional coincidence detectors downstream? Similarly, the mushroom body is currently the only reported case of increased cAMP triggering LTD rather than LTP, but how unusual is it really? Previously, it was thought that dopamine signals punishment in insects but reward in mammals, but it's now known to signal both reward and punishment in both phyla. Perhaps cAMP will prove a similar case; future studies may reveal coincidence detectors downstream of cAMP, or a role for increased cAMP in LTD, in other systems as well.

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Additional information

Competing interests

The authors declare no conflicts of interest.

Author contributions

K.B.: Conception or design of the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work. A.L.: Conception or design of the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work.

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Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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