of addressing the functional role of adult neurogenesis. However, their studies revealed surprising differences when they examined the impact of blocking hippocampal and olfactory neurogenesis on behavior. To assess hippocampal learning, they trained mice in spatial (Barnes maze) and fear learning tasks following ablation of hippocampal neurogenesis. In both tasks, mice showed learning and memory deficits, demonstrating that the addition of new neurons to the dentate gyrus is essential for normal functioning of hippocampal memory. Although this conclusion is consistent with a number of recent studies[8–10], the precise manner in which these new neurons contribute to hippocampal memory is still unclear.

To assess olfactory bulb function, the authors gave the same mice a battery of smell tests. Most surprisingly, blocking olfactory neurogenesis appeared to have little effect on olfactory-mediated behaviors. Mice could still readily discriminate between odors and learn to associate specific odors with a rewarding stimulus even at 6 months post-tamoxifen, when neuronal depletion in the olfactory bulb was very pronounced. These results suggest that, although the continuous replacement of dying olfactory bulb neurons is essential for maintaining olfactory bulb structure, it is not necessary for the maintenance of olfactory-mediated learning or behaviors. This conclusion is at odds with those of some previous studies[11], and, as the authors acknowledge, it is impossible to rule out the possibility that the behavioral analyses were insufficiently comprehensive to detect impairments. Indeed, evidence indicates that olfactory neurogenesis is important in maternal behaviors[12] and in mate selection[13] in female mice.

Together with previous work, this study provides us with clear evidence for the importance of adult neurogenesis in the normal adult brain. Adult stem cell–derived neurons are critical for the maintenance of the olfactory bulb, much as adult stem cell–derived progeny are essential for cell replacement in other parts of the body. When this process is perturbed, the tissue itself degenerates. In contrast, adult neurogenesis in the hippocampus is not required for maintenance, but is instead required for neuronal addition and hippocampal growth, thereby potentially contributing to the ability to accumulate new memories throughout life. Whether or not these, or other potential populations of nestin-positive adult NSCs, can be recruited for neural repair[14] can now also be addressed using the same elegant approaches.

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Pavlov's moth: olfactory learning and spike timing–dependent plasticity

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Spike-timing dependent plasticity is a favored synaptic mechanism for learning. However, a surprising new study by Ito and colleagues in the insect mushroom body suggests that it cannot account for a paradigmatic form of learning.

Pavlov and many others have noticed that timing is important in learning; for example, any action (such as ringing a bell) that typically precedes feeding tends to be quickly associated with an impending meal. Neuroscientists have long sought the circuit and cellular mechanisms that underlie such learning. Much attention has been focused on so-called Hebbian mechanisms[1], particularly a form known as spike timing–dependent plasticity (STDP) (reviewed in ref. 2), as these mechanisms mimic, at a cellular level, the requirement for a predictive association between stimulus (ringing the bell/presynaptic activity) and reward (food/postsynaptic spike). In this issue, Ito *et al.*[3] report their studies of this question in a setting, olfactory conditioning of moths, that bears a notable similarity to Pavlov's original experiment. Surprisingly, they found that the timing of physiological activity in the mushroom body, a center for olfactory learning[4], is all wrong: odor-induced activity largely disappeared well before the optimum time for delivery of the reward. This violates the central prediction of STDP and might indicate that, at least for this archetypal example of learning, key pieces of the overall puzzle of memory remain to be elucidated.

STDP typically (but not always[5]) manifests as a strengthening of a synapse when presynaptic activity precedes postsynaptic depolarization and an attendant weakening of the synapse when presynaptic activity follows postsynaptic depolarization[2,6]. In modeling studies, this simple rule endows synapses, cells and circuits with a number of very attractive properties for both development and learning, allowing plasticity and circuit optimization without 'runaway' positive feedback[7]. Several experiments have also shown that STDP can be induced *in vivo* and correlative evidence for STDP-induced alterations in sensory processing has been obtained using natural stimuli (reviewed in ref. 8).

The timing requirements for STDP are fairly tight, typically a few tens of milliseconds[2,7]. Although the evidence for neuronal activity with this degree of timing precision is widespread, the temporal gap between 'stimulus' and 'reward' in classical conditioning can be many seconds, causing some doubts about the ubiquity of STDP for tasks on behavioral time scales[9]. One attractive proposal, therefore, is the notion that a short-term memory of the stimulus,

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specifically in the form of persistent neural activity, might bridge the temporal gap between stimulus and reward[9].

To test this idea, Ito *et al.*[3] studied neurons in the olfactory processing circuit in moths. Moths and other insects can be trained with classical Pavlovian conditioning procedures, using single odorant compounds as conditioned stimuli and sucrose solution as the unconditioned stimulus[10]. Following training, moths learn to extend their proboscises following odorant presentation in anticipation of the reward (the so-called proboscis extension reflex[10]) (**Fig. 1**). In the insect olfactory circuit, two well-studied neuronal populations are the projection neurons in the antenna lobe and their downstream synaptic partners the Kenyon cells in the mushroom body, a structure that is required for associative olfactory learning[4].

Similar to previous findings in the locust[11], projection neurons in the moth antenna lobe were relatively promiscuous in responding to odors, whereas moth Kenyon cell responses were 'sparse'. A given odor excited only a small subset of Kenyon cells, and most (although not all) Kenyon cells were quite selective in their responses to odors. It has been argued that sparse sensory coding is well suited for learning, as sparse codes both enhance storage capacity and facilitate learning by simple synaptic rules such as STDP[12]. Enticingly, another Kenyon cell synapse, with neurons from the mushroom body's β lobe, had already been shown to display typical STDP, with a computational role in synchronizing population activity[13]. It would therefore seem that all of the necessary ingredients were in place for a detailed study of the role of STDP in forming associative memories.

However, one crucial detail, the timing of the Kenyon cell responses, ultimately led the authors to question the role of STDP in this form of olfactory learning. Kenyon cells displayed brief 'on' and 'off' responses at odor onset and offset, often consisting of as little as a single action potential. The small, brief spiking events elicited in Kenyon cells during odorant delivery led the investigators to test the hypothesis that the optimum timing of the unconditioned stimulus (sucrose reward) should coincide with Kenyon cell spiking, specifically at odor onset or offset. Using an odor delivery system that was identical to their electrophysiology setup, the authors trained moths using temporal variants of associative memory procedures. They paired short (0.5 s) and long (4 s and 20 s) odorant presentations with sucrose rewards starting 0.25 to 20 s after odor onset. They found, surprisingly, that moths learned to associate individual odors with the reward even when reward stimuli were temporally separated from the time windows of Kenyon cell spiking by many seconds (**Fig. 1**). This implies that precise STDP-like learning rules do not apply for Kenyon cells in this Pavlovian conditioning procedure.

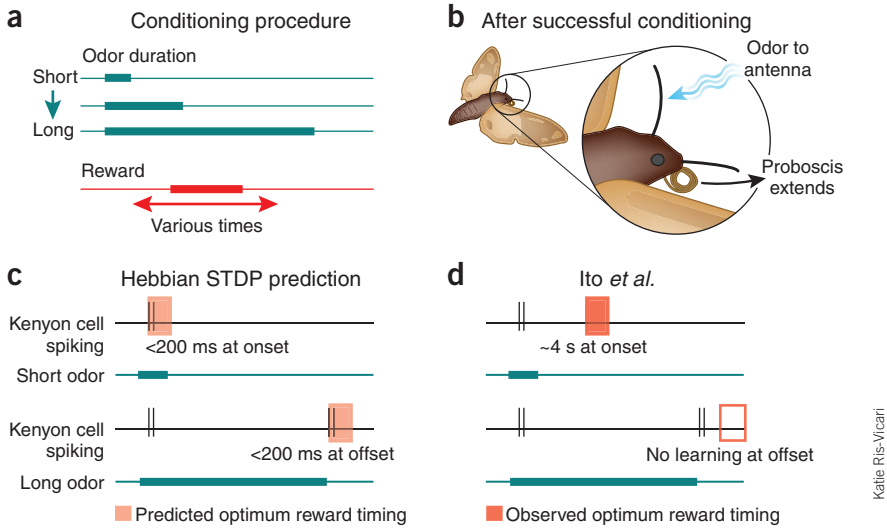

Figure 1 Testing spike timing–dependent plasticity in a moth associative learning procedure. (**a**) Conditioning procedures used to train moths to extend their proboscises in response to odors typically pair odors with rewards, usually with rewards coinciding with or shortly following odors. (**b**) After training, a moth will extend its proboscis in anticipation of a reward when the trained or 'conditioned' odor is presented to the antennae. The reliability of the proboscis extension following training is thought to correlate with the strength of the memory. (**c**) Hebbian STDP predictions would necessitate reward-based inputs to temporally coincide with odor-driven Kenyon cell spikes within several hundred milliseconds. (**d**) Ito *et al.*[3] instead found that optimum proboscis extension learning occurred when the reward cues followed the epoch of Kenyon cell spikes by several seconds, indicating that STDP rules cannot strictly account for this particular form of associative learning. Please note that timing indicators are for illustrative purposes and are not to scale.

As is typical for the large and difficult question of memory, there are alternative interpretations of the authors' findings. Although there is considerable evidence for the necessity of Kenyon cells in insect olfactory learning[4], it remains possible that some other cell type forms the crucial associative link; if so, the question of timing and STDP may ultimately need re-examination. Even in the absence of spike correlation, there are other potential physiological substrates that might link odor and reward in Kenyon cells; for example, subthreshold depolarization of Kenyon cells via persistent projection neuron synaptic input might be sufficient to permit synaptic potentiation without sodium action potentials[14], a sort of 'subthreshold timing–dependent plasticity'.

Despite these caveats, Ito *et al.*[3] have given us one of the best looks yet at an expected role of STDP in learning with natural stimuli. For this particular form of olfactory conditioning, they have come to the surprising conclusion that STDP simply does not fit the bill. Their results indicate that other neural substrates for learning may still be waiting to be discovered.

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