Learning: The Good, the Bad, and the Fly

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Olfactory memories can be very good—your mother's baking—or very bad—your father's cooking. We go through life forming these different associations with the smells we encounter. But what makes one association pleasant and another repulsive? Work in deep areas of the *Drosophila* brain has revealed the beginnings of an answer, as reported in this issue of *Neuron* by Owald et al. (2015).

Flies form Pavlovian associations with smells. In fact the first learning mutant ever discovered was identified by its inability to associate an odor with electric shock, and accordingly named *dunce*. *Dunce* was accompanied by a host of vegetable-named mutants of dubious intellectual quality, like *rutabaga* and *turnip*, some of whose expression patterns pointed to a particular brain area called the mushroom body (MB) as a likely site of memory formation. Mushroom bodies are appropriately named structures, with a stalk composed of parallel axons, and a cap made up of the dendrites and cell bodies of principal neurons, known as Kenyon cells (KCs). MBs are widely conserved structures in the invertebrate brain, and it was honeybee researchers who first showed that MBs are involved in olfactory learning by locally cooling this brain area.

Fine dissection of the neural circuitry involved in learning has been driven by the genetic toolkit available in *Drosophila*. Long before the optogenetic revolution, fly researchers were using thermogenetic means to either block or activate specific neurons to test their role in learning. Using different promoter elements to drive expression of these temperature-triggered effectors in specific populations of cells, it is possible to establish which circuit components are necessary and/or sufficient for learning. These types of experiments showed that synaptic output from Kenyon cells is essential for learning. They also identified many dopaminergic neurons (DANs) that are required for memory formation. The association of shock and odor was proposed to occur via a coincidence detection scheme, where KC depolarization paired with DAN input triggers plasticity (Heisenberg, 2003). In this scheme, KC depolarization is driven by olfactory input, while DANs convey punishment/reward-related signals.

Studies of neural activity in the KCs show that this simple model has some appealing features. Individual KCs have highly odor-selective response properties. Different odors evoke responses from sparse and largely non-overlapping sets of KCs (Campbell et al., 2013). Modifying the synaptic weights of KCs onto the downstream MB output neurons (MBONs) could therefore produce a very odor-specific memory. According to this model, memory would somehow be represented by changes in MBON activity, while the pattern of KCs responding to different olfactory stimuli would remain the same following learning, a useful feature for odor recognition.

But a big part of the puzzle that has been largely missing has been the downstream cells, the MBONs. The first extensive survey of these neurons showed that they extend dense thickets of dendrite into the parallel fibers of the KC axons (Tanaka et al., 2008). A recent effort to systematically identify all the MBONs found a collection of drivers labeling a total of 34 different MBONs, falling into 21 discrete anatomical classes (Aso et al., 2014a). The dendrites of these different MBONs tile the length of the KC axons in a non-overlapping manner, leaving essentially no gaps. In fact, this turns out to be all the MBONs there are. Exhaustively labeling MBONs using a pan-neuronal photoactivatable GFP and photoconverting the entire output region of the MB revealed no additional cells (Aso et al., 2014a). This represents a massive convergence from the 2,000 KCs down to only 34 different MBONs.

The other main innervation of the MB lobes comes from dopaminergic neurons, but in this case the projections are axonal. The DANs tile the MB lobes in a corresponding manner, dividing the lobes up into a series of compartments, each containing the dendrites of a particular MBON and a cognate DAN(s) with overlapping axonal projections (Figure 1A). This suggests that each of the MBONs can be independently modulated by signals coming from the DANs.

Although there are many pieces missing from this puzzle, it is possible to see the outlines of how an odor-specific memory could be formed by coincidence detection within each of these modules. But how is the attractive/repulsive quality of the association achieved? The valence of the association appears to be dictated by the dopaminergic inputs—different DANs are required for aversive versus appetitive conditioning. Owald et al. (2015) followed the trail of these DANs to find circuitry likely to differentiate between good and bad memories of odor. The DANs important for appetitive learning are located in a cluster of ~120 neurons. A subset of these are actually sufficient for learning—instead of training animals by pairing odor and reward, pairing odor and DAN activation was enough for them to learn (Burke et al., 2012; Liu et al., 2012). In other words, activation of this small set of DANs could substitute for a reward. These cells project to a particular zone of the output lobes of the MB. Owald et al. (2015) identified candidate MBONs in this compartment by searching for drivers that label neurons projecting to this area. They then used molecular

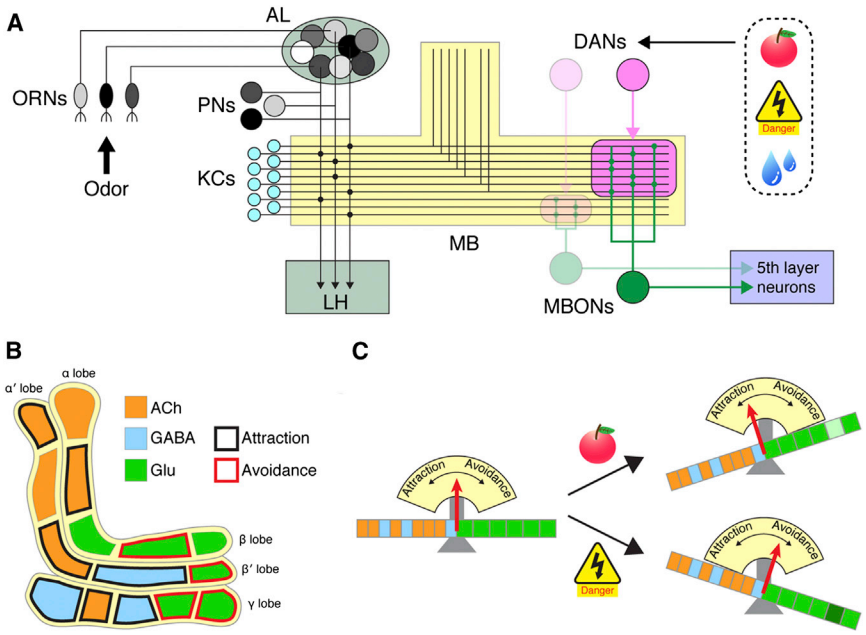

Figure 1. Adjusting Odor Valence with Associative Conditioning in the MB Circuit

(A) Schematic of the olfactory learning pathway in *Drosophila*. Projection neurons (PNs) from the glomerular layer of the pathway project to two different third-order olfactory areas, mushroom body (MB) and lateral horn (LH). Each Kenyon cell (KC) in the MB receives combinatorial inputs from PNs. The parallel axons of the 2,000 KCs form the MB lobes, where they synapse onto 34 MB output neurons (MBONs). Each MBON has dendrites that occupy a specific compartment in the lobes; as a population those dendrites tile the lobes in their entirety. Each compartment also receives inputs from a corresponding type of dopaminergic neurons (DANs), which is believed to carry information about reward or punishment.

(B) Relationship of MBON compartments to behavior. MBON dendrites define a total of 15 different compartments in the lobes. Colors indicate neurotransmitter released by each MBON. Behavioral consequences of MBON activation are shown by the color of the thick contours around different compartments (Aso et al., 2014b). Note that the directions of those evoked behaviors are segregated based on MBON neurotransmitter.

(C) MBON-balance model for adjusting odor valence during learning. In the naive state, odor responses of different MBONs are balanced, and there is no net driving force for attraction/avoidance behavior (left). After appetitive conditioning, the balance can be tipped toward attraction by decreasing odor responses of MBONs signaling negative valence (indicated by lighter compartment color), as observed in Owald et al. (2015). After aversive conditioning, a stronger response in the same MBON swings the balance to avoidance. Other ways of swinging the balance have also been observed recently (see main text).

markers to establish that that these cells were truly output neurons. Their dendrites were in a position to receive KC input, while their axons traveled to another, poorly understood part of the brain.

To prove these neurons are functionally important for learning, they tested the effects of inactivating them. They found that they were essential both for appetitive associations, and a little surprisingly, for aversive ones. So they are clearly integral to the process of memory formation. But does their activity actually change when a fly learns? They addressed this using in vivo two-photon calcium imaging. They trained flies to form an association, captured them out of the training apparatus, exposed their brains, and imaged odor responses from the large dendritic regions of these cells. As expected, these cells responded to odor, both the odor they had been conditioned with and an unconditioned control odor. But the exciting thing was that they observed bidirectional changes in the responses of one neuron, based on the valence of the association. When they looked at the responses in animals trained to form an aversive association, the responses went up, while in animals that formed an appetitive association, responses went down.

So far, so good: these MBONs are required for learning, and forming associations of opposite valence gives opposite effects on odor response magnitudes in this cell. But how does this all relate to behavior? Owald et al. (2015) make this connection using both thermogenetic and optogenetic approaches. They found that inactivating these neurons converts an aversive response to odor into an attractive response. This is consistent with the decreased odor response following appetitive conditioning. Conversely, stimulating these neurons drives an aversive response from the flies, again matching the direction of the effects from aversive conditioning. So Owald et al. (2015) go through the full span of "see it, block it, move it" to provide a compelling explanation for how valence can be modified during learning.

One thing to note about these results is that learning does not change these responses very much—it is around a 15% change in response magnitude. Owald et al. (2015)'s manipulations of neural activity that produce attraction/avoidance behavior are probably much stronger than that. In addition, they observed bidirectional changes in activity only in one type of neuron of several that are sufficient for driving the behavioral effects. So, although these changes clearly contribute to the behavior, they probably do not fully explain it. More likely, the activity of many MBONs is modified, and across the whole population small magnitude changes can add up to a relatively big effect. Previous studies have indeed identified other compartments involved in appetitive memory formation (Perisse et al., 2013; Yamagata et al., 2015).

A Framework for Assigning Valence to Memory

The wider implications of Owald et al. (2015)'s results become clear when you zoom out to a global view of the MBONs. An exhaustive study of this layer of the circuit by Aso et al. also found that activating particular MBONs evokes attraction/avoidance, depending on the cell type (Aso et al., 2014b). The drivers Aso developed are extremely specific, in some cases just a single neuron in the entire fly brain. This specificity warranted a close look at the behavior, by tracking locomotor responses of flies as they transition over a border into a cone of light used to stimulate cells optogenetically.

They found that activating a particular MBON does not trigger one specific, stereotyped behavioral response. But it does influence the probability that flies will turn around to avoid an odor or persist when heading toward an attractive one. So rather than triggering particular motor reflexes, they instead bias the likelihood a fly will choose one action over another. In fact, mapping the behavioral effects onto the different MBON-DAN compartments in the lobes shows a strong segregation, where compartments in the vertical lobe typically map onto attractive responses, and those in the horizontal lobes go onto aversive behavioral outcomes (Figure 1B). This seems in general to correlate with the MBON neurotransmitter for each compartment, with cholinergic and GABAergic MBONs driving attraction and glutamatergic neurons signaling avoidance. Aso et al. also explicitly showed that different MBONs interact so that, for example, stimulating multiple attractive neurons gives a largely additive effect on the levels of attraction. Taken together, these results suggest that several MBONs act together to reflect the bias to particular action plans. The summed activity across those different MBONs probably sets the overall balance between attraction and aversion (Figure 1C).

These connections to behavior in naive animals link up well, but not perfectly, with learning-related changes in MBON activity reported throughout the literature. Learning an appetitive association has been reported to decrease activity of glutamatergic MBONs (Owald et al., 2015) and increase activity of cholinergic MBONs (Plaçais et al., 2013). Conversely, learning an aversive association is accompanied by an increased odor responses in glutamatergic MBONs (Owald et al., 2015) and a decrease response in one cholinergic neuron (Séjourné et al., 2011). However, a different study found an increased response of cholinergic neurons (Pai et al., 2013), breaking the symmetry. So, the results do not hang together perfectly, but there is an intriguing theme. It seems that the valence of an odor may reflect a balance of the relative levels of activity among the MBONs. Tipping the balance so that cholinergic activity dominates shifts the behavior toward attraction, while tipping toward the glutamatergic side flips the behavioral response toward aversion (Figure 1C).

Topics for the Future

A major issue for the future is understanding how dopamine acts in the compartments within the lobes. Owald et al. (2015)'s work leaves open the question of how these bidirectional changes arise. There are two different types of DANs that innervate this part of the MB: one required for aversive learning, and one for appetitive. But since they are both dopaminergic, how do they have these different effects? Is it in the amount of dopamine released, or the timing of that release? Or perhaps there are subtle differences in the sites of dopamine release so these different inputs couple to different downstream signaling cascades. These questions will have to be resolved in the future. Additionally, it is rather unclear why there need to be so many (15!) MBON-DAN compartments for a fly simply to learn an odor is good or bad. Perhaps this diversity reflects the variety of motivational drives for the animal that govern learning in different contexts (Lin et al., 2014). Certainly, there is a lot more work to be done to connect sensory pathways related to reward and punishment to these modulatory cells; the pathways are barely mapped at present.

Nonetheless, with this conceptual framework firmly in hand, and the tools to label and manipulate all the circuit elements, understanding associative learning in this system seems like a truly solvable problem.

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