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The *Drosophila* Mushroom Body: From Architecture to Algorithm in a Learning Circuit

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Abstract

The *Drosophila* brain contains a relatively simple circuit for forming Pavlovian associations, yet it achieves many operations common across memory systems. Recent advances have established a clear framework for *Drosophila* learning and revealed the following key operations: *a*) pattern separation, whereby dense combinatorial representations of odors are preprocessed to generate highly specific, nonoverlapping odor patterns used for learning; *b*) convergence, in which sensory information is funneled to a small set of output neurons that guide behavioral actions; *c*) plasticity, where changing the mapping of sensory input to behavioral output requires a strong reinforcement signal, which is also modulated by internal state and environmental context; and *d*) modularization, in which a memory consists of multiple parallel traces, which are distinct in stability and flexibility and exist in anatomically well-defined modules within the network. Cross-module interactions allow for higher-order effects where past experience influences future learning. Many of these operations have parallels with processes of memory formation and action selection in more complex brains.

Contents

INTRODUCTION

Drosophila have proved to be a valuable subject for those seeking to understand the algorithms governing signal processing in a simple brain, including its hallmark plasticity. Flies are separated from Pavlov's dogs by 800 million years of evolution (Hedges et al. 2015), but they learn similar associations (Tully & Quinn 1985). The main difference is that flies are trained using a smell instead of a bell, and the experimenter simply measures whether they approach the odor source, rather than going to the trouble of measuring salivation in a fly. The relative simplicity of the fly brain makes it possible to examine how signals propagate across successive layers of a circuit. Comparing and manipulating activity patterns across layers is a powerful approach to describe how signals are transformed and get to the core algorithm at each layer (Wilson 2013). This approach has been extremely successful in *Drosophila* thanks to decades of effort to develop driver lines to express effector proteins in cells of interest. These driver lines have progressed in specificity to the point where it is now routine to tinker with individual neurons in the circuit.

Drosophila rely on a brain area known as the mushroom body (MB) for the formation of memories (Heisenberg 2003). The MB has a three-layered expand-converge architecture, which is found in many learning networks ranging from the cerebellum to the perceptron, an early artificial neural network (Stevens 2015) (**Figure 1*a–c***). This expand-converge architecture was the basis for one of the most influential algorithm-level descriptions of a learning network, the Marr-Albus model (Albus 1971, Marr 1969). According to the model, the expanding side of the network performs the function of pattern separation (Cayco-Gajic & Silver 2019). The circuit takes overlapping patterns of input activity and separates them into distinct patterns in the intermediate, expanded layer of the network. Work in *Drosophila* has revealed many mechanistic aspects of how this pattern separation is achieved to ensure the accuracy of memory formation.

By contrast, the operations carried out by the converging side of such networks are less understood. However, in *Drosophila*, the converging side of the circuit was cracked open with the revelation that the 2,000 intrinsic neurons of the MB converge onto a total of only 34 different mushroom body output neurons (MBONs), which appear to play an important role in guiding behavior (Aso et al. 2014a, Tanaka et al. 2008). This is also where reinforcement signals enter the network to drive synaptic plasticity. Modularity is a prominent feature at this layer of the circuit,

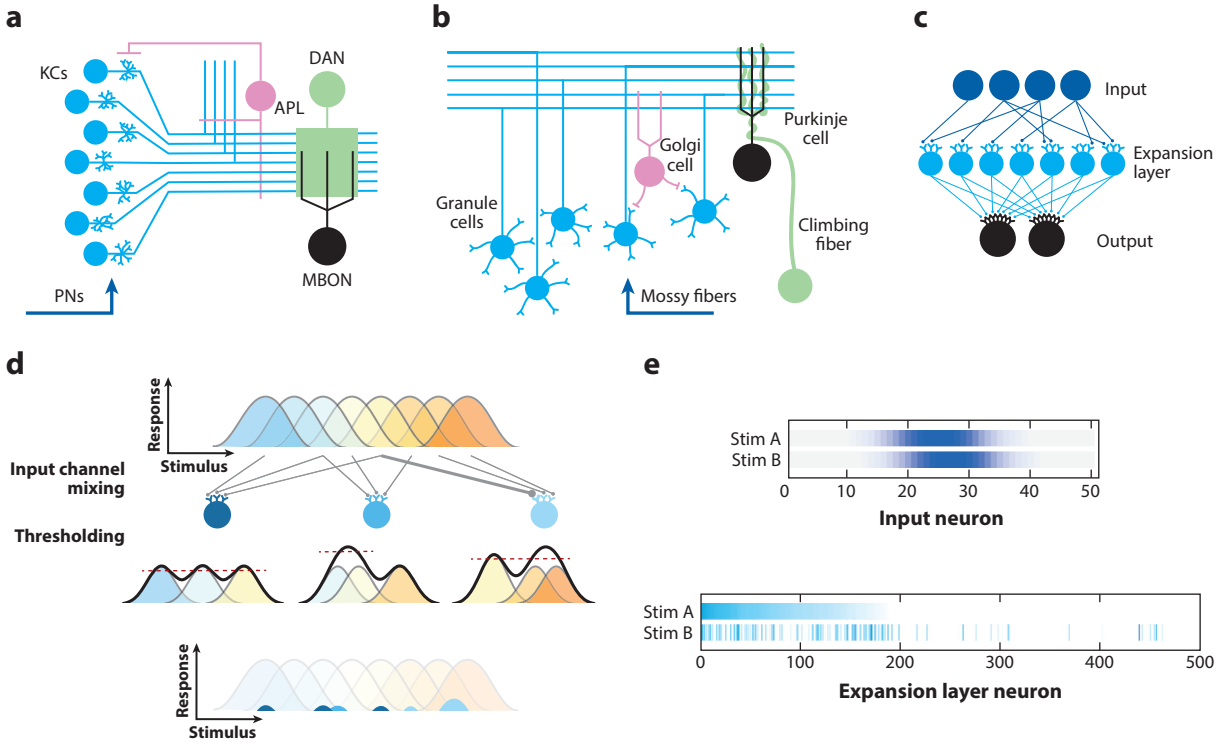

Figure 1

Three-layer networks and pattern separation. (*a*) Mushroom body (MB) circuit. Olfactory input arrives at the dendritic claws of Kenyon cells (KCs) (*light blue*) from projection neurons (PNs) (*dark blue*). KCs project axon bundles to the lobes, where they contact an example mushroom body output neuron (MBON) (*black*). Input from a dopaminergic neuron (DAN) (*green*) drives plasticity at KC>MBON synapses in this compartment. The APL neuron (*pink*) conveys feedback inhibition to KCs. (*b*) Cerebellar circuit. Granule cells (*light blue*) receive input from mossy fibers (*dark blue*) and send parallel fibers to synapse onto Purkinje cells (*black*). Climbing fibers (*green*) wrap around the dendrites of Purkinje cells and carry plasticity-inducing input. Golgi cells (*pink*) provide inhibition to granule cells. (*c*) Perceptron. A small number of input units (*dark blue*) project to a large number of hidden expansion layer units (*light blue*). This expansion layer then converges onto a small number of output units (*black*). (*d*) Channel mixing and thresholding operations. (*Top*) Depiction of tuning curves of multiple input neurons. These are idealized for illustration purposes; actual olfactory tuning properties are more complex. Expansion layer neurons (*light blue*) summate input from different combinations of inputs with different synaptic strengths (*thick line*). Dotted lines represent firing thresholds. (*Bottom*) Illustration of resulting tuning curves. (*e*) Simulation of pattern separation across layers. (*Top*) Responses of 50 input neurons to two stimuli (Stim A and Stim B) chosen to be strongly overlapping. (*Bottom*) Responses of 500 expansion layer neurons created by randomly integrating seven input neurons and thresholding so 5% of expansion layer neurons are active. Response patterns here are nonoverlapping and decorrelated.

supporting the formation of multiple parallel memory traces in anatomically distinct subdivisions of the MB. Examining these modules has revealed how they can contribute multiple features to what appears to be a simple memory. Additionally, interactions between modules increase the complexity of the system, making future memory formation dependent on past learning. The opportunity to examine plasticity in these neurons and relate those changes to behavior has brought the focus of the fly learning field onto the converging side of the circuit.

At this stage, the circuit is approaching the point where sensory coding transitions into motor encoding, and so processing in areas involved in action selection like the basal ganglia may have some conceptual overlap. In particular, we discuss how both the MB and the basal ganglia

incorporate plastic changes driven by learning with current motivational state to guide action appropriately.

CIRCUIT ARCHITECTURE

The three-layer motif in the fly learning circuit starts immediately downstream of the olfactory periphery (**Figure 1*a***). Sensory inputs come to the calyx (cap) of the MB from 50 different types of projection neurons (PNs) in the glomerular layer of the olfactory system. This is the expanding stage, as these 50 input channels project to 2,000 Kenyon cells (KCs). PN>KC contacts occur as large ball-claw synapses, and each KC has a relatively simple dendritic tree with typically seven claws (Caron et al. 2013, Strausfeld et al. 2003). KC axons then bundle together to form the stalk and lobes of the MB. The circuit then converges heavily, as the 2,000 KCs contact a population of only 34 different postsynaptic neurons, the MBONs (Aso et al. 2014a, Tanaka et al. 2008). Each MBON innervates a zone within the lobes, with the dendrites of different MBONs tiling the lobes. Dopaminergic neurons (DANs) arborize in corresponding zones, matching up to the different MBONs to form a series of 15 different compartments composed of DAN-MBON modules (Aso et al. 2014a). This repeating structure is central to the parallel processing that goes on in the circuit, discussed below. The DANs convey reinforcement signals to the MB and are key to synaptic plasticity in the system.

Anatomically, there is a genuinely surprising level of similarity to the cerebellum. The sensory inputs to the cerebellum come from the mossy fibers—in this case, they are carrying signals from many different brain areas (Raymond & Medina 2018) (**Figure 1*b***). The mossy fibers project to the granule cells where there is a 30-fold expansion, compared to 40-fold in the MB (Litwin-Kumar et al. 2017). Granule cells are remarkably reminiscent of KCs, having simple dendritic trees and typically five inputs, which also occur on dendritic claws. Again, like KCs, granule cells extend axons in a bundle of parallel fibers, which course through the dendritic trees of Purkinje cells. If a Purkinje cell's two-dimensional arbor is like a comb in the strands of the parallel fibers, an MBON is like a brush, innervating a zone rather than a plane. Climbing fibers wrap around the main dendritic trunk of the Purkinje cell and provide the depolarization required to induce plasticity.

EXPANSION RECODING AND PATTERN SEPARATION

The Marr-Albus model proposed that this circuit architecture was designed to solve a general problem of learning and memory networks: pattern separation. Of course, memories have to be accurate to be useful, but the fly olfactory system has only 50 types of sensory neurons to recognize thousands of different odors, so inevitably many different smells will elicit similar patterns of input activity. The overlap between those activity patterns is what makes accurate memory formation difficult.

In the Marr-Albus model, this problem is solved by the intermediate layer of the circuit via a process termed expansion recoding (**Figure 1**). By virtue of the expansion in cell numbers, each of the neurons in the intermediate layer can combine different receptive fields from the input layer and build more complex response properties. As a result, there are more distinctly tuned neurons in this layer. In other words, the dimensionality of the system increases because there are now more independent variables to form the stimulus representation (Litwin-Kumar et al. 2017). This echoed the importance of a hidden layer between input and output that enabled early neural networks like the perceptron (**Figure 1*c***) to solve previously intractable problems.

This appears to be exactly the same transformation that happens at the PN>KC transition in the circuit. The expansion in cell number is accompanied by an increase in response selectivity in the KCs, where only 5% of cells respond to a given odor, compared to 50% in the PNs (Honegger et al. 2011, Turner et al. 2008, Wilson et al. 2004). Consequently, odor responses in the KC population consist of relatively sparse activity patterns and less overlap than the odor representations in the PNs. In fact, the accuracy of memory formation can be predicted by the degree of overlap between KC response patterns, even very near the flies' psychophysical limit of odor discrimination (Campbell et al. 2013).

Sparsening and tuning diversification arise via a combination of input channel mixing on KC dendrites and thresholding (**Figure 1*d***). The approximately seven dendritic claws allow each KC to sample from a set of different PNs. Current evidence suggests that PN>KC connectivity is very loosely specified, if at all (Caron et al. 2013, Eichler et al. 2017). Random PN>KC connectivity is of course an effective means of generating independent odor tuning properties among the KCs. A thresholding operation is also essential. In a multilayer network, if all the cells just sum linearly on their downstream targets, having multiple layers does not add any complexity—the ultimate output is always just a linear sum of the inputs. Adding a threshold enables the system to separate tuning curves that would otherwise be overlapping (**Figure 1*d***). It also provides a means to generate KCs that respond selectively to odors that are on the shoulder of individual PN tuning curves.

Although KCs have only seven or so dendritic inputs, these cells cannot be driven to spike by activating only one of them (Groschner et al. 2018, Gruntman & Turner 2013, Li et al. 2013). Beyond the intrinsic spike threshold, inhibition is also important. Marr and Albus originally noted that feedback inhibition would be important to cope with the wide variations in overall input strength that a network encounters. Such inhibitory circuit elements are present in both the MB and cerebellum (**Figure 1*a*,*b***). In the MB, there is a single large inhibitory neuron, termed APL, that innervates the entirety of the MB lobes and calyx (Liu & Davis 2009). This neuron receives input from KCs and feeds it back to the KCs, providing the adaptive inhibition posited by the Marr-Albus model (Inada et al. 2017, Papadopoulou et al. 2011). A global inhibitory signal can in theory support winner-takes-all dynamics in the KC population, where the strongest and/or earliest-responding KCs laterally inhibit other cells, ensuring that decorrelation can be maintained at different levels of input.

The manipulability of the fly circuit has made it possible to test the expansion recoding model. One approach directly altered the degree of overlap between response patterns by blocking APL, the inhibitory neuron that controls activity levels across the KC population (Lin et al. 2014a). Inactivating this neuron resulted in more extensive and overlapping KC response patterns. Parallel behavioral experiments showed that this inactivation impaired learned discrimination of similar odors, thereby providing a direct link between the degree of overlap and learning accuracy. A second series of experiments that examined plasticity at the KC>MBON synapse directly tested the notion that overlaps degrade the specificity of synaptic changes (Hige et al. 2015a). Plasticity was induced with one odor, and then the specificity of those changes was evaluated using a series of off-target odors with different degrees of overlap in the KC population. The greater the overlap, the greater the off-target plasticity.

Despite odors activating highly overlapping patterns of activity at the sensory periphery (Hallem & Carlson 2006), just two synapses downstream in the KCs, these patterns become separate and decorrelated via mechanisms envisioned 50 years ago by Marr and Albus. In fact, the MB appears to adhere to this model more closely than the cerebellum, where recent experiments indicated that granule cells have broader response properties than expected (Badura & De Zeeuw 2017).

THE KENYON CELL TO MUSHROOM BODY OUTPUT NEURON TRANSITION: BUILDING A VALENCE MAP

The transition from 2,000 KCs to 34 MBONs means that, inevitably, there is a reduction in dimensionality of the system at this layer (Aso et al. 2014a). This raises an important general question: How is an information-rich, sparse code in a higher brain area transformed into a lower-dimensional representation downstream? In contrast to the strongly decorrelated odor representations in the KCs, different MBONs typically have very similar odor-tuning properties (Hige et al. 2015b). This is in line with electron microscopy measures of connectivity at this layer. Reconstructions of the α lobe of the adult MB (Takemura et al. 2017), as well as the entire larval MB (Eichler et al. 2017), showed essentially complete connectivity; every KC passing through a compartment that was extensively innervated by an MBON made synapses with it. Summating across overlapping sets of KCs would tend to produce similar odor-tuning properties in different MBONs.

However, one intriguing observation was that the two groups of odors that elicited the most distinct MBON activity patterns were of opposing valence. In a set of ten odors, the two food odors gave rise to similar response patterns, which were most well-separated from the two strong repellents included in the stimulus set (Hige et al. 2015b). The emerging picture is that distinct representations of odor identity in the KCs become less distinct in the MBONs, where they are rearranged to reflect less what the odor is and more what the fly is going to do with it. In other words, the dimensionality reduction may be along the lines of behavioral output, as many different sensory patterns in the KCs start to be mapped down onto the comparatively few behavioral responses the animal will make.

Direct evidence that the MBONs play an important role in signaling valence comes from experiments manipulating MBON activity in freely behaving flies. This has been done both in the T-maze typically used for learning experiments (Owald et al. 2015) and in an arena adapted for optogenetic stimulation (Aso et al. 2014b). In the latter case, an illumination field was created to examine how flies react to the boundary between light and dark zones. Flies expressing red-shifted channelrhodopsin in certain MBON types such as MBON-γ5β′2a responded with an avoidance response at the border, typically slowing and then turning to remain on the dark side of the arena. Stimulating other MBONs such as MBON-γ1pedc elicited the opposite response; flies did not cross from the light into the dark. These results suggest that individual MBONs signal qualitatively different valence values of an odor—its attractiveness/repulsiveness—not by triggering particular motor reflexes but by biasing an inherently probabilistic action selection process.

How do valence signals from different MBONs interact to drive coherent behavior? There are 15 different compartments, and it has so far been possible to attribute valence to nine of them (**Figure 2*a*,*b***), with stimulation of the remaining six compartments not producing an obvious phenotype (Aso et al. 2014b). Stimulating multiple MBONs has been shown to produce a sublinearly additive effect on behavioral levels of attraction or repulsion (Aso et al. 2014b). This suggests that the integrated activity across the MBON population likely sets the overall balance between attraction and aversion (**Figure 2*c***). How the neurons downstream of MBONs integrate MBON activity and use it to guide behavior remains an open question. Intriguingly, there is a strong correlation between the valence signaled by an MBON and its neurotransmitter. Cholinergic and GABAergic MBONs drive attraction, while glutamatergic neurons signal avoidance. Acetylcholine is excitatory, while glutamate is likely inhibitory at most synapses in the central nervous system of adult flies (Liu & Wilson 2013). This immediately suggests a simple mechanism where downstream neurons integrate opposing valence signals of opposing sign and generate a coherent behavioral output (Dolan et al. 2019).

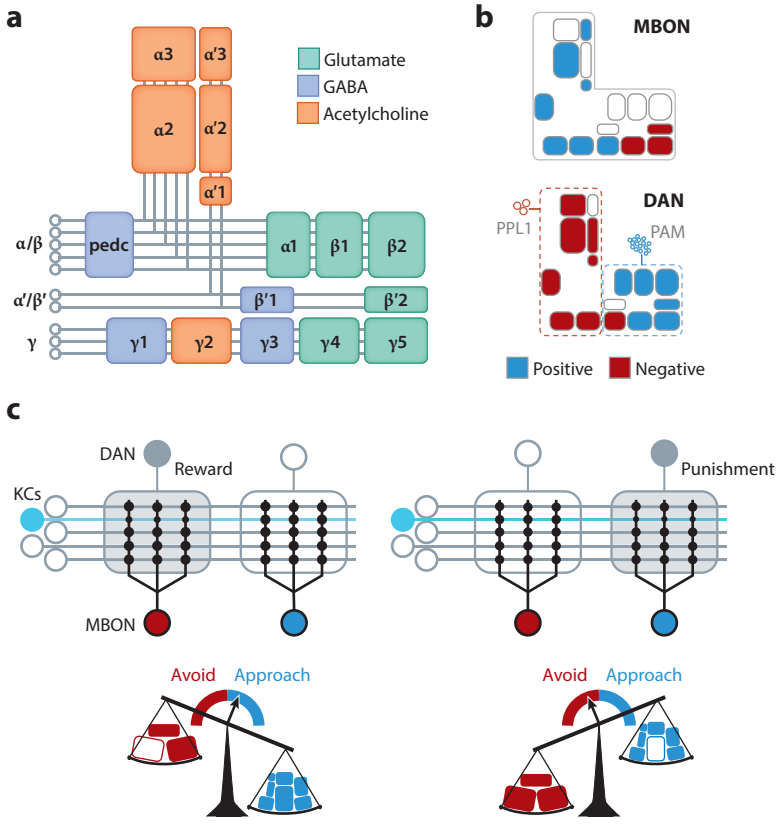

Figure 2

Valence remapping. (*a*) Mushroom body (MB) compartments. The three different Kenyon cell (KC) types (α/β, α′/β′, and γ) make contact with dopaminergic neuron (DAN)–mushroom body output neuron (MBON) modules in 15 different compartments (γ1pedc forms one compartment). Color indicates MBON neurotransmitter type. (*b*) Inverted valence maps of MBONs (*top*) and DANs (*bottom*). MBONs that signal positive valence (*blue*) are located in the compartments innervated by DANs that signal punishment (*red*). Conversely, negative-valence MBONs are paired with reward-signaling DANs. (*c*) Coincident activation of a KC (*light blue*) by odor and a DAN (*gray shading*) by reward depresses KC synapses onto a negative-valence MBON (*left*). This shifts the balance of overall MBON activity toward approach. Conversely, pairing odor with punishment activates DANs that project to a different compartment containing a positive-valence MBON (*right*). Depression in this compartment shifts MBON output toward avoidance.

Overall, these findings have coalesced into a clear framework for learning and memory in this system: A highly specific representation of odor identity in the KCs is mapped down onto a much lower dimensional representation of valence in the MBONs. Synaptic plasticity can then change that mapping as part of the learning process.

DOPAMINERGIC REINFORCEMENT SIGNALS REMAP VALENCE

Beyond KC-MBON synapses, a third factor is critical for learning: the reinforcement signals conveyed by dopamine. The dopamine receptor as well as many signaling pathway components involved in learning have the KCs as their site of action (Davis 2005, Kim et al. 2007, Margulies et al. 2005, Qin et al. 2012). This was the basis for the guiding model of the field, that coincidence

detection of odor and reinforcement occurs in the KC axons (Heisenberg 2003). Calcium influx from olfactory input and G protein signaling initiated by dopamine receptors were proposed to combine synergistically to activate cAMP production, which leads to plasticity (Gervasi et al. 2010, Tomchik & Davis 2009).

Dopamine is released to the MB lobes from two clusters of DANs that are distinct in their lobe projections (**Figure 2*b***). Aversive learning requires the approximately 10 DANs from the PPL1 cluster, while reward learning is mediated by DANs from the PAM cluster, composed of about 120 neurons, which project primarily to the horizontal lobes. Calcium imaging experiments show that these two clusters of DANs respectively convey punishment and reward reinforcement to the MB (Berry et al. 2018, Cohn et al. 2015, Lin et al. 2014b, Liu et al. 2012, Mao & Davis 2009, Riemensperger et al. 2005, Yamagata et al. 2015). In fact, it is possible to train animals by pairing the odor cue with direct activation of DANs (Aso et al. 2010, 2012; Burke & Waddell 2011; Claridge-Chang et al. 2009; Huetteroth et al. 2015; Liu et al. 2012; Schroll et al. 2006, Yamagata et al. 2015). Such optogenetic reinforcement has shown that DANs innervating even only one of the 15 compartments is sufficient to train an animal (Aso & Rubin 2016, Hige et al. 2015a). This compartmental approach also highlighted the fact that the valence of the association formed was opposite to the valence of the MBON in that compartment (**Figure 2*b***). In other words, learning a negative association involves modifying activity of an MBON that signals positive valence, suggesting that reinforcement should depress KC>MBON synapses and reduce the MBON response (**Figure 2*c***). Learning-related changes in several compartments generally follow this scheme, whether reinforcement is optogenetic or physical (Berry et al. 2018, Cohn et al. 2015, Hige et al. 2015a, Owald et al. 2015, Perisse et al. 2016, Séjourné et al. 2011; but see also Plaçais et al. 2013). However, it is noteworthy that real reinforcement, either sugar reward or shock punishment, evokes a broad and sometimes complex activation pattern among dopamine neurons (Mao & Davis 2009; Yamagata et al. 2015, 2016). Thus, learning likely elicits changes distributed widely across the network, and the sum of those changes is what drives behavior.

Although learning seems to primarily involve synaptic depression, simply changing the timing can flip the sign of learning and also the sign of plasticity. When reinforcement arrives before the odor cue, rather than following it, the odor switches from predicting upcoming reinforcement to predicting the end of reinforcement, and so the opposite association is formed (Aso & Rubin 2016, Gerber et al. 2014, Tanimoto et al. 2004). In this case, MBONs are potentiated instead of depressed (Handler et al. 2019). The two types of plasticity can be induced by the same dopamine neurons but exhibit different dopamine receptor requirements and activate different second messenger systems (**Figure 3*a*,*b***). Depression requires DopR1 and is accompanied by elevated cAMP levels, while potentiation depends on DopR2 and triggers calcium release from internal stores. How differences in relative timing are translated into differential activation of these two signaling pathways is an interesting topic for future study. However, it seems likely that more than just relative timing can control their differential recruitment. Potentiation can also be induced by prolonged (or intense) activation of dopamine neurons in the absence of an odor cue (Berry et al. 2018, Cohn et al. 2015, Hattori et al. 2017), and behaviorally, similar protocols are effective at inducing forgetting of earlier installed memories (Aso & Rubin 2016, Berry et al. 2018). In addition, potentiation has been observed in MBONs involved in courtship conditioning (Zhao et al. 2018).

Overall, these observations again highlight the similarity between the MB and cerebellar cortex. Just as in the MB, the synaptic connections between granule cells and Purkinje cells on the converging side of the circuit are the primary site of plasticity during learning. And again, there is a third circuit element required for plasticity in the cerebellum: the climbing fibers that also drive synaptic depression. However, there are important differences. Unlike the fly MB, the cerebellar cortex is often discussed in the context of supervised learning (Raymond & Medina 2018). The

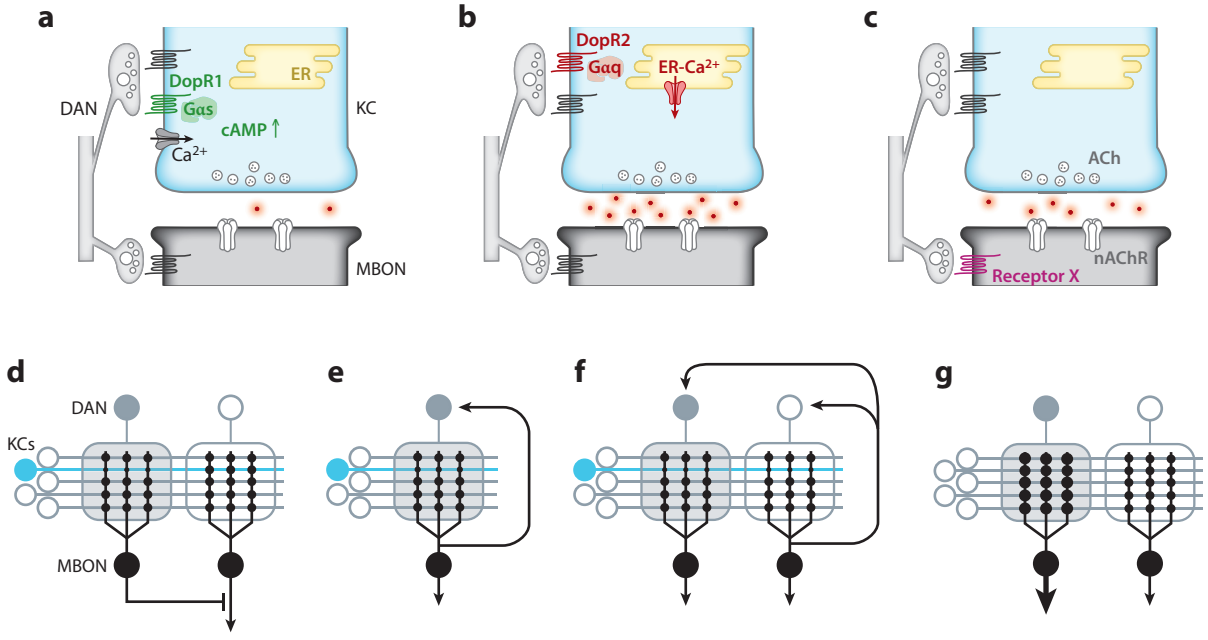

Figure 3

Modes of action of dopamine and memory module motifs. (*a*) Depression of Kenyon cell (KC)>mushroom body output neuron (MBON) synapses requires the DopR1 pathway and is accompanied by elevated cAMP levels. (*b*) Potentiation depends on DopR2 signaling and involves calcium (Ca^{2+}) release from the endoplasmic reticulum (ER). (*c*) Dopaminergic neurons (DANs) also provide direct excitatory input to MBONs. (*d*) Feed-forward inhibition between MBONs of opposing valence acts as a toggle switch. (*e*) Recurrent feedback of an MBON onto its cognate DAN is proposed to support persistent DAN activity that can drive associations into long-term memory. (*f*) Cross-compartment feedback to DANs allows MBON activity in one compartment to influence learning in another. This motif is involved in memory extinction and reconsolidation. (*g*) Switchboarding involves state- and context-dependent DAN activity that modulates signal transmission between KCs and MBONs, routing the same sensory input to different output channels.

climbing fiber teaching signal has long been thought to be triggered by error detection, that is, the mismatch between intended and actual motor output (Ito 2013). [However, recent work indicates the teaching signal can be modulated by ongoing behavioral state (Carey & Regehr 2009, Lawrenson et al. 2016) and even the expectation of reward (Kostadinov et al. 2019).] In the fly MB, DAN activity is primarily thought to convey reward or punishment signals, and learning governs which action plan the fly pursues rather than tuning the accuracy of its execution. However, more work is required to understand signaling properties of these neurons during the learning process, including whether they reinforce the fine motor patterns the fly performs.

PARALLEL PROCESSING BY MEMORY MODULES

The highly parallel structure of the MB output circuitry is apparent in both coarse anatomy and fine-scale connectomics. There is the obvious modularity of the DAN-MBON units, which tile the entirety of the MB lobes. Beyond this, however, connectomics has revealed that each KC makes synapses in every compartment, making an explicitly parallel structure where KC inputs are read off down the length of their axons by a series of different MBONs (Takemura et al. 2017). Each MBON then likely has access to similar information about odor identity from the KCs. What is different between the modules are the reinforcement signal inputs to each compartment and the sites of MBON output that actuate different behaviors.

This modularity not only is the basis for forming memories of different valence but also supports the diversification of memories in other ways. One example is the construction of parallel memories with the same valence but different time course. Pairing a typical food reward such as sucrose with odor forms a memory that lasts for days (Krashes et al. 2009). But clever experiments using sweet-tasting but noncaloric sugars showed that this memory is composed of both short- and long-term components (Burke & Waddell 2011, Fujita & Tanimura 2011). The sweet taste is only sufficient to produce a memory that lasts a few hours; long-term memory (LTM) requires the caloric benefit of a metabolizable sugar. There are two parallel pathways underlying these two memory phases, separable in terms of both the KC subtypes involved (Trannoy et al. 2011) and the DAN-MBON modules (Huetteroth et al. 2015, Yamagata et al. 2015). Surprisingly, the short- and long-term phases of memory are so independent that LTM can form in the absence of any behaviorally measured short-term memory (STM) (Trannoy et al. 2011, Yamagata et al. 2015).

The differences between DAN-MBON modules extend beyond memory duration, however, and include the rate of acquisition, the susceptibility to disruption, and the flexibility to learn a new association (Aso & Rubin 2016). It has been possible to link some of these differences to the underlying synaptic plasticity rule by targeting optogenetic reinforcement to different compartments. This has shown that the level of training required to induce plasticity is very different across compartments (Hige et al. 2015a). There are compartments that are relatively flexible, with a lower threshold for inducing plasticity, but the memory is short-lived; other compartments require more training to undergo plasticity, but the resulting memories are more enduring. Dopamine receptor expression is heterogeneous across both KCs and MBONs (Crocker et al. 2016, Croset et al. 2018), so the differences could lie in the signaling cascades that drive plasticity or in the mechanics of adjusting synaptic vesicle release. Another important factor is of course DAN activity, which is often very different across compartments (see, for example, Berry et al. 2012, Mao & Davis 2009, Yamagata et al. 2016).

The circuit's parallel structure is most evident in the fact that it is possible to form aversive and appetitive memories with the same odor. When flies are conditioned using an ingestible reinforcer composed of both a bitter tastant and a sweet sugar (Das et al. 2014), the result is a composite of two memories—a short-lived aversive memory entrained by the bitter compound and a long-lasting appetitive memory from the caloric sugar. Thus, flies are initially repelled by the odor, but later this flips and the odor becomes attractive. This biphasic memory profile was also observed when ethanol was used as reinforcement (Kaun et al. 2011). Two opposing memories can even be implanted using optogenetic reinforcement (Aso & Rubin 2016). How these memories interact is not clear in all cases, but some of the relevant compartments are connected. MBON-γ1pedc, a site for aversive memory, sends inhibitory input to multiple other compartments, including those involved in appetitive memory, e.g., MBON-γ5β′2a, which signals negative valence (Aso et al. 2014a, Felsenberg et al. 2018, Takemura et al. 2017). This MBON acts as a toggle switch in the circuit (Perisse et al. 2016). When an aversive memory is formed and MBON-γ1pedc is depressed, this lifts the inhibition on MBON-γ5β′2a, tipping overall MBON activity more toward negative valence (**Figure 3*d***).

In short, the modular arrangement of this part of the circuit funnels in different reinforcement signals to synapses carrying equivalent olfactory information and links those to different outputs. By laying down a series of parallel memory traces, the system can diversify the features of those memories, creating different thresholds for induction, different durations of persistence, and even dynamically modifying the valence of an association over time. An even greater level of complexity comes from the fact that there are interconnections between MB compartments, as discussed below.

CROSS-MODULE INTERACTIONS AND MEMORY UPDATING

Despite the parallel structure of DAN-MBON compartments, there are extensive interconnections between modules (Aso et al. 2014a, Takemura et al. 2017), including recurrent connections from MBONs to DANs. Intermodule interactions enable learning in one part of the network to be contingent on changes in another part of the circuit. This can increase the complexity of the memory formation process (Cognigni et al. 2018). In particular, intermodule interactions are an essential aspect of how an animal updates the state of its memory network, incorporating new information in a manner shaped by past experience.

Extinction is a classic example of memory updating. After forming an odor-reward association, repeated exposure to the odor without reward eventually extinguishes the learned association. This process requires DAN-MBON modules involved in aversive memory formation (Felsenberg et al. 2017). This model posits that the missing reward is represented as a type of punishment, and extinguishing an appetitive association involves forming an aversive memory in a separate compartment to counterbalance it. A similar but complementary process supports the extinction of aversive memories (Felsenberg et al. 2018). How the omitted reinforcement or the mismatch from expectation is computed is not fully understood. However, in both cases, connections from MBONs to DANs involved in the learning of opposite valence appear to be critical, consistent with recent theoretical results (Jiang & Litwin-Kumar 2019).

Another example where past experience influences future learning is reconsolidation. When a memory is recalled, the recall process itself makes that memory labile. The process of reconsolidation reestablishes the memory, often slightly updated based on the animal's experience during that recall event (Lee 2008). Reconsolidation requires the action of two different DAN-MBON modules, likely driven through excitatory input from MBON-γ2α′1 (Felsenberg et al. 2017). The current working model is that recurrent recruitment of PPL1-γ2α′1 is necessary for the induction of reconsolidation, and a subsequent reactivation of multiple rewarding DANs projecting to other compartments is likely to reinstate the original memory trace (**Figure 3*f***).

There is likely much more to learn about intermodule interactions and how they contribute to behavior. Recurrent connectivity appears to be very extensive at this layer, as activating individual MBONs can evoke broad and complex activity patterns among the DANs (Cohn et al. 2015). However, the framework for understanding the current work is clear: By either within- or across-compartment connections from MBONs to DANs, the circuit is able to shape its own propensity for plasticity. This contributes to both the acquisition of new memories and the updating of old ones.

PERSISTENCE AND FORGETTING

What governs the persistence of memory in the MB network? This question breaks down into two related issues: the process of forgetting itself and the process whereby labile memories are consolidated into a more long-lasting form. DANs play key roles in both processes.

The act of forgetting is not merely a homeostatic process that slowly erases synaptic changes. It has an active component governed by molecular pathways distinct from those involved in learning, and the process of memory retention reflects an interplay between both positive and negative regulators (Davis & Zhong 2017). The dopaminergic system is central to setting this balance and dictating the time course of memory (Berry et al. 2012, Shuai et al. 2015). This was first shown by examining memory decay while manipulating a set of PPL1 DANs that innervate a small number of compartments (γ1pedc, γ2α′1 and α′2α2) (Berry et al. 2012). Memory could be prolonged by blocking these DANs after training, while stimulating the same cells accelerated memory decay. Interestingly, these cells seem to overlap with the set of cells involved in acquiring the memory

in the first place (Aso et al. 2012). However, one notable difference is that forgetting requires the DopR2 receptor (Berry et al. 2012, Cervantes-Sandoval et al. 2016), which drives potentiation (Cohn et al. 2015, Handler et al. 2019). Perhaps because of its higher affinity for dopamine (Himmelreich et al. 2017), DopR2 could be selectively activated by low levels of dopamine released by ongoing DAN activity (Berry et al. 2012, 2015) and potentiate previously depressed KC>MBON synapses. This is consistent with observations that forgetting an aversive association is accompanied by restoration of MBON responses that were depressed by previous learning (Berry et al. 2018).

Only a small fraction of learned experience can enter into LTM, and DANs play a central role in governing this process. In *Drosophila*, the formation of aversive LTM requires multiple rounds of training with a resting interval in between each training session (Tully et al. 1994). A series of experiments from Preat and colleagues (Plaçais et al. 2012, 2013; Scheunemann et al. 2018) have shown that the pattern of activity in the DAN population is an important factor influencing LTM formation after spaced training. Examining a small population of DANs primarily from the PPL1 cluster, the authors observed that a subset of these cells exhibited spontaneous oscillations of activity, whose amplitude and frequency were increased by the spaced bouts of training. However, the authors traced the critical time window of DAN activity back to the training period itself. By blocking DAN activity in between each of the bouts of spaced training and very shortly after the training, they prevented the formation of LTM. It should be noted that the ongoing oscillatory activity of these DANs after memory acquisition was not essential, as prolonged blockade of DAN activity in the period between training and testing did not affect LTM formation. Rather, the manipulations during the spaced training period are what matter. Although it was not possible to track DAN activity during the training protocol, these results indicate that spaced training establishes a unique pattern of DAN activity, which triggers the signaling events that drive long-term synaptic changes.

Another prominent hypothesis is that consolidation into LTM is achieved via persistent activity of the DANs. Long-term appetitive memories have been mapped to the α1 compartment and/or require output from the α1 compartment (Bouzaiane et al. 2015, Plaçais et al. 2013). Inactivating the cognate DAN well after training prevents the memory from transitioning into LTM (Ichinose et al. 2015). In this case, persistent DAN activity may simply reflect the kinetics of the caloric value signal itself, which likely has a slow onset and is long lasting. Nevertheless, it seems that this prolonged activity is capable of inducing long-term plasticity at KC>MBON synapses, while the relatively transient activity during associative pairing is only sufficient for short-term changes. Persistent activity is also the leading hypothesis for LTM consolidation in courtship conditioning, a paradigm where a male fly learns to suppress its courtship display toward a mated female after he experiences rejection (Keleman et al. 2012). Consolidation of such associations requires the activity of DANs innervating γ5β′2a in a time period following training (Krüttner et al. 2015), and sleep seems to play an important role in modulating the reactivation of this group of DANs (Dag et al. 2019). Notably, both the α1 and γ5β′2a compartments have a feedback motif where the MBONs project to the dendrites of the DANs in the same compartment, potentially supporting reverberant activity (**Figure 3*e***). However, one unknown is why posttraining DAN activation drives consolidation in these two compartments, while in many other compartments DAN activation leads to forgetting.

The DANs, therefore, not only are essential to induce plasticity but also dictate how long that plasticity will last. In setting the balance between these two processes, the DANs can be considered a filter for the system to retain the most relevant and meaningful information, based on factors like recency, reliability, and impact.

FLEXIBLE ROUTING OF SIGNALS IN THE MUSHROOM BODY: A LINK TO ACTION SELECTION?

Recent studies have broadened our understanding of the role of the MB to include adaptive control of behavior based on internal state and environmental context (Grunwald Kadow 2019). This is encapsulated in the idea that the circuit acts as a switchboard (Cohn et al. 2015). According to this scheme, signal transmission through the MB is modulated on a moment-by-moment basis by DAN activity. This presents a more dynamic picture of the MB, where the same olfactory input can be coupled to different outputs depending on the contextual signal provided by dopamine (**Figure 3*g***).

This combination of flexible routing overlaid on more persistent memory-related changes suggests a coarse conceptual similarity to processing in the basal ganglia. The basal ganglia have a well-established role in dopamine-mediated reinforcement learning (Joel et al. 2002). They also serve as the central switching area for action selection in the mammalian brain. The corticobasal ganglia-thalamocortical loops form largely segregated, parallel channels that carry competing cortical inputs (Alexander et al. 1986), and the most salient action command is established via dopaminergic inputs that increase signal transmission as part of the process of channel selection (Bar-Gad & Bergman 2001). In comparison, the MB can be seen as a relatively simple version of such a switchboard circuit, with 15 different compartments in the lobes constituting the output, and DAN inputs providing the salience-based routing signals.

Several observations make the switchboard routing hypothesis for the MB both plausible and appealing. First, DAN activity is very dynamically coupled to a fly's behavioral state (Berry et al. 2015, Cohn et al. 2015), indicating that dopamine may modify circuit function on a very short timescale. Examining DAN inputs to four compartments in the MB horizontal lobe showed that these cells take on two complementary activity patterns. Activity switched between these two patterns every few seconds, each time the fly flipped between flailing its legs and being still (Cohn et al. 2015). Second, dopaminergic input can affect signal transmission in the MB via multiple mechanisms (**Figure 3*a–c***). In addition to inducing depression and potentiation of KC>MBON synapses, DANs also form direct excitatory connections to MBONs (Takemura et al. 2017). Therefore, either dopamine or possibly a cotransmitter (Aso et al. 2019) can directly activate MBONs and potentially alter their excitability. Third, dopaminergic action is highly spatially specific. The plasticity induced in one compartment does not spill over to neighboring compartments (Hige et al. 2015a), and the evoked second messenger signals are largely confined to the region of DAN activation (Boto et al. 2014, Handler et al. 2019). These features of the dopaminergic inputs endow the circuit with a high degree of flexibility for signal routing.

Although many aspects of the switchboard hypothesis have yet to be evaluated, the proposal that dopamine mediates motivational salience is borne out by studies examining state and context dependence of odor responses. In flies, the prime example of an internal drive is hunger. Satiety state can affect DAN activity through either neuropeptidergic or other neuromodulatory pathways (Krashes et al. 2009, Tsao et al. 2018, Yamagata et al. 2016). Among the different DANs, PPL1-γ1pedc is most extensively studied and shows higher activity, both spontaneous and odor evoked, in fed flies (Plaçais et al. 2013, Tsao et al. 2018). Consistent with DANs representing the satiated state, stimulating PPL1-γ1pedc prevents starved flies from seeking out an attractive odor, whether it is a reward-associated odor (Krashes et al. 2009) or, interestingly, an innately appetitive one (Tsao et al. 2018). In this case, state-dependent DAN activity serves as a gate controlling attraction responses (Perisse et al. 2016).

Beyond internal state, responses to a given stimulus also depend on environmental context. In flies, the potent aversive response to carbon dioxide, a stress pheromone (Suh et al. 2004), is

considerably reduced by the aroma of vinegar. This effect comes from the action of a small set of PAM neurons that are activated by vinegar. These PAM neurons dampen responses of the corresponding MBONs, reducing the repellent effects of carbon dioxide (Lewis et al. 2015). How this gating is implemented, via changes in synaptic strength or modulation of intrinsic excitability, remains to be established. However, in all these studies it is clearly the case that the DANs are not carrying reinforcement signals but instead serve a role in routing information through the network.

The MB output layer and the basal ganglia both mark a point in the sensory to motor pathway where the network is funneling down. They both receive a very large and diverse set of inputs, and despite having very different anatomical organization, the two circuits both have to perform the task of dimensionality reduction. What is shared between the two is the need to shape this dimensionality reduction based on past experience (Joel et al. 2002) and to contextualize the results according to the current internal or environmental state (Klaus et al. 2019). Dopamine is the common thread, playing a critical role in both processes. How the function of dopamine in motivation and reinforcement learning is separated is an important question to consider in both systems (Berke 2018).

SALIENCE

The involvement of the MB in motivational salience and flexible behavioral control also raises an interesting issue for speculation—that of attention and stimulus salience. There is evidence that, when presented with competing stimuli, flies suppress responses to the less relevant of those stimuli (van Swinderen 2007) and that dopamine and MBs are involved in these types of decisions (Zhang et al. 2007). A priori, it seems that even flies should have some capacity to selectively orient toward certain sensory inputs in their environment. Recent studies have offered some insight into how circuit elements in the MB may contribute to this process.

Often, the salience of a stimulus is borne of its novelty—we react most strongly to whatever item is new in our environment. This is the case with MBON-α′3, where odor delivery at first evokes a strong response, which becomes weaker with repeated presentations (Hattori et al. 2017). This weakening is driven by synaptic depression from DAN input to this compartment, and the activation of these DANs by odor itself appears to be the key. As the novelty response wears off, the odor also loses its ability to elicit an alerting response. In this way the network filters out familiar odors so that novel olfactory stimuli are the ones to drive behavior. This novelty-assessing module has interesting parallels with Bloom filters, data structures used in computer science that efficiently store and allow lookup for previously encountered patterns (Dasgupta et al. 2018).

A sensory stimulus also becomes salient when it is associated with reinforcing stimuli that carry strong intrinsic meaning. A recent study (Boto et al. 2019) found that the salience of a conditioned odor may be modulated by a group of DANs innervating the MB calyx, the PPL2-DANs. Pairing odor with the activation of these DANs increases calcium responses in the KCs. However, the pairing effect is different than with DANs innervating the compartments in the lobes. For PPL2-DANs, there was no observed effect on odor valence. Instead the pairing enhances the strength of the memory formed. PPL2-DAN modulation can thus be regarded as a way to specifically augment the sensory processing of the conditioned odor, which may support efficient recall or facilitate subsequent learning.

CONCLUSION

Three-layer neural networks have been central to our thinking about how memory systems address the problem of pattern separation. The MB actually works much like Marr and Albus originally proposed for the cerebellum. But what happens once patterns are separated has been more difficult to answer. In the fly circuit, we now have the beginnings of an answer: The

representation of odor identity in the KCs is mapped down onto a representation of valence in the MBONs, and adjusting KC>MBON synapses modifies behavior by changing that mapping.

The 15 parallel DAN-MBON modules build flexibility into the circuit by enabling memories to form over different timescales, with different training requirements, different sensitivities to updating, and different rates of decay. These module specializations give the circuit the capacity to establish predictive relationships but not be a slave to them. For example, motivations change based on internal state and the external world changes on many different timescales, and the system has to adapt accordingly.

DAN activity is central to this process, as DANs not only implement learning-related plasticity but also dictate how long memory lasts. As the field moves forward, one important question will be how DANs and the signaling pathways they control contribute to module specializations in different compartments. It is clear that these cells have multiple modes of action, and important components of the molecular pathways they act through have now been identified. Connecting these pathways to the genes and molecules identified by the extensive genetic study of memory in *Drosophila* will be important to get to the molecular origin of the learning rules and various other features in different DAN-MBON modules.

As the field digs deeper into how these modules give rise to changes in behavior, we will inevitably move closer to the motor end of the circuit, where the basal ganglia look like a useful guidepost for understanding. It would be a mistake to look too closely for analogies between brain areas of such evolutionarily distant species, but on a conceptual level, the process of selecting different actions based on learned experience, overlaid with internal state, must be shared. Certainly it is the case that in both systems, dopaminergic inputs are the mediators of motivational salience, flexibly routing information to different behavioral outputs.

Any efforts to link DAN-MBON modules to motor output will face the question of what valence really is. How can valence signals from MBONs be used in situations where the animal's context requires very different motor outputs to achieve an appropriate behavioral response? Rapid progress in connectomics means that this will become a very concrete question in the near future, as we start to see how MBONs are connected to circuits driving very different types of motor output, such as feeding, walking, and flying. How context is imposed, so that abstract valence signals trigger the correct motor output for whatever situation the fly is in, will be critical to a real understanding of how learning modifies behavior. However, the simplicity of the fly brain has allowed us to take the first steps beyond standard learning models toward an understanding of the circuit, from sensory to motor.

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