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Dopaminergic rules of engagement for memory in *Drosophila*

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Dopamine is associated with a variety of conserved responses across species including locomotion, sleep, food consumption, aggression, courtship, addiction and several forms of appetitive and aversive memory. Historically, dopamine has been most prominently associated with dynamics underlying reward, punishment, or salience. Recent emerging evidence from *Drosophila* supports a role in all of these functions, as well as additional roles in the interplay between external sensation and internal states and forgetting of the very memories dopamine helped encode. We discuss how cell-specific resolution and manipulation are elucidating the rules of dopamine's involvement in encoding valence and memory.

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Introduction

Contemporary recording, tracing, and manipulation approaches in mammals have resulted in tremendous progress towards identifying dopaminergic networks involved in the coding and processing of reward and aversive stimuli. This has also led to the discovery of a diverse population of neuronal subtypes within dopamine networks. Although the *Drosophila* brain contains orders of magnitude fewer dopaminergic neurons, there are several shared anatomical and functional features of dopamine systems in mammals and flies [1]. In mammals, dopamine is part of a highly interconnected network of modulators and transmitters important for complex behaviors such as reward seeking (Figure 1a). These include serotonin, norepinephrine (octopamine), acetylcholine, glutamate, and GABA, and many neuropeptides. A similar neuronal architecture mediates comparable behaviors in *Drosophila* (Figure 1b). Also shared across species are broad dopaminergic arborization throughout the brain and compartmental tiling onto specific brain structures. In the adult central nervous system of *Drosophila* there are 11 clusters of dopamine neurons, projecting to over 12 broadly defined neuropil structures [2••,3–6] (Figure 2). The improved ability to characterize and manipulate these neurons at the single cell level has allowed for unprecedented precision and insight into their diverse functions. Here we describe recent work in *Drosophila* that illustrates the timing and cell-type specific requirements of dopamine neurons in forming and forgetting memories. We consider how changes in internal state can affect these dopamine circuit dynamics and its implications for understanding memory and addiction.

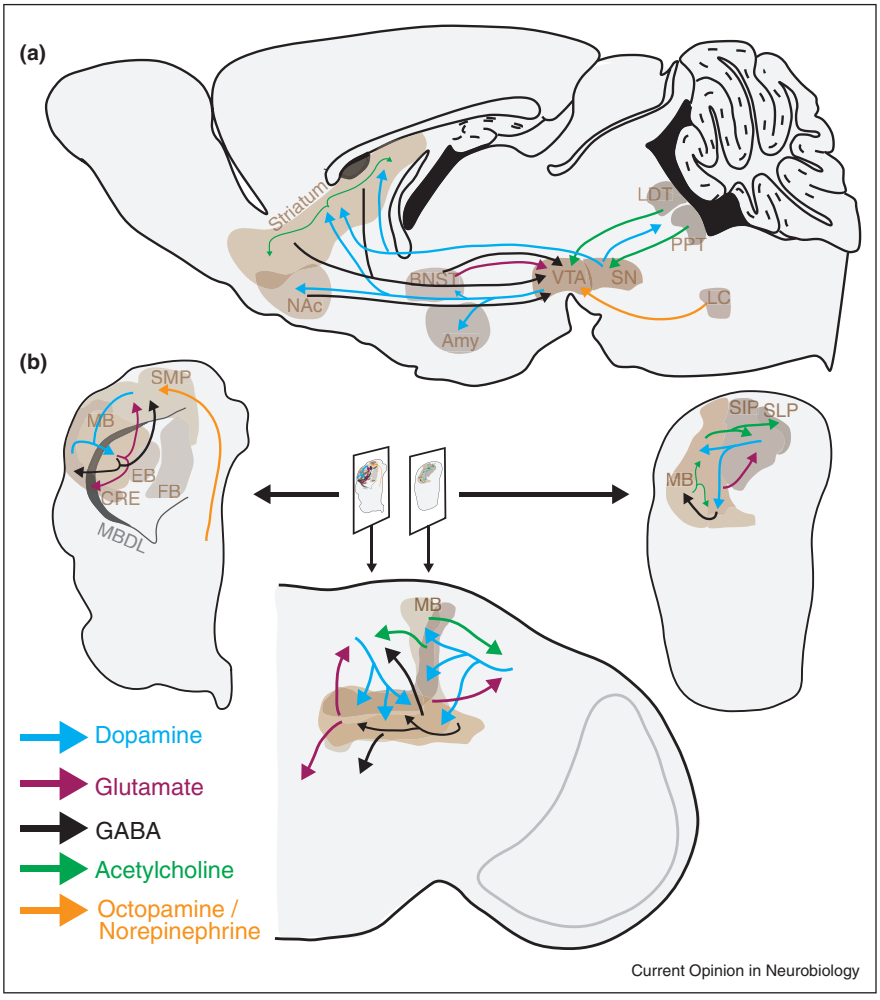
Distinct dopamine neurons assign opposing valences

In order to survive, animals need to sense their environment, evaluate the surrounding stimuli, and initiate an appropriate behavioral response. Flies, like other animals, learn the predictive value of stimuli to guide future behaviors. Most of the recent insight into the ability to do this derives from mushroom body (MB) circuitry. The MB receives information from multiple primary sensory centers in the brain, most notably the olfactory system [7]. The axons of these ~2000 MB intrinsic cells form three distinct MB lobes. Tiled on to these lobes are the axons of 20 types of dopamine neurons which originate from two clusters, PPL1 and PAM, consisting of 12, and roughly 100 neurons respectively (Figure 2a). Pioneering findings suggested that the PPL1 dopamine neurons innervating the vertical MB lobes relay aversive stimuli [8,9,10•] whereas the PAM cluster of dopamine neurons innervating the horizontal MB lobes relay appetitive stimuli [11,12]. Together, these data suggest that the punishing or reinforcing nature of a stimulus is largely conveyed by distinct clusters of dopamine neurons, and the respective MB lobe they innervate. In mammals, the indirect and direct pathways of midbrain dopamine projections to the basal ganglia intriguingly parallel this separation in behaviors for punishment avoidance and reward seeking [1].

Memory is compartmentalized

More recent data suggest that two dopaminergic neuron clusters are elaborately subdivided and segregate into distinct compartments along the MB lobes. Dopaminergic projections align with dendrites of glutamatergic, cholinergic, or GABAergic MB output neurons (MBONs),

Figure 1

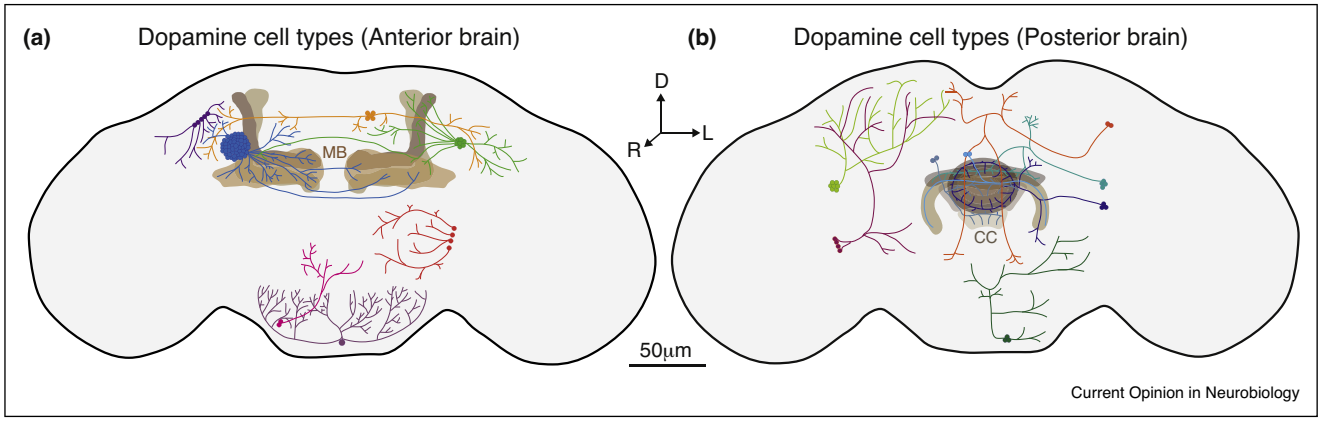


Dopamine networks in flies and mammals. In flies and mammals, complex feedback and feedforward networks between dopamine (blue) glutamate (magenta), GABA (grey), acetylcholine (green), and norepinephrine/octopamine (yellow) regulate reinforcement memory [1]. **(a)** A saggital section of a rodent brain shows dopamine projections from the ventral tegmental area (VTA) to the striatum, nucleus accumbens (NAc) and bed nucleus of the stria terminus (BNST), which send GABAergic and glutamatergic projections back to the VTA. These VTA dopamine neurons are modulated by norepinephrine from the locus coeruleus (LC). Another subset of dopamine neurons from the substantia nigra (SN) project to the pedunculopontine nucleus (PPN), which in turn sends cholinergic projections back to the SN. **(b)** In *Drosophila*, PAM dopamine neurons project from the superior medial protocerebrum (SMP) and crepine neuropil (CRE) to the horizontal mushroom body (MB) lobes, where GABAergic and glutamatergic MB output neurons project back to the SMP and CRE. These PAM neurons are modulated by octopamine. PPL1 dopamine neurons project from the superior intermediate protocerebrum (SIP) and superior lateral protocerebrum (SLP), to the vertical MB lobes and cholinergic neurons project from these areas to the SIP and SLP.

subdividing the MB into 15 distinct compartmental units [2••] (Figure 3). For example, blocking activity of the PPL1 dopamine input or corresponding GABA output of the γ1ped compartment blocks odor memory for electric shock [8,13]. Experimental activation of the γ1ped PPL1 neuron, in conjunction with odor presentation, causes a reduction in spiking of the corresponding γ1ped MBON consistent with MB-intrinsic long-term depression (Figure 4a,b) [14••]. Since activating the γ1ped GABA output signals positive valence, it is likely that dopamine modulates behavior by inducing a long-lasting depression in this neuron. Moreover, dopamine released in one compartment induces robust plasticity in that compartment, but not its neighbor [14••], suggesting that these anatomically defined compartments of the MB lobes are functionally independent (Figure 4b).

Spatial modularity is an ideal way to allow the fly to form a series of different memories, that cause distinct behavioral reactions. This is apparent in dissociating compartments important in different forms of appetitive memory. Two dopamine-MBON compartments (β′2 and γ4) are

Figure 2



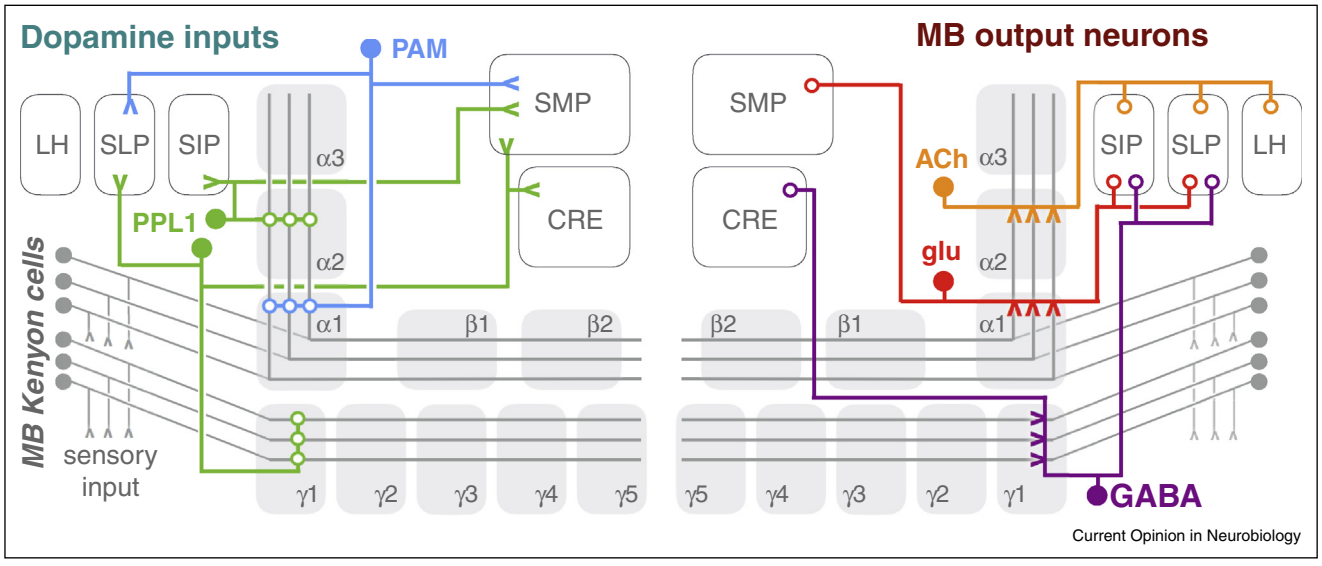
Dopamine neurons in the adult brain. Representative schematic of dopamine neuron motifs in **(a)** anterior and **(b)** posterior areas of the adult *Drosophila* central nervous system (largely based on data from the Virtual Fly Brain [4], FlyCircuit [5] and FlyLight [6] databases). The neurons are located bilaterally, but for clarity, only unilateral examples are depicted. Dopamine broadly innervates many brain areas, although subsets of neurons compartmentalize structures such as the mushroom body (MB) and central complex (CC).

involved in the short-term reinforcing effects of the sweet taste of sugar [15•,16•]. Conversely, the long-term reinforcing effects that are induced by sugar's calories engage dopamine neurons projecting to two different compartments (γ5 [15•] and α1 [16•]). This compartmentalization makes it possible for a fly to make the complex, context-dependent choices to survive in a changing environment.

The parameters of DA activation affect the outcome

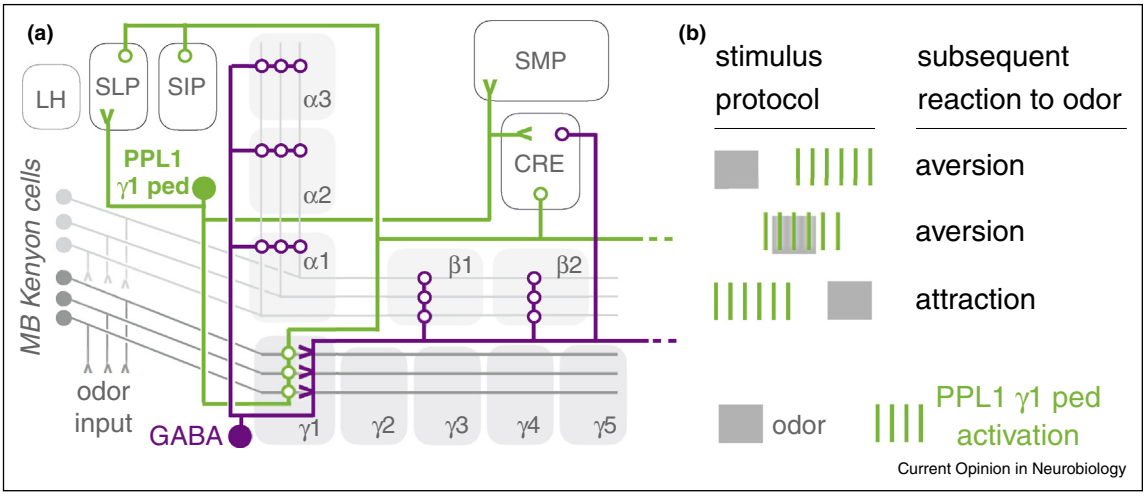
As mentioned above, different dopamine neurons induce short-term or long-term memory formation, thereby affecting the kinetics of memory retention. But what are the consequences of dopamine modulation at the level of the MB and MBONs? Different dopamine

Figure 3



Mushroom body circuitry in *Drosophila*. Sensory information projects to the mushroom body intrinsic neurons (MB Kenyon cells), where it is integrated with dopaminergic information about stimulus valence. Approximately 130 dopamine cells consisting of 20 cell types project axons from two clusters (PAM, and PPL1) that tile across MB axons [2••]. Three representative dopamine neurons are shown on the left. Dopamine axons project to glutamatergic (glu), GABAergic (GABA) or cholinergic (ACh) mushroom body output neuron (MBON) dendrites. The 34 MBON fall into 21 distinct types effectively, and together with the dopamine inputs they divide the MB into 15 compartments. Three of those are shown at the right. MBON axons project to five discrete neuropils outside of the MB where dopamine neuron dendrites and MBON axons converge: lateral horn (LH), superior intermediate protocerebrum (SLP), superior intermediate protocerebrum (SIP), superior medial protocerebrum (SMP) and crepine neuropil (CRE) [2••]. Note that for simplicity's sake, the α′β′ lobes of the MB and its five compartments were omitted from the schematic.

Figure 4



Location, timing and intensity of dopamine neuron activation determine formation and valence of memories. **(a)** Schematic of the γ1ped PPL1 MB dopamine-GABA microcircuit. Axons of the dopamine neuron align with dendrites of the GABA neuron in the γ1ped compartment. Both neurons send projections to the CRE and the contralateral side of the brain. The dopamine neuron also projects to the SMP, SLP and SIP [13]. The complex innervation patterns, including possible feedback, of the γ1ped dopamine and GABA neurons may contribute to how timing predicts valence, formation, or expression of memory. **(b)** Timing between experimental activation of the PPL1 γ1ped dopamine neuron and odor stimulus affects the behavioral outcome. When odor is presented before experimental γ1ped PPL1 dopamine stimulation, or concurrently, an aversive memory is formed. However, if the odor is presented following dopamine neuron activation, an appetitive memory is induced [17••]. Concurrent odor presentation and dopamine stimulation also results in long-term depression of the MB-GABA synapse, in a compartment-specific manner [14••]. Odor presentation after dopamine stimulation, however, did not change this synapse [14••], suggesting that the activation of other compartments via feedback loops might underlie the behavioral attraction induced.

neurons have distinct rules in regards to the intensity and repetition of activation required to produce synaptic plasticity and memory [14••,17••]. Interestingly, for one PPL1 dopamine neuron (γ1ped), the timing of its experimental activation relative to the odor presentation produces opposite behavioral effects. This neuron provides information about an aversive stimulus, such as an electric shock [8,9,10•]. When odor presentation precedes γ1ped PPL1 activation, the odor predicts punishment and the flies avoid the odor. Conversely, when the odor follows γ1ped PPL1 activation, the odor predicts relief from punishment and becomes attractive (Figure 4b) [17••]. This switch of valence is also observed with other PPL1 neurons (α′2α2, γ2α′1). In contrast, when appetitive PAM neurons are activated with different timing relative to odor presentation, a switch in valence is not observed [17••]. This suggests that the relative timing between dopamine neuron activation and cue presentation plays a more qualitatively instructive role in punishment, than in appetitive reinforcement [17••]. This provides a mechanism through which stimuli that predict punishment are valued differently from stimuli that predict the end of punishment, with the former being avoided, and the latter becoming attractive. Clearly, when and how dopamine neurons are activated matters not only for the strength and persistence of an induced memory, but also for its valence.

Parallel and additive processing

Within each cluster, a combination of dopamine neurons innervating multiple compartments along the same lobe may be required to signal the full extent of a stimulus and its resulting memory. In support of this, taste memory for an aversive stimulus is dependent on activation of combinations of PPL1 neurons [10•]. Similarly, experimental activation of dopamine neurons that innervate multiple MB compartments, rather than a single compartment, is more likely to produce memories [17••]. This quantitative multi-compartment coordination suggests that parallel and additive processing is built into the circuit response, which might provide flexibility for responses to multiple, and/or competing reinforcing or punishing stimuli.

Crosstalk and feedback in dopamine circuits

MBON axons and dopamine neuron dendrites show substantial connectivity in convergence zones outside the MB, resulting in a high degree of feedback and interconnectivity between these circuits [2••] (Figure 3). Activating dopamine neurons in one compartment can cause a concomitant increase in activity in another compartment and a decrease in yet others [18••]. This involves MBON-mediated feedback within the lobes or via the convergence zones outside the MB. For example, the γ1ped GABA MBON sends projections along the γ lobe, and its activation decreases odor-evoked response

of other γ MBONs (β′2γ5) [18••,19•,20]. Similarly, the α1 glutamate MBON sends projections to dendrites of the α1 dopamine PAM neurons, and activation of this MBON drives activity of the α1 PAM neurons in a feed-forward loop during appetitive memory expression [21•].

A role for dopamine in forgetting

The findings summarized above suggest a role for dopamine in stimulus reinforcement during memory formation, but other recent data suggest that it may also play an active role in forgetting. Silencing the majority of dopamine neurons after aversive odor conditioning increases avoidance memory 3 hours after training [22••]. This increased memory is a consequence of reduced forgetting of the 3-min avoidance memory and requires the DAMB (D1-like) dopamine receptor in the MB [22••]. Similarly, activating the γ1ped PPL1 neuron in the absence of odor causes a reduction in odor avoidance that was previously induced by pairing odor with activation of that very same γ1ped PPL1 [17••].

Why would activation of the same dopamine neuron cause aversion when paired with the odor, but cause forgetting of that very memory when presented alone? One intriguing suggestion comes from the finding that the same manipulations that lead to reduced forgetting also suppress reversal learning [23]. In this paradigm the odor A + shock versus odor B training is reversed to odor B + shock versus odor A and flies re-learn to avoid odor B instead of A. This behavioral flexibility allows for different context-specific associations so that the same odor can be associated with reward in one context and with punishment in another. Thus, a low level of dopaminergic activity may signal 'pay attention, things are happening in the environment'. Although this promotes forgetting of memories [17••,22••], it also facilitates reversal learning to prime animals for new experiences and valence associations that are most relevant. Intriguingly, subsets of dopamine (PAM-β′1) and MBON (γ4 > γ1γ2) involved in forgetting did not affect reversal learning, suggesting a complex interplay within the dopamine MB circuitry that provides opportunity to learn and re-learn in multiple contexts [24].

Internal state affects dopamine dynamics

The information that an animal pays attention to and learns is dependent on its internal state. If a fly is hungry, it is more likely to brave the heat and feed on a fermenting apple located in a sunny spot. Work in *Drosophila* suggests that these decisions are gated through dopamine neurons. Activation of the γ1ped dopamine PPL1 neuron suppresses the expression of memory for sucrose when flies are hungry [25]. Odor-evoked activity of the corresponding γ1ped GABA MBON is elevated in hungry flies [19•]. Thus, internal state changes basal dynamics within a dopamine memory circuit, which can, in turn, enhance its response to reinforcers like sucrose or shock [26].

This is not specific to this PPL1 neuron, but appears to be a more general phenomenon. Long-term memory formation from caloric reinforcement involves dopamine neurons projecting to the β′2 and γ4 MB lobes [15•,16•]. These PAM neurons receive input from a Gr43a-positive neuron that senses internal fructose levels therefore making them reactive to the internal nutritional state of the fly [16•]. Indeed, sucrose feeding in hungry flies increases the activity of appetitive dopamine neurons projecting to the γ5 and γ4 MB lobes [18••], as does water feeding, albeit only in thirsty flies [27]. Similarly, the response to appetitive odors is enhanced in dopamine neurons projecting to the β′2 and γ5 lobes in hungry animals [28]. This suggests that internal states not only cause activation of dopamine neurons, but can also modulate the effectiveness of the external reinforcer.

Relevance to addiction

A common theme in addiction is that drugs of abuse hijack the dopaminergic 'reward' circuit and thus these artificial rewards reinforce associated behaviors. Many drugs of abuse, including alcohol, are complex stimuli with both aversive and appetitive properties. Naïve flies' initial response in an alcohol consumption choice is to avoid it [29•]. Similarly, an odor associated with alcohol intoxication initially causes avoidance of that odor [30••]. This response is succeeded by attraction to the odor the next day [30••]. Because the PAM and PPL1 dopamine neurons are required for oviposition preference and avoidance for ethanol [31], we propose that they play critical role in providing aversive and appetitive alcohol reinforcement. Thus, ethanol-induced activation of PAM dopamine neurons would provide the appetitive intoxication effects whereas ethanol-induced activation of PPL1 neurons would provide both the punishing aversive effects, (when an odor predicts punishment) and relief from punishment (when an odor predicts PPL1 inactivation). We speculate that the simultaneous PAM neuron activation and PPL1 neuron inactivation provides an additional 'boost' to the appetitive nature of ethanol memory. Perhaps this explains why more flies will walk over a 120 V electric shock to seek an odor previously associated with ethanol than with sucrose [30••].

Further complicating dopaminergic involvement in addiction is how drugs of abuse can modulate internal state of the animal, thus affecting DA reinforcement. Many drugs of abuse stimulate locomotion, and that is true too for alcohol, which induces *Drosophila* hyperlocomotion [32]. This response is dopamine-dependent and requires activity in the PPM3 dopamine neurons that project to the ellipsoid body of the central complex, a known pre-motor center [32,33]. Changing the activity and arousal state of the animal, could potentially shift DA neuron dynamics in the MB as is observed when flies shift from low activity sucrose feeding to high activity flailing [18••]. This also predicts a high level of interconnectivity

between the central complex and mushroom body. Ultimately, the drug-induced engagement of the dopaminergic system leads to various, and even competing responses, which may have different strengths and decay kinetics, and the emerging summation of these responses and memories therefore appears complex, including shifts in valence [30••].

Conserved principles of neuromodulation

The last few years have revealed that flies use circuits defined by segregated compartments containing localized dopamine modulation layered on to sensory input—compartment-specific output neurons, and that memories are formed when dopamine changes the synaptic plasticity of these circuits. Given the compartmentalized nature of many mammalian brain structures, it seems likely that these basic rules are conserved. In addition to this compartmentalized circuit architecture, we are also continuing to learn how distinct dopamine neurons can mediate behavioral changes with opposing valence, and how they are involved in the expression/interpretation of the animal's internal state. Layered on this is the role for tonic dopaminergic activity serving as a signal for salience, priming the MB for learning, and re-learning. The possibility to study these complexities with both increasing behavioral sophistication, and anatomical precision, makes *Drosophila* an effective model organism to continue to unravel the ways in which dopamine guides and modulates memories and behavior.

Conflict of interest statement

Nothing declared.

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