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Dopamine reveals neural circuit mechanisms of fly memory

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Abstract

A goal of memory research is to understand how changing the weight of specific synapses in neural circuits in the brain leads to an appropriate learned behavioral response. Finding the relevant synapses should allow investigators to probe the underlying physiological and molecular operations that encode memories and permit their retrieval. In this review, I discuss recent work in *Drosophila* that implicates subsets of dopaminergic neurons in aversive reinforcement and appetitive motivation. The zonal architecture of these dopaminergic neurons may reveal the functional organization of aversive and appetitive memory in the mushroom bodies. Combinations of fly dopaminergic neurons might code negative and positive value consistent with a motivational systems role proposed in mammals.

Introduction

The neuromodulator dopamine has been ascribed many important roles in the vertebrate and invertebrate nervous system. In mammals these functions include motor control, reinforcement, motivation, memory, arousal and attention [1–4]. Consequently a dysfunctional dopaminergic (DA) system has been linked to a number of neurological and psychiatric disorders such as Parkinson's disease, schizophrenia, depression, drug addiction and attention deficit disorder [3–5]. The organization of the mammalian DA system is extraordinarily complex, with neurons clustered into nine major nuclei and projecting broadly throughout the brain [6]. Evidence suggests that DA neurons from at least two of these clusters in the ventral tegmental area (VTA) and substantia nigra process information from rewarding stimuli and distribute it to areas of the brain involved in motivation and goal-directed behavior. In the mouse, there are approximately 20–30,000 DA neurons that innervate the striatum, limbic and cortical areas and each brain structure receives a mixture of projections from both the VTA and substantia nigra. It is therefore apparent that these major brain regions, are innervated by a large heterogeneous population of DA neurons. This complexity represents a considerable hurdle to understanding how the dopamine system precisely influences nervous system function.

Perhaps surprisingly, dopamine has also been implicated in the control of movement [7–9], reinforcement [10], motivation [11], memory [10,12,13], arousal [14–18] and stimulus saliency [19] in the fruit fly *Drosophila melanogaster*. However, in the *Drosophila* brain these processes are orchestrated by a total of only 600 DA neurons whose cell bodies are organized into fifteen

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Supplementary data

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clusters [20,21]. This relative simplicity provides an obvious advantage to understand the functional organization of a complete DA system. In addition, the sophisticated genetic manipulation in *Drosophila* allows one to interrogate the molecular and cellular properties of the DA system with spatial and temporal precision. Taking advantage of this genetic arsenal (see Table S1 in the Supplementary Material online), investigators have recently described the anatomy of parts of the DA system in the fly at single-cell resolution [22,23] and have functionally dissected the role of subpopulations of DA neurons in locomotion [9,24], reinforcement [11,23–25; Glossary] and motivation [11]; in some cases to the level of a single morphological type of DA neuron [9,11,25]. This analysis has indicated that distinct fly DA neurons may influence these neural processes by innervating discrete anatomical zones.

The ability to identify and genetically label small groups or individual fly DA neurons that execute particular tasks would allow one to determine their underlying physiological operations. Precise and reproducible cell-specific expression permits live-imaging of genetically encoded reporters of neural activity and aids placement of pipettes for direct electrophysiological recording. Pioneering studies of appetitive conditioning in monkeys, pairing auditory or visual cues with a small piece of apple or fruit juice, have established a popular model for midbrain DA neurons in reward learning [26]. In this reinforcement learning model, phasic firing of midbrain DA neurons drives changes in synaptic strength of receptive circuits by signaling deviations or ‘errors’ between predicted reward and actual reward, rather than simply coding the occurrence of a rewarding stimulus. The magnitude of DA neuron activity scales to the unpredictability of the reward until the animal has learned an accurate prediction. At that point, reinforcement recedes to zero and the DA neurons instead fire to the stimulus predicting reward, which is proposed to guide action selection. Importantly, if no reward is presented following the predictive stimulus, DA neuron activity is suppressed below baseline at the time of the expected reward. Mammalian midbrain DA neurons therefore appear to constantly provide an update of the environment and code a positive prediction error if actual reward is more than predicted, no error if prediction matches actual reward and a negative error if actual reward is less than expected [26]. Although prediction error models have a strong quantitative and theoretical framework in temporal difference (TD), or TD algorithms of machine learning [27,28], DA neurons are not universally accepted to function this way [29]. Finding that certain fly DA neurons operate like their mammalian counterparts, would both validate the use of the fly as a model for DA neurons, and permit a genetic dissection of the underlying neural algorithms. In this review, I will illustrate how the fly DA system is being dissected by focusing on recent work investigating a role for DA neurons in reinforcement [22,24,25] and motivation [11] in the context of fly olfactory memory.

Teaching fruit flies

A number of assays have been developed to address how experience is represented in the fly brain. Here, I focus on the study of variations on two classic associative olfactory learning paradigms [30,31]. Presenting flies with an odor paired with electric shock forms a labile aversive odor memory [30], whereas multiple spaced training trials form an aversive long-term memory that lasts for days [32]. Alternatively, conditioning hungry flies by pairing an odorant with sugar forms appetitive odor memory [31] and a single 2 minute training session can form long-term appetitive memory that lasts for days [33,34].

Memory in either paradigm is observed at given times after training as a selective avoidance of, or attraction to, the previously reinforced odorant, when given a choice between that odorant and another. Understanding the difference between aversive and appetitive memory processes has been a driving force in the field for several years with the prevailing model being that unlike mammals, dopamine mediates aversive reinforcement whereas reward is carried by octopamine [10,35]. A collection of recent papers [11,22,24,36,37] have added to the

neuroanatomical resolution of our understanding of aversive and appetitive memory processes and together they challenge a strict dissociation between the monoamines and reinforcement processing in the fly. Coincidentally, recent work has suggested that discrete populations of mammalian DA neurons convey appetitive and aversive signals [38]. In this review I discuss these findings with the intent of illustrating that we are fast approaching a detailed understanding of the neural circuit mechanisms of fly learning and that these mechanisms may turn out to be more comparable to mammals than previously appreciated.

Odor memories in mushroom body neurons

Mushroom body (MB) neurons (Kenyon cells) are some of the third order neurons of the insect olfactory system, and a large body of literature points to these neurons as the likely location for olfactory memories [39] (Figure 1). The ~2,500 MB neurons can be functionally subdivided based on morphology, molecular markers, [40–43] and by their roles in memory processing. Experiments blocking synaptic output from different MB neuron populations suggest that the $\alpha'\beta'$ neurons are required during learning, for early memory retrieval and for memory consolidation [33,44,45] whereas the $\alpha\beta$ neurons are required for early retrieval [46,47] and are particularly critical for later memory retrieval [33,46,48,49]. There is some indication that olfactory memory processes may be distributed between $\alpha\beta$ neurons and the unbranched γ neurons [49–53] but experiments have revealed little or no consequence of blocking γ neuron output for short-term memory (STM) [46,52] or long-term memory (LTM) [49] respectively. Therefore their contribution to guiding behavior is presently enigmatic. A plausible model that is supported by most of the data obtained from testing the requirement of different subsets of MB neurons is that initial memory is laid down in parallel in $\alpha'\beta'$ neurons and $\alpha\beta$ neurons and it is refined to only $\alpha\beta$ neurons through consolidation involving $\alpha'\beta'$ and Dorsal Paired Medial (DPM) neuron activity [33,36,39,44].

Electro- and opto-physiological recordings at the level of Kenyon cell bodies suggest that, similar to the locust [54], odors are sparsely coded as a few action potentials in a small number of Kenyon cells [55] – a mode that would endow the MB with the capacity to store a large collection of highly specific olfactory memories. The key to models involving ‘parallel memory traces’ is that each odor is represented across the ensemble and in each class of Kenyon cell. Unfortunately, direct evidence for such a model is currently scant. Electrophysiological recordings from naïve flies found that $\alpha'\beta'$ neurons were more broadly tuned and responded more vigorously to odors than $\alpha\beta$ neurons and γ neurons – with γ neurons being the least responsive and mostly responding below threshold [55]. In addition, a recent detailed structural study of the MB [56] showed that dendrites from $\alpha'\beta'$ neurons and $\alpha\beta$ neurons were predominantly localized to a peripheral zone in the MB calyx whereas γ neuron dendrites mostly occupied the center. These data are consistent with prior studies [43,57] and the notion that some projection neurons could synapse with $\alpha'\beta'$ neurons and $\alpha\beta$ neurons in the same microglomeruli in the calyx [56]. This anatomy would allow some olfactory information to be simultaneously delivered to certain $\alpha'\beta'$ neurons and $\alpha\beta$ neurons.

It is possible that odor-evoked activity is more widespread across the MB neuron population and that even subthreshold or biochemical signals can be coded into memory as suggested by a study in the moth [58]. In addition, odor-representations in the MB may be altered by learning. It has been proposed that learning recruits new spiking neurons to the odor representation in the fruit fly antennal lobe [59] and it is therefore possible that γ neurons become more responsive after training. Others have proposed that learning increases the reliability of odor-evoked Kenyon cell firing and instead further sparsens odor representations in the Kenyon cells of the locust [60]; which might also be expected to increase the level of discrimination between odors. Assuming technical barriers can be overcome, it should be helpful to image activity in ensembles of MB neurons with more sensitive genetically-encoded reporters and with the

spatial resolution required to distinguish individual neurons to differentiate between these possibilities.

An important question is whether olfactory memories are represented at MB input and/or output synapses. Olfactory input is most evidently received from cholinergic projection neurons in the MB calyx whereas output is believed to occur from the MB lobe region. However it is clear that the MB lobes also receive input and therefore both pre- and post-synaptic plasticity could occur in the lobe region. Nevertheless, most current models of memory formation in flies favor presynaptic mechanisms of plasticity in MB neurons. To resolve the mechanisms and loci of memory we need to be able to localize memory-relevant plasticity to individual MB synapses rather than to entire groups of neurons. Recent work suggests an additional sophisticated anatomical and functional layer of subdivision within the individual MB lobes [11,22,24,61] that may guide microscopic study to the relevant places in the MB.

Dopaminergic reinforcement pathways intersect the MB

It is reasonable to expect that the odor-activated MB neurons that also receive innervation from the relevant reinforcement systems harbor synapses where memories are written [35]. The general model is that neurotransmission from DA neurons provide the reinforcing effects of punitive electric shock [10,35]. So where do the DA neurons project within the MB? A recent detailed clonal analysis demonstrated a remarkable and unforeseen complexity and diversity of DA neurons that innervate the MB [22]. Eight different types of individual DA neurons were identified that project from three discrete clusters of cell bodies to non-overlapping zones that decorate most of the MB. Furthermore, there is substantial heterogeneity within a given cluster. For example, the Protocerebral Posterior Lateral (PPL) 1 cluster was found to contain five types of DA neuron projecting to exclusive zones on the MB lobes including some that specifically innervate the α' and α lobes (Figure 2). This type of circuit element is an expectation of models predicting the formation of parallel early memory traces. Functional imaging of the DA neurons innervating these different zones revealed a variety of response parameters [22] consistent with the idea that each zone serves a different function.

Another recent study employing a new single-fly version of the olfactory conditioning assay identified the DA neurons in PPL1 to be required for negative reinforcement [24]. Silencing most DA neurons in the fly impairs aversive conditioning whereas blocking most DA neurons, but not those in the PPL1 cluster, has no effect. More impressively, by expressing a light-addressable neuronal trigger in DA neurons it was shown that flies can be aversively conditioned by coupling odor exposure with remote activation of the DA neurons, but only if the PPL1 neurons are included. These experiments suggest that the PPL1 DA neurons can carry reinforcement. As mentioned above and described in Figure 2, PPL1 includes DA neurons that innervate the peduncle, heel, junction, stalk, α' tip and α tip zones on the MB [22,24]. A different recent study using a heat-activated neural trigger determined that specific activation of the MB-MP neurons (named after the region of the MB they innervate –Medial Lobe and Peduncle) in the PPL1 cluster could substitute for aversive reinforcement [25] although acutely blocking these neurons did not impair aversive memory formation [11]. It therefore seems likely that aversive memories lie underneath one of the MB zones innervated by PPL1 neurons and that if the MB-MP neurons are involved in aversive reinforcement, they work in concert with other types of PPL1 neuron.

Finding that neurons projecting to the vertical α' and α lobes may be those carrying aversive reinforcement is consistent with two previous live-imaging studies that identified a vertical branch-specific plasticity after training. An early 30–60 minute increase in odor-evoked Ca^{2+} and synaptic transmission was observed in the projections of Dorsal Paired Medial neurons on the vertical lobes consistent with the time at which their output is required to

consolidate memory [62]. Furthermore, 3–9 hours after LTM training odor-evoked Ca^{2+} increased in only the α projections of $\alpha\beta$ neurons [48] (Figure 1). Taken together with the critical requirement for $\alpha\beta$ neurons in LTM [33,48,49], it seems reasonable to conclude that aversive LTM must be represented in the synapses of the α projections. Given the finding that flies lacking the vertical MB α projections have no LTM, but retain STM [63], it is conceivable that labile memory is served by the β projections of the same $\alpha\beta$ neurons, and perhaps the γ neurons (Figure 1). Individual DA neurons from the Protocerebral Anterior Medial (PAM) cluster are likely to innervate comparably complex zones within the horizontal β' , β and γ lobes [22] (Figure 2C) and recent work using a heat-activated neural trigger showed that activation of the MB-M3 neurons from the PAM cluster can substitute for an aversive stimulus in conditioning [25]. The MB-M3 neurons (3 neurons named after the region of the MB they innervate –Medial Lobe) primarily innervate the tip of the β lobe and sparsely innervate a small zone near the tip of the β' lobe. Importantly, blocking MB-M3 neuron output seems to specifically disrupt a component of labile memory consistent with MB synapses in the β lobe coding labile aversive memory.

Compartmentalization of dopamine-dependent intracellular signaling

Do intracellular cascades downstream of dopamine tell us more about the organization of memory? There are four dopamine receptor genes in *Drosophila*, DmDOP1 (dDA1) [64], dopamine receptor in mushroom bodies (DAMB) [65], DD2R [66] and DmDopEcR [67]. The dDA1 and DAMB receptors both stimulate cyclic adenosine monophosphate (cAMP) synthesis and are known to be expressed at high levels in the MB [64,65,68]. However, they appear to be distributed across the MB and are not obviously organized into the zones demarcated by the afferent DA neurons. It therefore seems likely that the same type of dopamine receptor localized to different zones in the MB neurons is fulfilling a variety of roles by responding to distinct DA source neurons.

Two recent live-imaging studies [36,37] using genetically encoded fluorescent reporters monitoring cAMP production or cAMP-dependent protein kinase (PKA) activity suggest that although the dopamine receptors may be evenly distributed, the signals they generate are not uniform. Surprisingly, bath application of dopamine to an isolated brain *in vitro* caused an increase in cAMP synthesis across the MB lobes whereas dopamine perfused on the brain of a living fly caused a vertical α lobe-specific PKA activation suggesting that DA receptors and the cAMP and PKA dynamic are differentially coupled and/or regulated within different branches of the same neurons [36,37]. Further experiments suggested the *dunce* gene encoded cAMP phosphodiesterase constrains the observable PKA dynamic to the vertical lobes. Strikingly, learning defective *dunce* mutant flies exhibited PKA activation across the entire MB when dopamine was applied to the brain [37]. These experiments are consistent with a model wherein cAMP levels and PKA activity are transient in β projections of $\alpha\beta$ neurons due to the rapid turnover of cAMP by Duncce phosphodiesterase whereas in the α projections cAMP levels accumulate and activate PKA. Experiments combining acetylcholine (ACh) with dopamine application, designed to mimic coincident MB neuron depolarization and reinforcement signaling, lead to a synergistic rise in cAMP and PKA activity [36,37]. Furthermore, stimulating cAMP synthesis was sufficient to enhance ACh-evoked Ca^{2+} influx [36]. Prolonged cAMP signals have long been considered a prerequisite for LTM formation [69] and therefore the imaging data revealing that a prolonged cAMP signal is facilitated in the LTM-relevant α lobe are entirely consistent with this model.

In addition to spatially and temporally regulating cAMP turnover, cells are known to organize PKA signaling. PKA is tethered to specific subcellular locations, at certain times and in close proximity to select substrates by assembling multiprotein complexes mediated by an interaction between the PKA regulatory subunits and a large number of A-kinase anchoring proteins

(AKAPs) [70]. AKAPs can also nucleate distinct collections of adenylate cyclases, phosphodiesterases, Epacs (cAMP stimulated guanine nucleotide exchange factors) and phosphatases as well as other kinases such as protein kinase C. The spatial and temporal complexity of intracellular PKA signaling is therefore likely to be extremely complex and dissecting it apart will be tricky. However, in addition to the live-imaging studies mentioned above, two other reports have indicated a role for PKA-anchoring in memory. Interfering with PKA binding to AKAPs by overexpressing a blocking peptide in MB neurons specifically disrupted an intermediate form of aversive memory [71] whereas flies that are mutant for the *Yu* encoded AKAP have defective aversive long-term memory [72]. Determining the distribution of the different AKAPs in MB neurons and understanding the consequences of their misregulation could prove highly informative.

It will be also be important to establish the organization and regulation of cAMP signaling that results from dopamine released by afferent DA neurons onto a specific MB zone. For example, will dopamine release onto the tip of the α lobe lead to cAMP synthesis that is precisely restricted to that zone and are downstream intracellular signaling complexes uniquely organized within that zone? Furthermore, the very different anatomy of the individual DA neurons in PPL1 and functional imaging data [22] suggests that these DA neurons receive discrete presynaptic input and may be differentially controlled. It will therefore be critical to investigate how DA neurons are regulated and whether particular groups of DA neurons are simultaneously controlled and if so, in what direction. In order for the DA neurons in the fly to code prediction error and switch from being driven more strongly by aversive stimuli than odorants in the naïve state to being preferentially driven by the conditioned odor in well-trained flies they must be efferent to both types of information. They are likely to be pre- and postsynaptic to Kenyon cells in the MB lobes because live-imaging showed that they respond when flies are exposed to odorant or shock [22,73].

What about appetitive memory in the fly?

We know less about the neural circuitry and reinforcement signaling of appetitive memory formation formed following pairing of odors with sugar. Appetitive memory is similarly impaired in mutant flies that disrupt the cAMP pathway and it is expected that the intersection between olfactory signals and positive reinforcement also occurs in the MB [10], although there is also an apparent role in appetitive learning for plasticity in projection neurons [74]. Furthermore, the same dissection of MB subfields seems to apply with $\alpha'\beta'$ neurons being required for consolidation and $\alpha\beta$ neurons for retrieval [33,44]. Current models propose that positive reinforcement is mediated by octopaminergic (OA) neurons [10,35,75] consistent with classic studies in the honeybee [76–78]. We do not know where the relevant OA neurons project to in the fly brain; innervation of the MB is notably scant, especially compared to that of DA neurons and is most evident in the calyx and heel region [61,79,80]. This positioning of input might be a critical element of spatial control, with innervation of the calyx perhaps modulating the incoming projection neurons and MB neuron dendrites [79].

Recent data suggest that the spatial organization of downstream OA signaling is remarkably broad. The only OA receptor studied so far, OAMB (octopamine receptor in mushroom body), appears to be all over the MB [42,81,82]. Furthermore, live-imaging experiments in which OA was bath applied to a brain *in vitro* or perfused on the exposed brain of a live fly suggest they are similarly coupled and regulated - cAMP levels were elevated and PKA activated all over the MBs [36,37]. In contrast to the application of dopamine, no branch specific PKA activation was evident after OA application. Furthermore, at the behavioral level genetic manipulations expected to disrupt PKA localization that impaired aversive memory, had no effect on appetitive memory [71]. Although it seems likely that appetitive memory will also be localized to specific synapses, so far investigating OA signals has not provided any resolution. However,

it could be that we are somewhat misled by focusing on OA alone in appetitive reinforcement. An important earlier study found a role for the dDA1 receptor in appetitive and aversive learning [83] (a result recently replicated in larvae [23]) challenging the straightforward DA versus OA dissociation between aversive and appetitive memory and suggesting a more complex involvement of DA. It is therefore entirely possible that DA is also involved in appetitive reinforcement (Figure 3).

Consistent with this, recent studies have implicated DA neurons in a different aspect of appetitive memory. A subset of DA neurons in the PPL1 cluster were found to be involved in a hierarchical inhibitory mechanism that controls appetitive memory performance [11]. Appetitive memory performance is acutely regulated by the relative satiety state of the fly and is most efficient in hungry flies [11,33]. The MB-MP neurons in the PPL1 cluster are required to suppress appetitive memory performance in satiated flies since blocking their output mimicked food-deprivation and released behavioral expression of appetitive memory [11]. Conversely, stimulating the MB-MP neurons in hungry flies with a hot or cold-sensitive trigger suppressed memory performance. MB-MP neurons project specifically to the heel and the $\alpha\beta$ neuron strata at the base of the peduncle [11,22,61] consistent with a role for these neurons in modulating the efficiency of memory retrieval through these sites.

As mentioned previously, activation of MB-MP neurons (Figure 2A, B) can substitute for reinforcement in aversive conditioning [25] but they are apparently not the PPL1 neurons that are critical to provide negative reinforcement value from electric shock. Blocking them during acquisition revealed that they are dispensable for aversive (and appetitive) memory formation [11]. Taken together with the extensive literature suggesting a localization of aversive memory to the vertical α lobes [22,48,62], it is likely that the other PPL1 neurons that innervate the MB vertical lobes will include those providing the negative reinforcing effects of electric shock [24]. It has been proposed that MB-MP neurons represent an inhibitory gate on MB function that is released in hungry flies to promote appetitive memory retrieval [11] and this model is compatible with the MB-MP neurons providing reinforcement and motivational control. It is perhaps worth noting that some mammalian midbrain DA neurons encode value-related reward signals [26] whereas others are activated by rewarding and aversive stimuli [38,84]. At least some of these DA neurons are inhibited by aversive cues [85], through neurons in the lateral habenula that have been proposed to code negative prediction error [86]. This organization suggests that the balance between the activity of the distinct midbrain DA neurons might code positive and negative value. Flies may have similar discrete populations of DA neurons that carry aversive value and others that carry appetitive value. If this is the case, aversive signals could excite one group and inhibit the other, and vice versa for appetitive signals, and the balance between the two systems would be critical to determine appetitive and aversive learning and memory-driven behavior (Figure 3). Such a balancing act might explain why dDA1 receptors are required for aversive and appetitive learning [83] because the fly is unable to encode the relative balance between aversive and appetitive signals. This antagonism between DA subsystems may in turn be regulated by hunger and satiety. In fact, it has been reported that sucrose produces mild aversive learning in fed flies and this switches to appetitive learning as the fly becomes more hungry [87]. The fly therefore seems to attribute different value to sucrose during learning [87] as well as to appetitive memory retrieval [11] depending on relative satiety state. Further detailed analysis of the MB-MP neurons and other individual types of DA neurons innervating the MB should resolve whether MB-MP neurons operate as part of a neural system that codes value and conveys motivational signals.

Dopaminergic zones and the control of other brain functions

The MB has been implicated in a large number of behaviors that do not involve olfactory stimuli and many of these also involve dopamine. These functions include sleep and arousal [17,88–

90], visual context generalization [91], salience-based choice behavior [19] and behavioral flexibility [92]. It therefore seems clear that the MB can no longer be simply viewed as a repository for olfactory memories and it might be better described as a brain center controlling action selection. The incredible diversity of DA neurons that project from a variety of places in the brain to the MB suggests that each of these functions might be served by a different MB zone and be regulated by a unique subset of DA neurons [22,61]. Mapping the input to each of these DA neurons and understanding when, where and how their released DA influences MB physiology is now one of the major challenges to be addressed (Box 1). We should soon be able to determine whether individual fly DA neurons operate like larger groups of DA neurons in mammals.

Outstanding Questions

- What is the high-resolution connectivity of dopaminergic (DA) neurons to mushroom body (MB) neurons? Do the DA processes synapse on MB neurons and/or other MB input and output neurons? Do particular MB neurons drive DA neurons? Do any of the DA neurons relay information from one MB neuron to others?
- What are the inputs to the different DA neurons?
- Are groups of DA neurons co-regulated? Does the activity of some increase while others decrease?
- What are the firing characteristics of DA neurons? Are some of them tonic and others phasic? If so, is there an interaction between tonic and phasic dopamine release?
- Do fly DA neurons code negative or positive prediction error? If not, what do the signals represent and where do the relevant neurons project on the MB?
- How does DA neuron activity alter the physiology of MB neurons?
- What DA receptors are involved and how are they coupled to downstream cell signaling pathways?
- Do the anatomically distinct DA neurons that innervate the MB perform different tasks? If so, does their anatomy also mark relevant MB output zones?

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Glossary

Action Selection	often referred to as choosing ‘what to do next’. The task of selecting between a number of behavioral actions to best achieve a goal.
Appetitive Memory	a memory whose expression involves approach behavior. In the fly experiments discussed here, this involves training flies by pairing one odorant with sucrose reward and another with nothing. When subsequently given the choice between the two odorants in a T-maze, the flies preferentially approach the previously sugar-paired odorant. Appetitive learning and memory performance is facilitated by hunger and suppressed by satiety and is therefore considered to be a goal-directed action.

Aversive Memory	a memory whose expression involves avoidance behavior. In the fly experiments discussed here, this involves training flies by pairing one odorant with electric shock and another with nothing. When subsequently given the choice between the two odorants in a T-maze, the flies preferentially avoid the previously shock-paired odorant. A new automated version of the assay also allows trial-and-error learning where single flies sample two odor streams and learn to avoid the odor stream triggering punishment.
Phasic Dopamine Release	transient release of dopamine evoked by neural activity in dopaminergic neurons.
Prediction Error	the difference between the value of the actual reward (or punishment) experienced and the expected reward (or punishment).
Reinforcement	depending on the context, reinforcement is either a rewarding or aversive stimulus or the neural representation of the reinforcing stimulus.
Reinforcement Learning	how artificial or neural systems learn by trial-and-error to maximize rewards and minimize punishments.
Temporal Difference (TD) Algorithm	a computational solution to calculating and updating a prediction error involving an ongoing comparison of present and predicted state/situation.
Tonic Dopamine Release	continuous and sustained 'background' dopamine release.

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Figure 1. Model of the fly brain detailing the position and anatomy of the mushroom bodies

Olfactory information enters the brain through axons of olfactory sensory neurons that synapse in the antennal lobes. Transformed information is then carried on the axons of projection neurons (blue) to the MB neuron dendrites in the calyx and to poorly characterized neurons in the lateral horn. The approximately 2,500 Kenyon cells in each MB are roughly subdivided into three morphologically distinct groups based on the bundling of their projections in the region of the MB called the lobes. Each Kenyon cell that contributes to the $\alpha\beta$ subdivision bifurcates and sends one axon branch vertically to the α lobe and one horizontally to the β lobe. Similarly, each neuron in the $\alpha'\beta'$ lobe bifurcates and sends one axon branch to the α' lobe and one to the β' lobe. The γ neurons send a single unbranched axon horizontally in the γ lobe. All three lobes are shown in the right hand MB whereas the γ lobe (magenta) has been removed in the left hand MB to reveal the position of the $\alpha'\beta'$ (green) and $\alpha\beta$ (red) lobes. Scale bar 100 μ m. Image constructed by Wolf Huetteroth from confocal stacks collected by Shamik DasGupta.

Figure 2. Zones of the mushroom body innervated by dopaminergic neurons

A. Projection view of a subset of dopaminergic neurons in the Protocerebral Posterior Lateral (PPL) 1 cluster. A brain from a transgenic fly expressing a red fluorescent protein in MB neurons and a green fluorescent protein in MB-MP neurons that innervate the MB-heel and peduncle region (shown as green in panel B) and also the neurons shown as magenta in panel B that seem to project around the MB. Image adapted, with permission, from Ref. [11]. Scale bar 20 μ m.

B. The PPL1 cluster. The Butcher's cut illustration summarizes data from Refs. [11,22,25]. The image shows one neuron projecting to the tip of the α lobe (red), one to the tip of the α' tip (orange), one to the upper stalk of the vertical lobes (MB-V1 neuron, yellow), one to the

lower stalk and junction region (MB-MV1 neuron, blue) and one to the heel and distal peduncle (MB-MP neuron, green). In addition, PPL1 includes a neuron that innervates the anterior superior and inferior medial protocerebrum (magenta), that runs along a similar path to the MB-MP neurons but does not appear to innervate the MB, and another neuron that innervates the region behind the α lobe and sends another branch into the central complex (grey cell body, projection not shown for clarity). All neurons shown have a projection to a similar zone on the contralateral MB (dotted lines with arrowhead). The cell body locations are not stereotyped. Although the number of neurons in each class has not been exhaustively determined, there appear to be at least two MB-MP neurons per PPL1 cluster [11]. There are also likely to be multiple neurons in some of the other classes because 4 of the 12 neurons in PPL1 remain to be accounted for. **C. The PPL2ab and Protocerebral Anterior Medial (PAM) clusters.** The illustration summarizes data described in Refs. [22,25]. At least two neurons from the PPL2ab cluster innervate the ipsilateral MB calyx (brown and blue). Tyrosine hydroxylase staining suggests that DA neurons project to discrete zones of the horizontal β , β' and γ MB lobes (marked with black dotted lines for β and β' and orange for γ) but the only individual PAM neurons described so far ramify on the tip of the β lobe (MB-M3 neurons, cyan) and a similar zone on the contralateral MB (dotted line with arrowhead) or further along the β lobe (MB-MVP1 neurons, purple).

A Aversive reinforcement – DA neurons engaged?

B OA neuron innervation of the MB

C Appetitive reinforcement – DA neurons relieved by OA?

Figure 3. Hypothetical models for the involvement of dopaminergic (DA) neurons in aversive and appetitive reinforcement

A. DA neurons representing aversive reinforcement. Studies from Refs. [24,25] suggest that neurons in PPL1 convey negative reinforcement whereas live-imaging data from Ref. [22] indicates that DA neurons innervating the lower stalk, junction and α tip regions are most strongly activated by shock. In this illustration, a selection of DA neurons innervating these specific MB zones, and the MB-M3 neurons on the tip of the β lobe [25], are activated (green). **B. Octopaminergic (OA) neuron innervation of the mushroom body (MB).** Single cell analysis has determined that OA-Ventral Unpaired Medial (VUM)a2 neurons innervate the MB calyx whereas OA-Ventral Paired Medial (VPM) 4 neurons innervate the MB heel [23]. Should

the OA neurons that innervate the MB be those that are important for appetitive reinforcement in *Drosophila*, they are appropriately placed to influence the activity of the DA neurons in the calyx and in the MB heel (the MB-MP neurons). **C. DA neurons that could be relieved by appetitive reinforcement.** OA neuron activation can substitute for sugar presentation in appetitive conditioning in *Drosophila* larvae [75] and in the honeybee [76]. In this model, OA neuron action would relieve the DA neurons in the heel (MB-MP neurons) and those in the calyx that can convey negative signals (now red rather than green in panel A). Hunger inhibits the MB-MP DA neurons to promote appetitive memory retrieval [11]. Therefore, learning could involve a similar MB-MP relief mechanism that relies on OA.