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Neuronal mechanisms underlying innate and learned olfactory processing in *Drosophila*

Running title: *Drosophila* olfactory processing

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Abstract

Olfaction allows animals to adapt their behavior in response to different chemical cues in their environment. How does the brain efficiently discriminate different odors to drive appropriate behavior, and how does it flexibly assign value to odors to adjust behavior according to experience? This review traces neuronal mechanisms underlying these processes in adult *Drosophila melanogaster* from olfactory receptors to higher brain centers. We highlight neural circuit principles like lateral inhibition, segregation and integration of olfactory channels, temporal accumulation of sensory evidence, and compartmentalized synaptic plasticity underlying associative memory.

Introduction

How does the brain translate olfactory input into appropriate innate and learned behavior, such as approach, avoidance, feeding, mating, and reproduction? Recent advances into olfactory discrimination have been enabled by new neurogenetic tools in *Drosophila*, especially highly specific driver lines for specific neurons in the fly brain [1,2]. Because the fly brain has only ~100,000 neurons, many of which are reproducibly identifiable across individuals, genetic access to specific neurons leverages modern neuroscience techniques (optogenetic/thermogenetic manipulation of neural activity; calcium imaging; targeted patch-clamp recordings; connectome reconstruction from electron microscopy volumes) to achieve unprecedented cellular resolution in defining neural mechanisms underlying sensory processing.

Early neural mechanisms enhancing separation of odor responses

Differential behavior for different odors requires differential neural activity, and many anatomical and physiological features of the first two layers of the fly olfactory system are best understood as mechanisms for producing reliably different patterns of neural activity for different odors.

Olfactory discrimination begins with the fly's repertoire of ~60 olfactory receptors, each of which binds to a unique profile of odorant molecules [3]**.** Olfactory receptor neurons (ORNs) typically each express a single olfactory receptor [4-6], allowing distinctive receptor binding for different odors to translate into distinctive ORN activity. ORNs synapse onto second-order projection neurons (PNs) in the antennal lobe in structures called glomeruli, in a roughly one-to-one manner (**Figure 1**). 10-65 ORNs expressing the same receptor converge on a single glomerulus, providing input to 1-8 PNs; likewise, each PN receives input from a single glomerulus [7]. ORN-PN convergence reduces noise (PN activity is less variable than ORN activity) because each PN can average the activity of many ORNs [8]. Reducing noise aids reliable olfactory discrimination, which requires that different odors elicit not just different neural activity, but *reproducibly* different activity across multiple odor encounters.

As in other species [9], odor discrimination in *Drosophila* is enhanced by lateral inhibition. In the fly olfactory system, lateral inhibition begins even before the first synapse: most ORNs are co-housed in the same sensillum with one or more other ORNs expressing a different olfactory receptor [5]. Co-sensillar ORNs inhibit each other through non-synaptic interactions (probably ephaptic coupling) [10], which enhances differences in neural activity for different odors. Ephaptic lateral inhibition might occur faster than synaptic inhibition and therefore work better in turbulent air with rapidly changing odor concentrations [5].

Lateral inhibition also acts at the first synapse in the olfactory system, between ORNs and projection neurons (PNs), where local interneurons inhibit release from ORN presynaptic terminals [11-13]. This 'input gain control' normalizes responses by total ORN activity, decreases correlations between odor response profiles of different PNs [12], and improves odor discrimination by a perceptron modelling neurons post-synaptic to PNs [14]. Interestingly, some glomeruli are more sensitive to this gain control than others, potentially allowing some glomerular channels to be concentration-sensitive and others to be concentration-insensitive [15]. At the next synapse, between PNs and neurons in the lateral horn (LH), lateral inhibition by inhibitory projection neurons enhances naive discrimination of

similar odors by increasing distances in 'neural activity space' between PNs' population responses to different odors [16,17].

Integrating olfactory channels in the lateral horn

PNs project to two higher-order brain centres, the mushroom body and the lateral horn, which integrate odor-specific patterns of PN activity to produce appropriate behavior (**Figure 1**). Traditionally, the mushroom body and lateral horn have been seen as regulating learned and innate behavior, respectively. This division has been complicated recently by findings that the mushroom body regulates some innate behaviors [18,19] and drives learned behavior in part via the lateral horn [20]; it may be more useful to think of the mushroom body as regulating 'flexible' or 'context-dependent' behaviors [21]. Regardless, in this section we discuss 'innate' olfactory processing by the lateral horn; we address mushroom body function in the next section.

Do some glomeruli 'encode attraction' while others 'encode repulsion'? Such dedicated channels, often called 'labeled lines', could wire specific odors directly to neurons in the lateral horn that drive approach or avoidance motor outputs. Indeed, some glomeruli do have clear behavioral functions, especially when their olfactory receptor is extremely selective for an ethologically relevant odor. For example, the male-specific pheromone 11-cis-vaccenyl acetate (cVA) is detected by Or67d-expressing ORNs, which project to glomerulus DA1 [22,23]. DA1 PNs form stereotyped synapses in the lateral horn onto the neuron aSP-f in males, and aSP-g in females [24-26]. Presumably, males and females react differently to cVA because aSP-f and aSP-g elicit different downstream behaviors. Other odors activating dedicated channels include repulsive odors from harmful microbes [27] or parasitoid wasps [28]. Less specialized glomeruli may also have clear behavioral roles. For example, Or19a-expressing ORNs respond preferentially to a group of odorants found in citrus fruits, a favored egg-laying site for *Drosophila melanogaster*. This pro-citrus egg-laying preference requires Or19a-expressing ORNs, and artificially activating Or19a-expressing ORNs promotes egg-laying [29]. Taking a broader view, correlating behavior with population activity of ORNs or PNs across many odors reveals that some glomeruli respond primarily to aversive (e.g. DL5/Or7a) or attractive (e.g., DM4/Or59b) odors [30-32], although most glomeruli do not show such a clear division. Interestingly, narrowly turned glomeruli have more PNs [7], raising the possibility that ethologically important odors are processed differently or over-represented in higher brain areas.

How is activity of different olfactory channels integrated to produce behavioral outputs? Optogenetic activation of one or two classes of ORNs revealed that behavioral effects of single glomerular channels sum, either linearly or sub-linearly depending on which two glomeruli are being combined [33]. The importance of integrating multiple channels of a combinatorial code is reinforced by findings that removing small numbers of PNs from the population generally affects behavior moderately rather than all-or-none, whether through experimental manipulations affecting behavior [16] or through removing PNs from a regression model affecting its prediction accuracy [32].

How does this integration occur at the circuit level? Some inter-channel integration occurs in the antennal lobe. For example, excitatory local neurons in the antennal lobe provide lateral excitation between glomerular channels via electrical synapses [34-37], allowing pheromone responses of DA1 PNs to be enhanced by food odors [38]. Conversely, with mixtures of odors of opposing valence, repellent-responsive glomeruli inhibit attractant-responsive glomeruli via inhibitory interneurons in the antennal lobe [39]. Further integration occurs in the lateral horn, where PNs' projections to particular zones [40-42] and synaptic connections to particular neurons [43-45] are stereotyped (unlike in the PNs' connections to the mushroom body; see below).

These stereotyped connections suggest that glomerular channels may be combined according to behaviorally-relevant categories. Large-scale mapping of PN inputs to identified types of LH neurons revealed that some pairs of glomeruli converge on common LH neurons more often than would be expected by chance. Some of these pairs respond to the same or similar odorants (e.g, DA1 and DL3 both respond to cVA), but other pairs respond to dissimilar odorants that have in common only that they should elicit similar behavioral responses, such as dissimilar odorants that are both found in food sources or both promote social behaviors [44]. Supporting the idea that the LH processes odor categories rather than odor identity, LH neurons respond more broadly to odors than their PN inputs do, but LH odor responses categorize odors by chemical class (amines, esters, etc.) more accurately than PN responses do [45]. Consistent with this, glomerular channels whose activation prevents egg-laying (presumably because their naturally activating odorants signal toxicity or predation) converge on the same ventral-posterior zone of the lateral horn, possibly a zone specialized for negative control of egg-laying behavior [46]. Indeed, EM reconstructions show that DA2 PNs (responding to the toxic mold odorant geosmin) and DL2 PNs (responding to parasitic wasp odors) synapse onto common lateral horn neurons [47]. Optogenetic activation of these LH neurons drives avoidance behavior, while blocking them prevents geosmin-evoked inhibition of egg-laying. Conversely, activating other LH neurons

drives approach behavior [2]. Together, these results suggest that hard-wired connectivity between PNs and LH neurons determines innate odor preferences.

Kenyon cells encode odor identity for learned olfactory discrimination

Beyond innate olfactory discrimination, flies can also *learn* to discriminate between odors: if they experience a specific odor paired with reward (e.g., sugar) or punishment (e.g., electric shock), they learn the association and thereafter approach/avoid the trained odor, but not untrained odors [48]. Such classical conditioning requires flies to (1) hold unique representations of arbitrary odors, (2) assign valence (reward/punishment) to odors, and (3) generate the appropriate approach or avoidance behaviour. We first discuss odor representation.

Theoretical work suggests that learned stimulus discrimination should be aided by encoding stimuli sparsely (i.e., where only a few neurons in a population respond to each stimulus), which should reduce overlap between stimulus representations [49,50]. Such sparse coding occurs in Kenyon cells (KCs), the principal neurons of the mushroom body (MB). While PN inputs to KCs respond broadly to odors [8], only 5-10% of KCs respond to each odor [51]. Notably, the KCs' sparse coding scheme still allows flies to generalize learned associations to similar odors (which could be viewed as noisy variations of the same odor) [52-54], a property characteristic of a computer algorithm called locality-sensitive hashing, which resembles KC sparse coding [55].

Sparseness is maintained in part by feedback inhibition from the GABAergic APL neuron; blocking APL output broadens KC population odor responses and increases overlap between odor representations, thereby impairing learned discrimination of similar odors [56]. Sparseness is further aided by the fact that KCs require multiple simultaneous inputs from different glomeruli to generate spikes [57]. This connectivity is not stereotyped [58,59], but is also not purely stochastic, as PNs with similar odor tuning profiles, and from the same glomeruli, tend to converge on the same KCs [57,60]. This lack of stereotypy contrasts with the LH and allows the MB to complement the LH's innate responses with learned responses to arbitrary odors.

KCs' integration of dendritic inputs mirrors the fly's integration of sensory inputs, supporting the so-called 'drift-diffusion' model of sensory decision making. The drift-diffusion model posits that neurons accumulate sensory evidence until reaching a threshold that triggers a decision, in order to explain the characteristic reaction times of animals (including flies)

choosing between two sensory stimuli: fast for clearly contrasting stimuli where evidence accumulates quickly, slow for noisy or ambiguous stimuli where evidence accumulates slowly [61]. This phenomenon has been elegantly demonstrated in a subset of KCs called $\alpha\beta_c$ KCs, which accumulate information over time through integration of subthreshold synaptic inputs. The more ambiguous the stimulus, the longer $\alpha\beta_c$ KCs accumulate evidence before reaching the firing threshold. A mutation that causes abnormally slow reaction times reduces the intrinsic excitability of $\alpha\beta_c$ KCs, thereby making them depolarize abnormally slowly toward firing threshold. Indeed, the latency between stimulus onset and the first spike in this KC subset accurately predicts reaction times [62]. Through their sparse representations and evidence accumulation of olfactory sensory input, KCs lay the foundation for olfactory discrimination and subsequent learning.

The mushroom body integrates stimulus identity and valence to allow learned olfactory discrimination

How does the fly brain assign valence to these sparse stimulus representations, and how is stimulus identity and valence read out to guide experience-based decision-making? Current evidence suggests that stimulus valence is assigned by 'teaching signals' from MB extrinsic dopaminergic neurons (DANs) that encode punishment or reward. Many DANs respond specifically to punishment (electric shock) or reward (sugar) [63], and blocking DANs prevents memory formation [64-66], while artificial activation of DANs alone during odor stimulus is sufficient to induce memory formation [67-73] (see **Table 1**). The current working hypothesis in the field is that memories are read out by MB output neurons (MBONs), which are activated by KCs and bias the fly towards approach or avoidance [74-76]**.**

The three elements of the mushroom body – stimulus identity, teaching signals, and readout – are coupled together by an ingenious compartmentalized architecture. KC axons running in parallel make up the MB lobes, which are divided into 15 compartments, each of which is typically innervated by one type of DAN and one type of MBON paired according to opposite valence (i.e., reward DAN + avoidance MBON; punishment DAN + approach MBON) [1,77] **(Figure 2**). DANs depress KC-MBON synapses specifically in the same compartment. For example, when odor X coincides with punishment to create an aversive memory, punishment-responsive DANs depress synapses from odor-X-responsive KCs onto 'approach' MBONs but not 'avoidance' MBONs, thus making the fly avoid odor X in the future. Conversely, 'reward' DANs depress KC synapses onto 'avoidance' MBONs to create appetitive memories [53,63,78-80].

In other words, the current working hypothesis in the field posits the following: Olfactory learning in *Drosophila* works by suppressing the 'wrong' action rather than promoting the 'correct' action. This is possible because behavior is driven by the *balance* of competing 'approach' and 'avoidance' MBONs. MBONs that are depressed during learning are required for memory retrieval, at least for 'forced-choice' learning tasks where flies choose between the trained odor and an untrained odor, because it is the difference in MBON activity between the two odors that drives choice. Memories are odor-specific because learning depresses the output synapses of only KCs that responded to the trained odor, not KCs that respond to other odors.

Why are there so many compartments, rather than only 2 (reward/avoidance vs. punishment/approach)? The different compartments store memories that form and decay at different speeds and are differentially sensitive to being overwritten by new information [53,66,70,71]. This diversity in 'learning rules' could store multiple memory traces in parallel depending on the intensity and reliability of odor-valence pairing. Indeed, different 'reward' DANs are differentially required for learning to associate odors with different kinds of rewards (e.g., water vs. sweet taste vs. caloric value of food) that entrain memories of different stability [64-66,68]. In addition, MBONs do not only drive approach and avoidance. One MBON drives 'alerting' behavior and responds only to novel odors because its corresponding DAN depresses its responses to familiar odors [81]. Another MBON reduces flight bout durations, and its activity is reduced by a DAN that prolongs flight [82]. Similarly, MBON signaling is modulated not only by 'reward' and 'punishment', but also by internal states like arousal and hunger. DAN activity correlates with behavioral state even in the absence of external reward/punishment [63], and various DAN/MBON compartments regulate approach toward food odors depending on hunger state [19], or avoidance of carbon dioxide depending on presence of food odors [18]. These findings suggest that the diversity of MBON/DAN compartments allows multidimensional, flexible regulation of behavior.

A few additional features are worth noting. First, compartments communicate with each other. For example, aversive training can increase responses of 'avoidance' MBONs to the trained odor because it suppresses feedfoward inhibition from an 'approach' MBON [76,80]. Such inter-compartment communication may explain why some MBONs are required for memories of the 'wrong' valence (e.g., MBONs required for appetitive memory even though their matching DANs implant aversive memory [71,75,83,84], or vice versa [74]). Second, odor-valence associations are order-sensitive: odor+punishment only causes learned avoidance if punishment (or artificial activation of punishment-encoding DANs) follows the

odor within a few seconds. If punishment *precedes* odor, flies learn to *approach* the trained odor [71,85]. Indeed, DAN activation without odor potentiates KC-MBON synapses rather than depressing them [63,78]. Third, different subpopulations of KCs can be differentially required for aversive vs. appetitive memory, perhaps through different connectivity with DANs or MBONs [86,87]. Fourth, despite the basic picture outlined above, a single compartment can be innervated by multiple types of DANs signaling opposite valence (e.g., the β2 and β′2 compartments [64-66,69,71]).

Finally, how do MBONs guide motor output? One output path goes through the LH. MBON-α2sc is an 'approach' MBON required for aversive memory retrieval, whose response to punished odors is reduced after aversive training [53,79]. MBON-α2sc activates LH neurons called PD2a1 and PD2b1, which also are required for aversive memory retrieval and show reduced responses to punished odors after aversive training. PD2a1/b1 also receive input from PNs and other LH neurons and are required for innate attraction to some odors, suggesting that they integrate both learned and innate pathways to drive behaviour [20] (**Figure 1**).

Post-learning mechanisms involve recurrent activity and cross-compartmental signaling

Previously learned olfactory discriminations must be re-evaluated in changing conditions. What happens to memories after they are formed? Short-term memories can be consolidated into protein synthesis-dependent long-term memory (LTM), which can last >24 hours, depending on the nature of the training protocol (e.g., odor+food LTM arises from a single pairing, whereas odor+shock LTM requires multiple spaced training trials) [88-90]. The need for multiple spaced shock trials for aversive LTM may represent a requirement for more robust or persistent reinforcement, but it is unclear what naturalistic reinforcement this artificial protocol resembles. This consolidation to LTM requires recurrent signaling between DANs and MBONs, for example between the DAN and MBON of the α1 compartment [91]. In addition, LTM consolidation requires oscillatory activity in a DAN called MP1 or PPL1-γ1ped for ~30-60 min after training, but no longer [92]. This oscillating activity is shut off after 60 min by the MBON in the same compartment (γ1), called MVP2 or MBON-γ1ped>α/β [93]. The crucial LTM-gating oscillating activity in MP1 depends on activity from a pair of serotonergic projection neurons, whose activity in turn is stimulated by 'spaced' aversive training that induces LTM training, but not single aversive training sessions that don't induce LTM [94].

Memories that are acquired but not consolidated rapidly decay, discarding irrelevant information in a brain with limited resources [95]. Remarkably, forgetting, like learning, is triggered by DAN activity [78,96], suggesting that the same dopamine signal can trigger opposing parallel processes (forming and erasing memories), most likely through parallel biochemical pathways in KCs. In learning, dopamine receptor Dop1R1 (aka *dumb*) activates the G protein Gs, triggering cAMP signaling via a Ca^{2+}-dependent adenylyl cyclase, *rutabaga*, that detects the coincidence of odor (Ca^{2+} influx in the KC) and dopamine [97,98]. In forgetting, another receptor, Dop1R2 (aka *damb*), activates Rac1 and Scribbled via a different G protein, Gq [98-100]. These pathways likely cause opposing changes to KC-MBON synapses, although this remains to be demonstrated.

What happens when re-exposure to a previously conditioned odor leads to an unexpected outcome? When a fly smells a previously punished odor, now without punishment, the now-obsolete aversive memory undergoes 'extinction'. Yet the aversive memory trace (depression of 'approach' KC-MBON synapses) is not erased. Rather, the unexpected lack of punishment acts as a 'reward', creating a competing appetitive memory trace (depression of 'avoidance' KC-MBON synapses) that behaviorally cancels out the original aversive memory [80]. Similarly, appetitive memory extinction requires signaling by 'punishment' DANs [83]. When a fly instead is re-exposed to partial features of a previous conditioning trial (including the odor not paired with shock or sugar), the memory becomes labile, and can either be reconsolidated or erased, via MBONs signaling to DANs [83].

Future directions

Several questions remain under-explored. Certain fly behaviors suggest active sensing: Flies' flapping wings draw odors toward the antennae during flight [101] and flies move their antennae actively during flight [102]. Do flies use active sensing to enhance olfaction (akin to sniffing in mammals)? In addition, recent work has started to reveal how flies integrate olfactory input with wind direction to drive locomotion toward/away from odor sources [33,103-106], but we know little about the neural circuits between the MB/LH and motor outputs, especially about how they translate odor identity into behaviors more complex than attraction and repulsion (e.g., feeding, mating and egg-laying) [47]. Future work will shed light on these and other questions using new tools like whole brain connectomes [60,107,108] and recording from brains of freely behaving flies [109].

Conflict of interest statement

Nothing declared.

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References of special (*) or outstanding (**) interest

*[2] Dolan et al. describe the anatomy of the lateral horn and the behavioral effects of many of its neurons, by creating a library of split-Gal4 lines for the lateral horn analogous to Aso et al.'s library of mushroom body split-Gal4 lines [1]. Together with ref. [45], this work opens the lateral horn to detailed functional dissection.

**[20] Dolan et al. show that olfactory memory retrieval requires communication from the mushroom body to the lateral horn. An MBON required for aversive memory retrieval drives lateral horn neurons called PD2a1 and PD2b1, which are also required for aversive memory retrieval.

**[44,45] Jeanne et al. and Frechter et al. describe the anatomy and functional properties of LH neurons in unprecedented detail. They show that connectivity between PNs and LH neurons is stereotyped, and that LH neurons integrate olfactory channels to identify odors not by individual odorant but by behaviorally relevant category (e.g., food, pheromone, toxin, etc.).

*[46,47] Chin et al. and Huoviala et al. show that olfactory channels that drive flies to avoid laying eggs converge on a similar areas of the lateral horn, even on the same identified neurons, suggesting that there may be dedicated neurons or anatomical regions in the lateral horn for specific odor-driven behaviors.

**[62] Groschner et al. show that the abnormally slow reaction times of *FoxP* mutants are caused by extra K^+ leak channel expression in $\alpha\beta_c$ KCs that reduce their excitability and therefore slow down their odor-evoked depolarization to spike threshold. This suggests that evidence accumulation may occur through dendritic integration of synaptic inputs.

*[53,63] Hige et al. and Cohn et al. provided key evidence that associative learning in the mushroom body occurs through DANs locally depressing KC-MBON synapses in the same compartment.

*[71] Aso and Rubin show that optogenetic activation of DANs innervating different compartments of the mushroom body can implant artificial memories with different characteristics: different speeds for learning and forgetting and different sensitivities to updating for new information. These parallel memory traces could explain why there are so many MB compartments, and how different aspects of memory could be stored in parallel.

*[80] Felsenberg et al. show that when later experience shows a previously formed memory to be obsolete, the fly brain does not necessarily erase the previously formed memory trace but rather forms an additional opposing memory trace that cancels out the first one.

*[94] Scheunemann et al. show that the classical memory gene *dunce* also gates consolidation of long-term memories (LTM). Beyond its known role in learning, *dunce*'s phosphodiesterase activity inhibits activity of serotonergic projection neurons, thereby preventing the oscillatory activity in dopaminergic MP1 neurons required for LTM consolidation. Spaced training sessions that induce LTM inhibit *dunce* activity, thereby removing this gate and allowing LTM to be consolidated.

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Figure legends

Figure 1. Overview of *Drosophila* olfactory system.

Olfactory receptor neurons (ORNs) expressing the same receptor (different receptors indicated here by red, green, blue colors) converge on the same glomerulus in the antennal lobe, where they synapse on projection neurons (PNs). The ORN-PN synapse and the interneurons of the antennal lobe 'pre-process' the olfactory signals that PNs carry in parallel to their two targets in the central brain, the mushroom body (MB) and lateral horn (LH). The MB implements flexible behaviors: Kenyon cells (KCs) carry unique, sparse odor representations and their outputs to mushroom body output neurons (MBONs) can be modified by dopaminergic neurons (DANs), allowing experience or internal state to modify behavioral responses to specific odors (see **Figure 2**). The LH implements innate behaviors: LH neurons integrate PN activity through stereotyped connectivity to encode behaviorally relevant categories of odors (e.g., food, pheromones, toxins). These and other circuit principles from the main text are summarized in the small text on the figure.

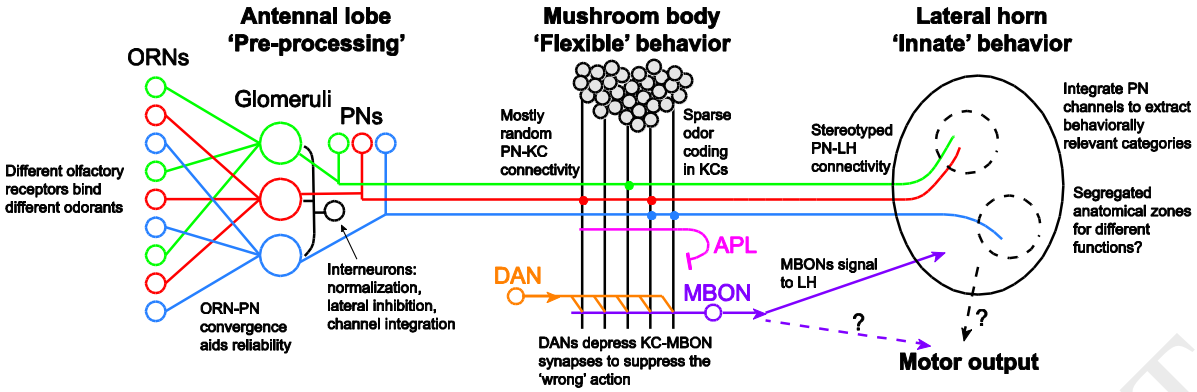

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Figure 2. Architecture of the mushroom body.

(A) Schematic of the compartmentalized architecture of the mushroom body (MB). KCs (gray) carry sensory identity information to the MB lobes, where they form local synapses with pairs of DANs and MBONs. When an odor is paired with reward, DANs activated by reward weaken KCs' excitatory drive to avoidance-MBONs, thereby biasing the fly's response to the trained odor toward approach. The converse happens when an odor is paired with punishment: punishment DANs weaken KC excitation of approach-MBONs, so the fly later avoids the trained odor.

(B) Schematic of MB anatomy, showing an example of a γ-KC (which receives input from PNs in the calyx and sends an axon into the lobes) and the γ1 compartment innervated by PPL1-γ1pedc (a 'punishment' DAN, also known as MP1) and MBON-γ1pedc>α/β (an 'approach' MBON, also known as MVP2). Anatomical axes: D, dorsal, P, posterior, M, medial.

(C) Schematic of the MB lobes divided into the three KC subsets (αβ, α′β′, and γ), and further segregated into compartments according to innervation by DANs signaling

punishment (red), reward (green), or familiarity (blue), or regulating flight (white). The 'familiarity' DAN in α′3 suppresses the novelty-encoding MBON-α′3. Red/green hatching on β2 and β′2 indicates multiple DANs that each signal reward or punishment, not a single DAN that signals both. References: see **Table 1**.

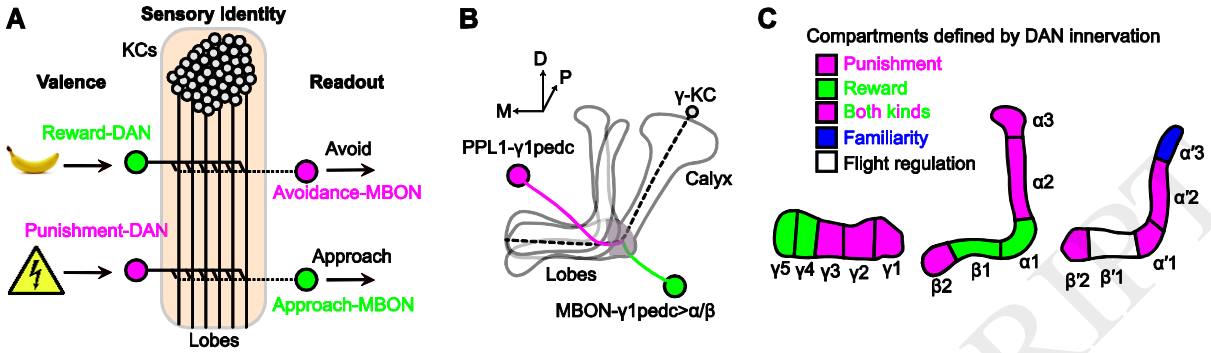

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Table 1. References for different types of evidence for the function of DAN/MBON compartments in **Figure 2C**. Notes: 1. Some DANs/MBONs innervate more than one compartment and some compartments are innervated by more than one type of DAN or MBON. 2. In some cases, the evidence comes from driver lines expressed in more than one type of DAN or MBON. Sometimes this is because DANs or MBONs have combinatorial effects such that activating two neurons might be sufficient to drive learning/behavior, while activating one alone is not; in other cases, it is because sufficiently specific drivers did not exist at the time of the study. 3. With the exception of [63], most studies have not directly tested if these DANs/MBONs function *selectively* in memory of only one type (e.g., responding to reward vs. punishment or necessary/sufficient for appetitive vs. aversive memory). 4. It is unclear how the β′1 compartment fits in the associative memory paradigm presented in this review but we include it here for completeness.

Compartment	Dopaminergic neuron (DAN)			Mushroom body output neuron (MBON)		
	Responds to 'training' feature (e.g., reward)	Artificial activation drives learning / plasticity	Required for learning / plasticity	Activity depressed by learning / matching DAN	Artificial activation drives behavior	Required for memory retrieval
γ1	[110,111]	[53,70,71]		[53,76,80]	[75]	[75,76]
γ2	[63,78,110,111]	[71,78]		[78]	[75]	[75,78,83,87]
γ3	[63]				[75]	
γ4	[63,64]		[64,65]		[75]	[75]
γ5	[63,64]	[71]	[64,65]		[74]	[74,75,87]
α1		[65,66,71]	[66]			[75,91]
α2		[53,71]		[53,79]		[79]
α3		[71]				[75]
β1		[65,71]				
β2		[65,69,71]		[74]	[74]	[74]
α′1	[63,78]	[71]		[78]	[75]	[75,78,83,87]
α′2		[71]				[75]
α′3	[81]	[81]	[81]	[81]	[81]	[81]
β′1			[82]	[82]	[82]	
β′2	[64]	[65,69,71]		[74]	[74]	[74,75,87]