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Mapping Odor to Action: (Dopaminergic) Timing Is Everything

Kristina V. Dylla[1] and Elizabeth J. Hong[1,*]
[1]Division of Biology & Biological Engineering, California Institute of Technology, Pasadena, CA, USA
*Correspondence: ejhong@caltech.edu
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Animal brains use the relative timing between sensory cues and behaviorally salient events to form predictive associations about their environment. Handler and colleagues provide new mechanistic insights into how differential signaling downstream of dopamine receptors couples this timing to the dynamic reweighting of synapses that link sensation to action.

The smell of fresh-baked cookies wafting from the oven is a reasonable predictor that a tasty treat may soon be on offer. As an animal navigates the environment, its brain constantly uses the relative timing between its encounters with sensory cues and important experiences to infer useful causal relationships among them. In a busy world full of potentially relevant cues, behaviorally significant events are marked by potent neuromodulatory signals in the brain, dopamine being chief among them. In this issue of *Cell*, Ruta and colleagues study olfactory learning in the mushroom body (MB) of *Drosophila melanogaster* to provide new mechanistic insights into how the timing of dopaminergic signals with respect to sensory cues shapes how stimuli are coupled to action (Handler et al., 2019). The MB circuit can be roughly understood to map a high-dimensional odor representation, encoded by the sparse population activity of its ~2,000 principal Kenyon cells (KCs), onto a low-dimensional representation of behavioral meaning, encoded by the ensemble activity of 21 mushroom body output neurons (MBONs) (Owald and Waddell, 2015). KC-MBON synapses are anatomically and functionally compartmentalized along the length of bundled KC axons (Figure 1B). Each MBON subtype typically pools inputs from 1 to 2 compartments and projects to distinct downstream targets, which couple to different actions. The axon terminals of each dopaminergic neuron (DAN) subtype, which signal positive or negative outcomes, are also typically restricted to just 1–2 compartments. This highly organized architecture allows each DAN subtype to selectively adjust the strength of the KC-MBON synapses in its target compartment(s) (Aso and Rubin, 2016) and thereby specify a remapping of the KC-MBON transformation (Hige et al., 2015; Owald and Waddell, 2015).

Whereas the neuromodulatory signals underlying olfactory learning have been intensely investigated, the timing effects of dopamine are less well understood. This study makes a major advance by developing a naturalistic behavioral assay that allows sensitive, trial-by-trial readouts of the fly's odor preference after precisely timed reinforcements. Flies were placed in a laminar airstream, and odor attraction was quantified by an increase in upwind walking in response to a pulse of odor. The unambiguous timing of reinforcement relative to the odor cue was achieved using a fictive reinforcer, the optogenetic activation of large subsets of DANs normally activated by either rewards or punishments. Interleaving individual training and testing trials, the authors observed that attraction to odor was bidirectionally modulated by exposing the animals to forward or backward conditioning (Figure 1A). Notably, the preference of the flies could be continuously updated and reversed on a trial-by-trial basis by simply reordering the odor cue and reinforcement. In a second remarkable advance, the authors developed a closed-loop virtual olfactory navigation system that allowed them to image MBON activity in a head-fixed fly walking on a spherical treadmill, while simultaneously making real-time measurements of odor tracking to evaluate conditioning. This experiment allowed them to directly correlate, on a trial-by-trial basis, bidirectional changes in MBON postsynaptic activity with bidirectional changes in odor preference. The dependence of behavioral and neural plasticity on the timing of reinforcement (relative to odor) were remarkably similar, both ranging over a timescale of several seconds.

The authors next investigate how the timing of dopaminergic reinforcement couples to KC-MBON synaptic strength. Physiological and genetic evidence strongly suggest that the locus of KC-MBON plasticity is presynaptic (Hige et al., 2015); thus, dopaminergic signals likely impact neurotransmitter release from KCs. Finding that DAN activity triggered by forward or backward conditioning was indistinguishable, the authors focused on signaling downstream of dopamine receptor activation. Two dopamine receptors, DopR1 and DopR2, were previously shown to have opposing roles in memory formation (Berry et al., 2012; Kim et al., 2007); each couples to a different G-protein signaling pathway that generates distinct second messengers. *In vivo* imaging of KC axons expressing optical reporters selective for these second messengers revealed that cyclic AMP (cAMP) downstream of $G\alpha_s$-coupled DopR1 required the arrival of cue and reinforcement close in time but was insensitive to their temporal ordering. However, inositol triphosphate (IP3)-dependent calcium release from the endoplasmic reticulum downstream of $G\alpha_q$-coupled DopR2 was selectively triggered by backward pairing (Figure 1C). Mutation of *DopR1* resulted in impairment of neural and behavioral plasticity to forward-pairing protocols, whereas mutation of *DopR2* resulted in selective impairment to backward conditioning. Collectively, these results support a model where differential signaling downstream of DopR1 and

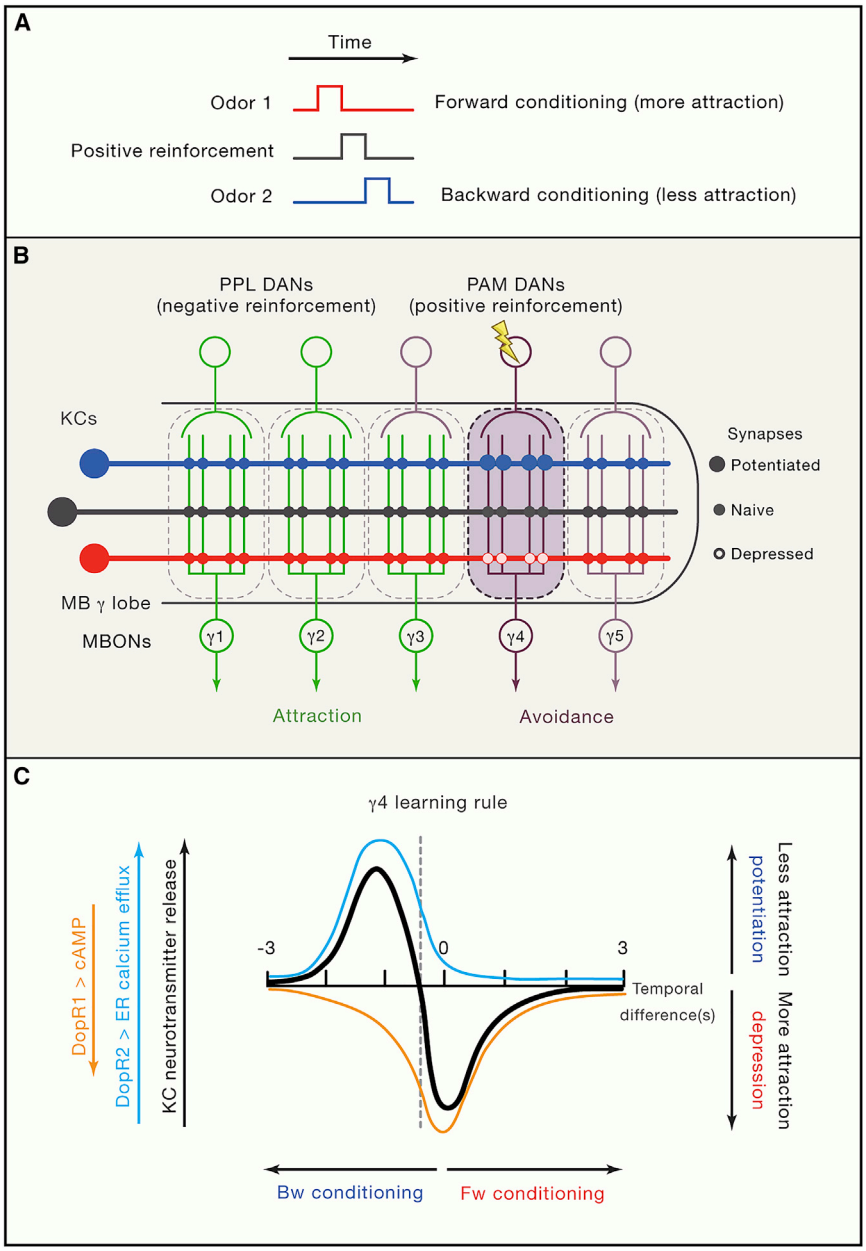

Figure 1. The Timing of Dopaminergic Reinforcement Bidirectionally Regulates Mushroom Body Synaptic Output

(A) Conditioning protocols for probing reinforcement timing. In this example, odor 1 is forward (fw) paired with positive reinforcement (sensory cue precedes reinforcement), and odor 2 is backward (bw) paired with positive reinforcement (sensory cue follows reinforcement). This training results in increased attraction by the animal to odor 1 and reduced attraction (avoidance) to odor 2.

(B) Compartmentalized modules in the mushroom body (MB) map odor representations to behavioral meaning. The red Kenyon cell (KC) represents the sparse ensemble of KCs (~5%–10% of the total population of ~2,000 KCs) that is activated by odor 1, and the blue KC represents the subset of KCs activated by odor 2. KCs form *en passant* synapses with mushroom body output neurons (MBONs) and dopaminergic neurons (DANs) all along the length of their axons, which traverse the multiple compartments of each MB lobe. Two major categories of DANs are the protocerebral posterior lateral (PPL) DANs, which predominantly signal punishment, and the protocerebral anterior medial (PAM) DANs, which predominantly signal reward. Within these two populations, DANs are further subdivided into compartment-specific subtypes. The dendrites of each MBON subtype, and the axon terminals of each DAN subtype, are segregated and typically restricted to just 1–2 compartments, forming 15 functional modules that coordinately map representations of different odors onto different behavioral meanings (Owald and Waddell, 2015). Depicted here is the MB γ lobe and, in particular, the γ4 MBONs, which couple to behavioral avoidance and are a major focus of Handler et al. (2019). The size and shading of the circles at the intersection of KC axons and MBON dendrites indicate the synaptic strength of the specific categories of KC-MBON synapses, following the example conditioning protocol specified in (A). For instance, forward pairing of odor 1 with optogenetic positive reinforcement (lightning bolt) depresses synapses between KCs activated by odor 1 (red) and the γ4 MBON, thereby reducing γ4 MBON activity and promoting attraction.

(C) Proposed model for the detection of reinforcement timing in the γ4 MB compartment. The colored curves schematize the activation of second messengers downstream of the dopamine receptors DopR1 and DopR2 in KC presynaptic terminals, which depends on the relative timing between odor cue and reinforcement. Temporal difference is defined as the arrival time of the dopaminergic reinforcement minus the arrival time of the odor cue; thus, forward pairing has a positive temporal difference. The direction of the amplitude axis for each second messenger is chosen to be congruent with its proposed function in promoting or inhibiting synaptic vesicle release. Note that, if cAMP- and ER-derived calcium signals were to converge with opposing signs on a common regulator of neurotransmitter release, a simple linear summation would predict a biphasic dependence of KC synaptic activity on temporal difference, which closely mirrors the bidirectional behavioral and γ4 MBON plasticity curves measured experimentally in Handler et al. (2019).

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DopR2 serves as the biochemical basis for the instructive role of dopamine timing in bidirectionally modifying synaptic strength and behavior (Figure 1C).

Like all exciting science, this study raises many new questions. First, what are the molecular mechanisms for the detection of the relative timing of dopamine receptor activation and sensory-driven calcium influx? The authors suggest that the timing rules may be governed by the intrinsic biophysical characteristics of adenyl cyclase, which integrates coincident $G\alpha_s$ and Ca^{2+}/CAM-signals (Tomchik and Davis, 2009), and of the IP_3 receptor, which requires priming by $G\alpha_q$-derived IP_3 for full activation by subsequent calcium (Marchant and Taylor, 1997). Second, how are these second messengers coupled to the presynaptic release machinery? Do they converge on a common target or act through independent parallel mechanisms? Third, what is the generality of the plasticity rule in the γ4 compartment (the primary focus of this study)across the other MB compartments? Indeed, studies of other γ lobe compartments suggest that reinforcement timing rules may vary among compartments (Aso and Rubin, 2016; Hige et al., 2015; König et al., 2018). Extending how the basic learning

rule, determined using highly controlled cues and reinforcement, translates into more naturalistic behavioral contexts will be important future work.

The bidirectional learning curves described by Hander et al. have intriguing parallelism to Hebbian synaptic learning rules, such as spike-timing-dependent plasticity (STDP). These forms of plasticity likewise rely on the inherent biophysical properties of a synaptic coincidence detector, the NMDA receptor, highlighting a core reliance on molecular computation. A key point of divergence, however, is the timescale of plasticity. Hebbian-like mechanisms act over short timescales of tens to hundreds of milliseconds, but the reinforcement-based plasticity described in this study occurs over several seconds. Intriguingly enough, this is also the timescale of most natural behaviors. Recently, a new form of non-Hebbian plasticity has been described in the formation of hippocampal CA1 place fields. During a seconds-long plasticity window defined by a powerful, global calcium plateau, co-active synaptic inputs were selectively strengthened and thereby bound together, in a process termed behavioral timescale plasticity (BTSP) (Bittner et al., 2017). The trigger for the calcium plateaus is poorly understood, but one possibility is that unexpected or otherwise behaviorally salient events increase their likelihood, perhaps via neuromodulators such as acetylcholine or dopamine. As more forms of plasticity are investigated in different circuits, we may discover more commonalities in the core logic for seemingly diverse forms of learning. Further investigation is required to know the extent to which they may also share conserved molecular mechanisms.

Finally, it's noteworthy that the core MB circuit module bears remarkable similarities to several vertebrate circuits underlying adaptive behaviors, including the cerebellum, hippocampus, and striatum. In the striatum, in particular, D_1-like and D_2-like dopamine receptors, coupling to distinct G-protein pathways, map onto two anatomically and functionally distinct modules: the direct and indirect pathway. Although precisely what midbrain DANs are encoding is incompletely understood, dopamine release in the striatum is linked to salient events like reward and threat. Striatal dopamine modulates opponent activity in the direct and indirect pathway, via D_1- and D_2-like receptors respectively, to dynamically reshape the mapping of cortical representations onto action selection. The study of phylogenetically distant neural circuits allows us to recognize these core structure-function relationships governing similar neural computations and highlights the importance of comparative neuroscience for deriving general principles of brain function.

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