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‘One must imagine Sisyphus happy’: unveiling the intimate secrets of a tenacious fruitfly.

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Abstract

Perseverance in foraging is a high risk/high gain strategy. In this issue of *Neuron*, Sayin and colleagues decipher the neuronal circuit that arbitrates this choice in *Drosophila*. The fly’s remarkable tenacity illuminates the interaction between working memory and decision-making.

Sensory cues indicating the presence of food are the most valuable type of behavioral drive for a hungry animal. However, foraging for food, which involves different types of locomotion, represents an important source of energy expenditure, and may expose the animal to danger. Therefore, making the decision to track an odor source requires the integration of metabolic state, motivation, and sensory inputs on a cost/benefit basis.

In this issue of *Neuron*, Sayin and colleagues performed an experimental assay to decipher the neuronal circuit involved in the onset and regulation of food odor tracking in the fruitfly *Drosophila melanogaster*. In a genuine laboratory version of the Sisyphus ordeal (see their graphical abstract), a tethered fly was allowed to walk on a spherical treadmill so that its virtual trajectory could be reconstructed. Flies were submitted to repeated presentations of a very appealing vinegar odor. At the odor onset, initially wandering (and hungry) flies were able to track the odor source by running upstream of the odor flux and decreasing their turning, although they never could reach the expected odor source.

There are three major features in this tracking behavior. First, it is strongly hunger-dependent. Strikingly, optogenetic activation of internal sugar-sensing neurons was sufficient to suppress tracking in starved flies, suggesting that post-ingestion signals following a meal (or the absence thereof) tune the foraging motivation.

Second, and perhaps more surprising, the tracking intensifies over repeated odor presentations, in terms of running speed, duration, and turning inhibition. Thus, hunger-driven motivation seems to overcome the failure to meet the expected outcome, i.e. the discovery of the odor source. Whatever its ethological significance, the short-term ‘memory effect’ demonstrated here (the whole sequence of repeated stimuli lasts ca. 20 minutes), which impacts decision-making, could be seen as a working memory equivalent in *Drosophila*.

Third, and consistent with the previous point, there seems to be no room for resignation in these Sisyphus flies. Only success in their food search can make them stop! In a closed-loop version of the assay where the animal can trigger odor offset by ceasing to run, hungry flies continued tracking the odor for hundreds of seconds without any loss in tracking velocity. While understanding why flies are so tenacious was beyond the scope of this study, one thrilling recent study has shown that zebrafish tend to give up after repeatedly failing to obtain the expected outcome in a locomotor assay (Mu et

al., 2019). As this relied on the slow integration of an error signal by astrocytes, it may be promising to investigate the role of glial cells in the surprising resilience of flies in their pursuit of food.

After characterizing this persistent tracking behavior, the authors made use of the genetic tractability of fruitflies to decipher the underlying neuronal circuits. Because mushroom body (MB) neurons were previously identified as a site for state-dependent modulation of olfaction, they focused on the role of MB output neurons (MBONs) in the tracking behavior. Two of these MBONs inhibited tracking when thermogenetically silenced. One of them is a feed-forward MBON (ff-MBON) that has two distinct input sites on distinct MB areas and inhibits downstream targets including other MBONs (Perisse et al., 2016). The second one belongs to the category of MBONs driving attraction (Aso et al., 2014) and is the only MBON that directly connects the MB to the lateral horn, another brain region receiving direct input from olfactory projection neurons (Figure 1). Using optogenetics, the authors showed that when this LH-connecting MBON (LH-MBON) was activated, flies pursued the odor at maximum velocity from the first trial. Since this MBON is a strong synaptic partner of the feed-forward neuron, the authors suggest that they act in series in the same neuronal pathway to drive the tracking behavior.

As the LH-MBON directly synapses onto neurons involved in the attraction to vinegar (Dolan et al., 2018), the rationale for its recruiting by vinegar odor tracking is straightforward. But what is the role of the ff-MBON? A first clue lies in the fact that activation of the ff-MBON reinstalled robust tracking in fed flies, whereas it is normally suppressed. This led the investigation towards the octopamine system (the equivalent of mammalian norepinephrine), known to regulate appetitive behaviors. Using fine anatomical labeling, high-resolution electron microscopy reconstruction and a functional connectivity assay, the authors identified a novel and direct inhibitory synaptic connection between a precise set of octopaminergic neurons and the ff-MBON. Consistently, activation of these neurons inhibited tracking in starved flies. Thus, the level of activity in these neurons can arbitrate between short-range exploitation of a food source and long-range food odor tracking. Bridging the gap between the onset of food intake and the suppression of foraging will require future studies to investigate how sugar-sensing neurons connect to these octopamine neurons.

Finally, the authors focused on the circuit underlying the ‘working memory’ that induces the progressive increase in tracking velocity over trials. Given the prominent role of MBONs in the tracking behavior, and the rich literature on dopamine modulation of the MB output synapses, they focused on dopamine neurons (DANs) that innervate the MB and found that upon silencing MP1 DANs, flies lost the memory of the past odor presentations and failed to exhibit persistent odor tracking. As the MP1 DAN is presynaptic to the ff-MBON, the ff-MBON receives direct modulation from the satiety state through octopaminergic neurons and from previous odor tracking outcomes via the DAN, integrating them to regulate subsequent tracking behavior by acting on the LH-MBON. The MP1 DAN is known to signal the perception of punishing stimuli during associative olfactory conditioning. It is tempting to reframe these findings in a lexicon of associative memory. The frustration from failing to reach the source of the odorant acts as a negative reinforcement signal through MP1 DAN. This adaptively enhances tracking behavior upon further odor presentation, by modulating the activity of the MBONs. It is worth delving deeper into the detailed comparison of the well-studied circuits involved in associative memory and the “working memory” circuits highlighted here, as it may challenge what we know about learning-induced plasticity in the MB.

The description of MB anatomy has introduced a model of how the MBON network can lead to adaptive olfactory behavior, where the excitatory drive from MB intrinsic neurons to valence-encoding MBONs could be locally modulated on a compartment-by-compartment basis by dopamine, biasing the outcome of the MBON network towards attraction or behavior. A notable (and slightly counterintuitive) feature of the hard-wired network architecture is the matching of positive DANs with avoidance MBONs and vice versa (Figure 1A). Yet, this makes sense if MB synapses are endowed with a plasticity rule of dopamine-driven depression (Handler et al., 2019), as it has been reported that aversive memory manifests in the depression of positive valence neurons, while attractive memory involves a depression of negative valence neurons (for review, see: Cognigni et al., 2018).

Here, however, flies are presented a situation in which a negative experience (failure to find the expected food source) results in an increased attractive behavior (tracking) of the odorant. How can it be, with these rules and architecture, that the MB network processes such an association? The response lies within the peculiar characteristics of the inhibitory ff-MBON (Figure 1B). One previous study (Perisse et al., 2016) described the role of the MP1 DAN and ff-MBON in the recall of odor/shock associative memory, where the depression of the ff-MBON leads to the activation of a downstream avoidance MBON. The same mechanism could be in effect here, assuming that the depressed ff-MBON could cause an increased activation of the LH-MBON. The existence of such inhibitory feed-forward connections can thus be seen as a way to enrich the repertoire of valence/outcome pairing within the MB network. Still, that persistent foraging recycles the circuit and mechanisms of aversive associative memory raises the following question: how can the same DAN – ff-MBON circuit selectively activate either an avoidance or an attraction MBON, which lead to opposite behavioral outcomes? This is an additional incentive for further subcellular study of the integrative properties of this intriguing feed-forward neuron.

There is a strong consensus that, independently of their valence, olfactory short-term memories involve a particular subtype of MB neuron (the γ neurons), while more persistent memories (lasting from hours to days) are encoded independently in a distinct MB neuron subtype (the α/β neurons). By contrast, the working memory described here is independent of the γ neurons. Instead, it relies on a circuit that closely matches the one involved in the negative long-term memory of odor/shock association. Interestingly, we reported that a memory component present immediately after odor/shock association relies on α/β neurons and the LH-MBON (Bouzaiane et al., 2015). Further investigation is needed to determine if this memory component is related to the working memory reported in this new study.

At the molecular level, there is a wealth of literature associating the dopamine receptor dop1R1 with associative olfactory learning. Bridging the behavioral and synaptic levels, a recent article (Handler et al., 2019) reported that dop1R1 is indeed involved in the dopamine-induced synaptic depression of MB output synapses, while another receptor, dop1R2, can on the contrary induce synaptic potentiation. Assuming that memory arises from the depression of targeted synapses, this seems consistent with the simplistic model that dopamine signaling acts on MB neurons through two distinct receptors, one for learning (dop1R1) and one for forgetting (dop1R2) (Berry et al., 2012). However, one final compelling finding from Sayin and colleagues is that the memory effect, and hence persistent odor tracking, relies on dop1R2 in α/β neurons, but does not require dop1R1. As is the case for the circuit underlying this memory, there is an interesting similarity with associative

long-term memory formed after repeated and spaced conditioning, in which dop1R2 in the MB is required after training to initiate consolidation (Plaçais et al., 2017). These findings make it clear that there is no such thing as a ‘learning’ dopamine receptor on the one hand and a ‘forgetting’ dopamine receptor on the other hand. Instead, the misnamed ‘forgetting’ receptor dop1R2 seems to be especially important for memory formation in a context where repetition matters. It would be interesting to investigate whether dop1R2 mediates working memory formation through local synaptic potentiation (Handler et al., 2019), and if so in which MB compartment(s) this plasticity happens. Alternatively, the mechanism involving dop1R2 may not be purely synaptic, but involve modulation of energy metabolism, as was shown for associative long-term memory (Plaçais et al., 2017).

These speculations emphasize that the fascinating functional exploration of how the MB network sustains adaptive behavior is far from complete. The local, compartment-based model of learning-induced plasticity may represent only the first layer of the complexity that this network is capable of. The reductionist approach based on compartment-restricted neuronal activation or silencing, which brings invaluable mechanistic insights, is not dissociable from integrated studies involving real and complex behavioral paradigms, like the present one from Sayin and colleagues. This is the last – and perhaps not the least – lesson from this work.

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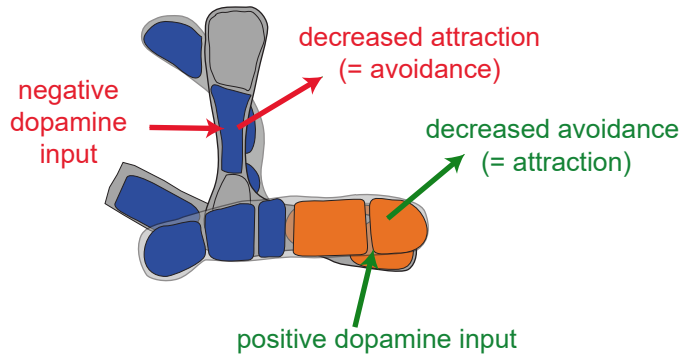
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Figure 1: Local (A) versus remote (B) modulation of MB output by dopamine.

A Learning through local plasticity

MB output neuron: ● driving attraction
● driving avoidance



B Working memory during food-odor tracking

