Converting Drosophobia into Drosophila

Meng-Fu Maxwell Shih & Josh Dubnau

Fruit flies seek water, but only when they are thirsty. And imbibing is rewarding only to water-deprived individuals. The effects of thirst on water seeking and on formation of associative memories of drinking water each are mediated by distinct sets of dopamine neurons that innervate restricted zones of the mushroom bodies in the fly brain.

The name of the genus of small flies Drosophila has an etymological root in the Greek words drosos, meaning dew or moisture, and philia, meaning loving. Drosophila melanogaster has a long and storied history in the fields of genetics and behavioral neuroscience. Ironically, Drosophila under standard laboratory conditions innately avoid water and prefer a dry environment. In this issue of Nature Neuroscience, Lin et al. uncover some of the key neurobiological underpinnings that cause D. melanogaster to seek water when thirsty—showing behavioral signs of liking water instead of avoiding it, thereby living up to their name—and to remember odors that were informative about where to quench their thirst. Notably, these neural mechanisms tap into the highly conserved dopamine system. Moreover, the naive water seeking and rewarding behaviors are each modulated by anatomically distinct and dedicated sets of dopaminergic neurons whose processes label different substructures of the mushroom body (MB), a known olfactory association center in insects.3,4

In laboratory conditions, wild-type adult fruit flies exhibit a strong innate preference for dry conditions over wet. This is likely an adaptation to avoid becoming caught in sticky food substrates. Lin et al. demonstrated, however, that a 6-h deprivation, which makes the animals thirsty enough to seek water, will overcome this moisture avoidance. Investigation of the neural circuits underlying this simple plasticity in responses to water provides insight into mechanisms by which past experience and internal state modulate behavioral choices.

The effect of thirst on water seeking is reminiscent of those of satiety on associative memory of sugar reward. With sugar reward learning, hungry animals learn which odors were present during sugar presentation. And hungry animals are able to retrieve and express such memories. In contrast, well-fed animals don’t remember which odors where present when food was offered.5,6

The known mechanisms by which satiety modulates sugar learning provided an entry point to uncover neural mechanisms regulating naive and conditioned water-seeking preferences. Both aversive and appetitive olfactory memory in flies relies on the MB brain structure, and specific sets of dopamine neurons provide instructive signals to MB both for aversive (electric shock reinforced) and appetitive (sugar reinforced) olfactory memory. Lin et al. decided to use water as a reward in place of sugar, with the assumption that this would likewise engage the MB olfactory learning center.

In the conventional sugar reward-learning task, hungry flies are sequentially exposed to two different odorants, one of which is paired with sugar reinforcement. Memory performance is then tested in a T maze, in which the animals choose between the two odors. To test whether water has rewarding value to thirsty flies, Lin et al. first adapted the assay to use water as the reinforcement in place of sugar. Indeed, pairing water with an odor yielded a robust, but short-lived, olfactory memory. As with sugar learning, performance in the water reinforced learning assay was enhanced in thirsty flies relative to that in water-satiated counterparts. These behavioral manipulations of thirst revealed plasticity in the valuation of water as a reward for olfactory learning, just as is true with hunger and sugar-reinforced learning. But we don’t eat when we’re thirsty or drink when we’re hungry. Similarly in flies: water-based memories weren’t influenced by hunger and sugar-based memories weren’t altered by their thirst. This deprivation state specificity immediately suggested distinct underlying neural representations of thirst and hunger. By investigating the neural circuits involved, Lin et al. were able to demonstrate that this is indeed the case. Moreover, distinct circuits also mediated naive water seeking, the rewarding value of water in olfactory conditioning and the expression of water memories.

To investigate the neural circuits mediating water reinforced learning, Lin et al. began at the sensory level. Water taste perception is mediated by an ion channel called pickpocket (ppk), which is expressed in sensory neurons in the proboscis. Indeed, Lin et al. found that these mutants were defective in water reward learning. However, water seeking by untrained thirsty flies was normal in ppk28 mutants. Thus, the taste of water mediates the rewarding value for learning, but the search for water in thirsty animals does not require water taste. Although the sensory mechanism used for naive water seeking remains unknown, Lin et al. demonstrated that it involves sensation of water vapor rather than the taste from drinking water.

In the case of sugar-mediated reinforcement, there are both sweet taste and nutritive value features to the reward and these involve distinct neural mechanisms. The insect noradrenaline analog octopamine mediates the sweet taste reinforcing effects, but not the nutritive value. Because water tasting through ppk28 was required for water-mediated reward learning, Lin et al. guessed that octopamine might also mediate the reinforcing effects of water taste in learning, but here the analogy with sugar reinforcement began to break down. Neither manipulations of octopamine biogenesis nor of octopaminergic neurons affected water-reinforced learning. They next examined a role for dopaminergic transmission

Meng-Fu Maxwell Shih and Josh Dubnau are at the Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA.
e-mail: dubnau@cshl.edu

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because dopaminergic neurons mediate the rewarding effects of both the nutritive value and sweet taste (driven by octopamine neurons) of sugar.

With both appetitive and aversive olfactory memory, dopaminergic neurons convey information to MB via DopR, a type 1 dopamine receptor. Not surprisingly, Lin et al. discovered that mutations in DopR also disrupted the water-reinforced olfactory memory. And as with aversive olfactory learning, expression of DopR in a specific subset of MB intrinsic neurons called γ neurons (but not α’/β or α’/β’ MB neurons) was sufficient to mediate dopaminergic inputs for water-reinforced learning. This finding focused the search for the relevant dopaminergic neurons onto those that innervate the MB, particularly dopamine neurons that contact the γ neurons, whose axon terminals are spatially separate from those of the other MB neuron cell types (Fig. 1).

To identify the relevant dopamine inputs, Lin et al. took advantage of a powerful suite of genetic tools that is available in Drosophila, which provides the means to target expression of a transgene of interest to individual neuronal cell types. Thermogenetic tools allow transient activation (with a temperature-activated channel) or silencing (with a temperature-sensitive shibire transgene) of neurons in vivo, and genetically encoded sensors such as GCaMP permit imaging of calcium responses to behavioral manipulation such as presentation of water.

Lin et al. identified several groups of water-responsive dopaminergic neurons innervating distinct regions or zones of the MB lobes (Fig. 1). For example, strong GCaMP responses to water were observed in the γ4 zone of the γ lobes and the β’2 zone of the β’ lobes. Somewhat lesser responses were seen in the γ5 zone of the γ lobes. To identify the specific dopamine neuron inputs mediating the effects of thirst and water, they used intersectional combinations of several transgene expression strategies to focus thermogenetic manipulations into subsets of dopamine neurons that label each of these zones. These manipulations revealed that a set of dopamine neurons called PAM-γ4 are necessary for mediating the rewarding effects of water during learning: transiently silencing them with a temperature-sensitive shibire transgene blocked water reinforced learning. Moreover, thermogenetically activating these same PAM-γ4 neurons with the TrpA1 channel was sufficient to substitute for water presentation, thereby implanting an artificial reward memory. Like water-reinforced memory, this implanted memory was reduced if the flies were permitted to drink water before testing. Together, these findings provide strong evidence that PAM-γ4 neurons convey the relevant water taste information to DopR expressed in MB γ neurons during water-reinforced learning.

Retrieval and expression of water memory were gated by thirst as well: only thirsty flies exhibited memory performance at the time of testing. One might think that the same water-activated dopamine inputs would be relevant to the retrieval and expression of water memory, but, remarkably, that was not the case. Instead, retrieval and expression of this memory required a different set of neurons that lies outside of the PAM cluster: blocking all of the PAM neurons during testing left memory intact. Similarly, blocking the PAM-γ4 of dopamine neurons in naive but thirsty animals did not prevent water seeking. So this effect of thirst also relies on a distinct neural circuit.

Which dopamine neurons mediate water attraction in naive thirsty animals? Lin et al. show that this is most likely mediated by the PAM-β’2 neurons, which target the β’2 zone of α’/β’ neurons rather than γ neurons (Fig. 1). Although no direct strategy exists to specifically target the PAM-β’2 neurons without also expressing in other dopamine neurons, Lin et al. used a series of manipulations of various overlapping groups of neurons to deduce the role of PAM-β’2. Together, the findings support the conclusion that the PAM-γ4 neurons convey the rewarding effects of water taste to γ MB neurons during learning, whereas the PAM-β’2 neurons convey the water-seeking drive to α’/β’ MB neurons in naive thirsty flies. Notably, the downstream dopamine receptor acting in these α’/β’ MB neurons also appears to be distinct: DopR mutants exhibited normal naive water approach.

Although the mechanism by which thirst gates memory retrieval and expression remains mysterious, Lin et al. do report one paradoxical hint about how it may work. It turns out that blocking a larger group of dopamine neurons after training and during memory testing actually enhances memory performance. This effect does not involve the PAM-β’2 neurons (which are included in the larger set of dopamine neurons). So although the circuits that mediate the effects of thirst on expression of odor cued water memory remain to be identified, there is
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a hint that some combination of dopaminergic neurons may supply the mechanism by which thirst gates memory expression. This idea is reminiscent of the known role for MB-MP1 dopamine neurons in gating sugar reward memory expression in satiated flies\textsuperscript{14}.

In *Drosophila*, water seeking only occurs in water-deprived animals. Drinking is rewarding to flies, but only when they are thirsty. Memories of previous water rewards are only expressed when the internal state is one of thirst. Moreover, when these new findings with water memory are viewed in the context of the larger literature on olfactory memory in flies, the theme that emerges is that olfactory memory relies on the same MB structure irrespective of the modality or value of the reinforcement. The specificity of the experience and the effect of internal state arise from the distinct neuromodulatory input neurons and their restricted zones of contact with different subsets of MB intrinsic neurons. The so-called lovers of dew are underestimated by their name. Instead of thoughtlessly yearning for the morning dew, fruit flies exhibit plastic responses to water and to their levels of thirst.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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**Bilingual neurons release glutamate and GABA**

Naoshige Uchida

A study finds evidence supporting co-release of glutamate and GABA, excitatory and inhibitory fast neurotransmitters, from a single axon terminal in neurons of the ventral tegmental area that project to the lateral habenula.

Dale’s principle, promoted by Sir John Eccles\textsuperscript{1}, postulates that the same chemical transmitter is released from all of the synaptic terminals of a neuron. In other words, each neuron produces a single neurotransmitter and the identity of this neurotransmitter never changes. Those neurons that release glutamate excite their postsynaptic partners, those that release GABA inhibit them and those that release dopamine ‘modulate’ them, for example, by modifying the efficacy of synaptic plasticity. Increasing evidence suggests, however, that Dale’s principle does not always hold true: some neurons release multiple neurotransmitters\textsuperscript{2,3} and some change their neurotransmitter identity\textsuperscript{4}.

In a study published in this issue of *Nature Neuroscience*, Root et al.\textsuperscript{5} add yet another astonishing case: a large fraction of rodent ventral tegmental area (VTA) neurons that project to lateral habenula (LHb) co-release glutamate and GABA, two main excitatory and inhibitory fast neurotransmitters, from single axon terminals.

The VTA is one of the main sources of dopamine in the brain, but it also contains neurons that release other neurotransmitters. The authors first examined the neurotransmitter identity of VTA neurons that project to the LHb (Fig. 1a) by retrogradely labeling neurons from LHb and staining for neurotransmitter markers at their cell bodies in the VTA. The authors found that most (~80%) of the LHb-innervating VTA neurons coexpressed markers for glutamate and GABA signaling: vesicular glutamate transporter 2 (VGlut2, an enzyme that loads glutamate into synaptic vesicles) and GABA transporter 3 (GAT3, an enzyme that loads GABA into synaptic vesicles). A study finds evidence supporting co-release of glutamate and GABA, excitatory and inhibitory fast neurotransmitters, from a single axon terminal in neurons of the ventral tegmental area that project to the lateral habenula.

**Figure 1** LHb-innervating VTA neurons co-release glutamate and GABA from single axon terminals. (a) VTA-to-LHb projection. (b) A Venn diagram illustrating the proportions of neurons that express markers for glutamatergic (VGlut2\textsuperscript{+}), GABAergic (GAD\textsuperscript{+}) and dopaminergic (TH\textsuperscript{+}) neurons. Areas shown are approximate. (c) Schematic drawing of the ultrastructure of a synapse between a VTA neuron axon and a postsynaptic LHb neuron. AS, asymmetric (putative excitatory) synapse; PA, punctum adherentium; SS, symmetric (putative inhibitory) synapse.