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Modulation of neural circuits: how stimulus context shapes innate behavior in *Drosophila*

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Summary

Remarkable advances have been made in recent years in our understanding of innate behavior and the underlying neural circuits. In particular, a wealth of neuromodulatory mechanisms have been uncovered that can alter the input-output relationship of a hereditary neural circuit. It is now clear that this inbuilt flexibility allows animals to modify their behavioral responses according to environmental cues, metabolic demands and physiological states. Here, we discuss recent insights into how modulation of neural circuits impacts innate behavior, with a special focus on how environmental cues and internal physiological states shape different aspects of feeding behavior in *Drosophila*.

Introduction

Innate behavior, programmed by genetically predetermined neural circuits, is robust and stereotyped. Although considered to be hardwired, innate behavior is also flexible and subject to modulation by internal states (e.g. satiety state) and external contexts of the stimuli (e.g. environmental cues) [1–6]. Dissecting the mechanisms whereby external and internal contexts of stimuli influence the behavioral outputs of a hardwired circuit might appear a daunting task. However, aided by powerful genetic tools, much progress has recently been made to address this fascinating question in genetic model organisms such as *Caenorhabditis elegans* and *Drosophlia melanogaster* (for review, see [1,2,4,7]). Here we will focus on recent advances in neuromodulation of *Drosophila* innate behavior.

Context-dependent modulation of innate behavior is particularly well described for fruit flies, which forage only when they are starved [8••], feed only when they verify that food is not spoilt [9••], and court vigorously only when they detect that a food source is nearby to sustain their progeny [10]. This inbuilt behavioral flexibility allows animals to mount appropriate behavioral responses to stimuli. At the circuit level, this flexibility is thought to be driven by information rerouting and neuromodulation [2]. The former involves

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reconfiguring information processing by alternative circuit pathways, and the latter refers to chemical neuronal communications that are neither simply excitatory nor inhibitory but serve to modulate the properties of existing synaptic connections [11].

Here we will first focus on how the context of an external stimulus influences innate behavior, using the example of how fruit odors suppress *Drosophila*'s natural aversion to carbon dioxide. In the second part of this review, we will highlight the neuromodulatory mechanisms by which internal physiological states, particularly satiety levels, regulate appetitive behavior in *Drosophila*.

External context: how fruit odors suppress *Drosophila*'s aversion to carbon dioxide

Carbon dioxide is a key component of the “stressed odors” released by agitated fruit flies, and is detected by the ab1C olfactory receptor neurons (ORNs) [12]. Activation of ab1C ORNs leads to robust aversive behavioral responses [12,13]. However, CO_2 is also present in different contexts of the natural environment for *Drosophila*. For instance, CO_2 is emitted from ripe fruits that are attractive to fruit flies [14]. Thus, CO_2 in the context of fruit odors does not trigger aversion. Given that CO_2 is detected primarily by a single class of ORNs, ab1C, how do fruit flies manage to avoid CO_2 robustly in one context but tolerate the same compound in another?

Several recent studies have shed light on the circuit mechanisms that underlie this intriguing context-dependent behavior. Interestingly, as we describe below, these mechanisms appear to operate at every layer of the olfactory circuit, from the peripheral sensory organ, to the first information relay center, and further on into multiple higher brain regions (Figure 1).

Direct modulation of CO_2 response in the antenna

In fruit flies, the CO_2-responsive ab1C ORNs are located in the primary olfactory organ, the antenna. Given that ORNs are the first neurons in the olfactory circuit, one effective means for flies to ignore CO_2 would be to directly suppress the response of ab1C ORNs in the presence of fruit odors. Indeed, two complementary mechanisms by which fruit odors inhibit ab1C response to CO_2 have been reported, one operating within ab1C and the other between ab1C and its neighboring ORN. The former mechanism acts on the CO_2 receptor complex (Gr21a/Gr63a) localized on the outer dendrites of ab1C [15,16]. Interestingly, certain fruity odorants can directly interact with Gr21a/Gr63a to inhibit the response of ab1C to CO_2, thus suppressing flies' behavioral aversion to CO_2 [17].

In addition, a second, novel mechanism of inhibition occurs, driven by lateral inhibition between neighboring ORNs housed in the same sensillum (Figure 1b). Transient activation of any given ORN robustly inhibits the chronic olfactory response of its neighbor via ephaptic coupling [18••,19]. Notably, in the ab1 sensillum, ab1C is grouped with another ORN (ab1A) which responds strongly to fruit odors [20]. As a result, strong activation of ab1A by fruit odors may attenuate the response of ab1C to CO_2, thereby making CO_2 more

tolerable in the presence of fruit odors [18••]. In this context, we consider ephaptic coupling as a means of neuromodulation.

Inhibition of CO_2–activated output by interneurons in the antennal lobe

Upon ab1C activation, CO_2 input is propagated by ab1C axons to a spherical neuropil structure called the V glomerulus in the antennal lobe [12,21]. In the V glomerulus, ab1C axon terminals form synapses with projection neurons (PNs), which are the main output neurons that relay CO_2 information to higher brain regions (see below). Also innervating the V glomerulus are the inhibitory GABAergic local interneurons, which receive excitatory inputs from a wide variety of ORN types, including those that respond to fruit odors (Figure 1c) [22–25]. Interestingly, it has been suggested that these local interneurons can attenuate the response of PNs from the V glomerulus [26], likely by activating $GABA_A$ receptors on PN dendrites [27]. Therefore, activation of this GABAergic inhibitory pathway by fruit odors may further dampen CO_2 signals by inhibiting PN outputs from the V glomerulus.

Inhibition of CO_2–activated output by projection neurons in the higher brain regions

In addition to ab1C ORNs and their corresponding PNs, a third layer of regulation has been proposed recently. Remarkably, multiple types of PNs innervate the V glomerulus [28••,29]. These PNs differ in their sensitivity to CO_2 and their axonal innervating patterns in higher brain regions. Among them, two PN types are largely responsible for flies' behavioral aversion to low (0.5%) and high (2%) concentrations of CO_2. The response of PN_v-1 to CO_2 saturates at a low concentration (0.5%), whereas PN_v-2 shows graded responses to different concentrations of CO_2 (0.5% ~ 2%) [28••].

Segregation of the CO_2 processing circuit into multiple pathways may allow differential modulation of each individual pathway [29,30]. Indeed, in a higher brain region named the lateral horn, some higher order neurons that receive PN_v-1 input can be inhibited by yet another PN type (PN_v-3) [28••]. Unlike most PNs, PN_v-3 is GABAergic and may belong to a reported parallel inhibitory pathway in the fly olfactory circuit [31•]. Notably, the dendrites of PN_v-3 innervate multiple glomeruli, including those that are activated by fruit odors (Figure 1d). Thus, fruit odors activate PN_v-3 to selectively inhibit the PN_v-1 output neurons without affecting the PN_v-2 pathway that mediates aversion to high concentrations of CO_2 [28••]. As a result, fruit odors may selectively inhibit the behavioral aversion to low levels of CO_2 present in ripe fruits [14], while the fly retains its ability to respond to high CO_2 levels that may signal danger.

Much remains to be learned about how these neural substrates work in concert to modulate the CO_2 olfactory circuit and to determine the contribution of each individual mechanism in shaping flies' behavioral response to CO_2. Moreover, other factors, such as the fly's locomotive state, can also impact whether they find CO_2 attractive or aversive [32•]. It will be of interest to know where locomotive information is integrated into the CO_2 circuitry. A broader question is to determine whether similar mechanisms govern context-dependent responses to other stimuli. Importantly, though, the multiplicity of neural substrates

highlighted here provides insight into the fundamental circuit logic that determines how environmental cues can alter the behavioral output of a hardwired neural circuit. In addition to these external cues, it has recently become clear that the internal states of the animal can also influence behavioral responses, as we explain below.

Internal context: how satiety state regulates *Drosophila*'s feeding behavior

Feeding is a highly regulated behavior; many factors influence an animal's decision to eat, such as the aroma, palatability and nutritive value of food, as well as the satiety state and metabolic demands of the animal [33]. To integrate these diverse cues, animals produce a variety of signaling molecules. These molecules code for different internal states that modulate different aspects of feeding behavior, from sensory input to behavioral output and several processing stations in between (see below). In the following sections, we will highlight recent discoveries on the molecular and cellular mechanisms whereby starvation regulates feeding behavior. In particular, we will focus on how starvation promotes feeding by enhancing olfactory and gustatory sensitivity and by regulating activity of central neurons that express internal nutrient sensors in *Drosophila* (Figure 2a).

Starvation modulates feeding by elevating olfactory input

In mammals, up-regulation of Neuropeptide Y (NPY) signaling in the hypothalamus increases food intake [34]. Interestingly, in addition to hypothalamic neurons, NPY is also expressed in the olfactory epithelium of a variety of vertebrates [35,36], suggesting that NPY signaling may regulate olfactory sensitivity to modulate food searching behavior.

Indeed, in flies, there is direct evidence supporting this hypothesis. Two fly homologs of mammalian NPY, neuropeptide F (NPF) and short neuropeptide F (sNPF), have been implicated in regulating feeding behavior in *Drosophila* [37–39]. Like its mammalian counterpart, fly sNPF is expressed in olfactory tissues [40]. Interestingly, sNPF and its receptor, sNPFR1, are both expressed in a subset of ORNs. Among them, DM1 ORNs are both necessary and sufficient to promote food searching behavior in *Drosophila* [20]. By down-regulating insulin signaling, starvation increases the expression of sNPFR1 at ORN axon terminals, thus strengthening ORN-PN synaptic transmission in DM1 (Figure 2b). Consequently, starvation enhances DM1 response to fruit odors, which triggers a more robust food searching behavior in hungry flies [8••]. These studies illustrate the profound impact of elevated olfactory input on the feeding behavior in adult flies.

A similar logic applies to *Drosophila* larvae, where the presence of fruity odorants, such as pentyl acetate, promotes feeding in larvae [41•]. Mechanistically, appetitive odorants appear to promote feeding by activating NPF receptors that are expressed in a subclass of dopaminergic interneurons in the lateral horn (DL2-LH neurons) [41•]. Similarly, another study shows food odors excite NPF neurons which are necessary to drive attraction to food odors in flies [42].

Taken together, these findings reveal a direct link between heightened olfactory activity and enhanced appetitive behavior. Remarkably, a recent study shows that endocannabinoid

signaling promotes food intake by increasing odor detection in mice [43•], suggesting that the link between olfaction and feeding may be evolutionarily conserved.

Starvation modulates feeding by enhancing gustatory sensitivity

Hungry flies show heightened sensitivity to sugar and are more prone to extend their proboscis when they encounter food. Here we will describe several recent studies that address how starvation alters gustatory sensitivity by means of metabolic hormone and dopaminergic signaling mechanisms.

Upon starvation, flies release adipokinetic hormone (AKH, the fly equivalent of glucagon) in the hemolymph to signal hunger [44]. Like insulin, AKH is implicated in regulating flies' feeding behavior. Interestingly, the AKH receptor is expressed in certain gustatory receptor neurons that respond to sugar (Gr5a GRNs) [45]. Activation of the Gr5a GRNs promotes proboscis extension response (PER) in fruit flies to facilitate food intake [46–48••]. Therefore, these findings suggest that starvation may increase the sensitivity of sugar-sensitive GRNs to promote feeding in hungry flies.

Additionally, starvation heightens gustatory sensitivity by dopaminergic signaling. At the level of sensory input, as measured by Ca^{2+} imaging, short-term starvation (~6 hr) enhances the response of the Gr5a GRNs in the subesophageal ganglion (SOG), the primary taste center of the fly brain. Mechanistically, starvation causes dopamine release in the SOG (see below), which activates dopamine receptors (DopEcR) at Gr5a presynaptic terminals to facilitate Ca^{2+} influx (Figure 2c) [48••]. Interestingly, DopEcR is dispensable for enhanced PER in the flies that are starved for more than 24 hrs [48••], suggesting that multiple neuromodulatory mechanisms acting at different time scales are involved.

Indeed, a group of dopaminergic neurons in the SOG, named TH-VUM (tyrosine hydroxylase positive, ventral unpaired medial neurons), were shown to enhance PER after 24-hr starvation [49•]. TH-VUM neurons are interneurons that likely release dopamine to activate DopEcR at Gr5a axon terminals as mentioned earlier (Figure 2c). In addition, TH-VUM may innervate yet another group of neurons in the SOG that express dopamine-2 receptor (D2R) to regulate PER. TH-VUM neurons are unusual in that their basal spike activity scales with the duration of starvation up to 24 hrs. Notably, upregulating TH-VUM activity enhances proboscis extension probability when flies are presented with low concentrations of sucrose, suggesting that TH-VUM sets the behavioral threshold for PER [49•].

Regulation of feeding behavior and food preference by internal nutrient sensors

Finally, we consider internal nutrient sensors as a novel mechanism by which satiety state regulates feeding. Although not strictly neuromodulators, internal nutrient sensors may play a key role in modulating circuit function by engaging existing neuromodulatory pathways [50].

In addition to metabolic hormones, satiety state is also encoded by hemolymph sugar level (i.e. blood sugar) [51••,52•]. Gr43a, a fructose receptor expressed in peripheral GRNs, is also expressed in a small cluster of the central neurons located in the posterior superior lateral protocerebrum (Figure 2a). These Gr43a-positive neurons can thus report hemolymph fructose level directly and, strikingly, their activation assigns opposing valence to feeding experience in a satiety state-dependent manner. That is, Gr43a neurons suppress feeding in satiated flies and promote feeding in hungry flies [51••].

In addition to Gr43a, another internal sugar sensor has been identified in a genetic screen. A mutant line was found to be insensitive to nutritive sugars, which are usually preferred by hungry flies in a taste-blind assay. The affected gene, named *cupcake*, encodes a putative sodium/solute co-transporter, which may function to transport glucose into a subset of brain neurons in the ellipsoid body (Figure 2a) [52•]. These neurons may thus function as an internal nutrient sensor to promote feeding in hungry flies.

In addition, an internal sensor for essential amino acids (EAAs) has been identified [50]. Flies tend to avoid food sources that are EAA-deficient. This behavioral avoidance is mediated by activation of the central neurons which express an amino acid sensor, GCN2, a kinase that promotes dopamine release from these neurons [50]. This finding illustrates how metabolic demands shape food preference via an internal nutrient sensor to promote a balanced diet by recruiting an existing dopaminergic neuromodulatory pathway.

Conclusions

To conclude, the multitude of signaling molecules featured in this review provide a glimpse of how satiety state and metabolic demands regulate aspects of feeding behavior but raise an equally exciting new set of questions for future investigation. For example, do other metabolic hormones, such as the recently identified fly homolog of leptin [53], also regulate the olfactory/gustatory sensitivity in *Drosophila*? What is the circuit mechanism that affords Gr43a central neurons the ability to encode opposing valence in a satiety-dependent manner? Is innate olfactory or gustatory aversion also regulated by satiety state? Why does *Drosophila* employ multiple internal nutrient sensors? Do they act on different time scales?

The neuromodulatory mechanisms featured in this review will serve as a foundation to delineate broader principles by which neural circuits incorporate internal state to shape innate behavior. The study of neural circuit function will benefit tremendously from a growing number of available high-resolution anatomical wiring diagrams [54–57]. Finally, we note that several neuromodulators important for regulating innate behavior, including dopamine, sNPF and NPF, have also been implicated in regulating appetitive olfactory learning [58–61], suggesting a shared neuromodulatory mechanism for innate and learned behaviors. Thus, a better understanding of how innate behavior is modulated at the circuit level may well provide new insights into the neuromodulatory mechanisms that impact complex behavior, such as learning.

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Highlights

- Innate behavior is flexible and subject to modulation by stimulus context.
- Fruit odors modulate *Drosophila*'s innate aversion to CO_2 by multiple mechanisms, operating at every known station of the CO_2 olfactory circuit.
- Satiety state regulates feeding behavior by altering olfactory and gustatory sensitivity.
- Satiety state is communicated by a variety of metabolic cues, which regulate neuromodulator signaling to influence neural circuit function.

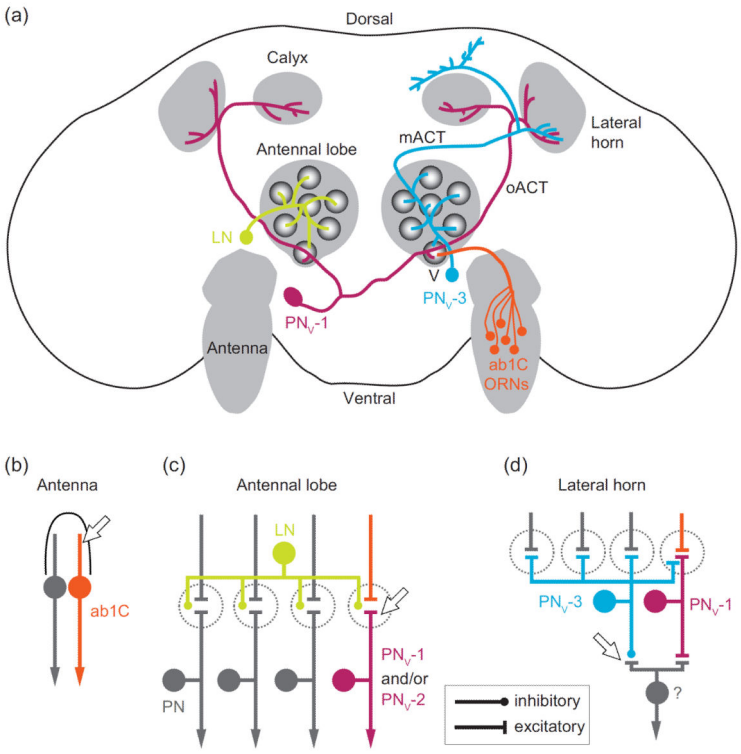

Figure 1.

Fruit odors suppress *Drosophila*'s aversion to carbon dioxide by inhibiting propagation of CO_2 information at multiple layers of the olfactory circuit. (**a**) Schematic of the fly olfactory circuit for CO_2 information processing. CO_2 is detected by ab1C olfactory receptor neurons (ORNs) in the antenna. In the antennal lobe, ab1C axons synapse with projection neurons (PNs) in the V glomerulus. GABAergic local neurons (LNs) innervate multiple glomeruli and suppress PN output from the V glomerulus via dendro-dendritic inhibition. Two types of PNs that innervate the V glomerulus (PN_v) are highlighted: PN_v-1 is excitatory and receives bilateral input from the V glomeruli. PN_v-1 projects to the lateral horn and calyx of the mushroom body via the outer antennocerebral tract (oACT); PN_v-3 is inhibitory and receives input from every glomerulus in the ipsilateral antennal lobe and projects to the lateral horn and other higher brain regions (not indicated) via the medial antennocerebral tract (mACT). Fruit odors inhibit the CO_2 olfactory circuit at the antenna via lateral inhibition in a sensillum (**b**), at the antennal lobe via LN feed-forward inhibition (**c**), and at the lateral horn via parallel inhibition of an unidentified output neuron by PN_v-3 (**d**). Arrows indicate sites of inhibition.

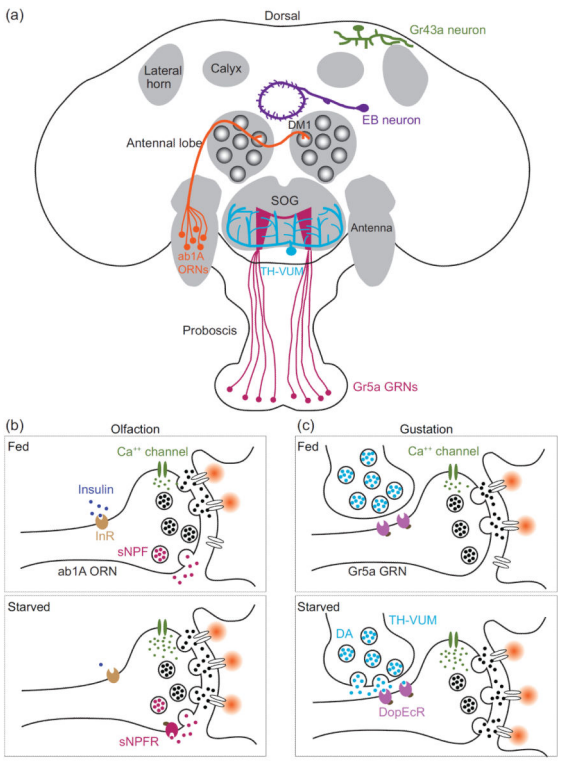

Figure 2.

Satiety state regulates feeding by a diverse array of neuromodulatory mechanisms. (**a**) Starvation regulates feeding in flies by enhancing olfactory and gustatory sensitivity and by regulating activity of central neurons that express internal nutrient sensors. These nutrient sensors include the fructose receptor, Gr43a, in the posterior superior lateral protocerebrum and a putative sodium-solute co-transporter, Cupcake, in some neurons in the ellipsoid body (EB). For simplicity, only one of the multiple, bilateral Gr43a- and Cupcake-neurons is shown. (**b**) By means of insulin and sNPF signaling, starvation enhances synaptic transmission between ORNs and PNs in several glomeruli. Among them, DM1 is crucial for flies' food searching behavior and receives input from ab1A ORNs that express Or42b receptor. Down regulation of insulin signaling promotes the expression of sNPF receptor (sNPFR) to enhance Ca^{2+} response at ORN synaptic terminals. We note that the precise subcellular localization of insulin receptor (InR) is unclear. For simplicity, InR is drawn near the synaptic terminal. (**c**) Starvation also promotes proboscis extension response (PER) in flies to facilitate feeding. Hunger elevates dopamine (DA) release from a class of interneurons in the SOG, named TH-VUM (tyrosine hydroxylase positive, ventral unpaired medial neurons), to activate a dopaminergic receptor (DopEcR) at the sugar-sensitive gustatory receptor neurons (Gr5a GRNs). Elevation of dopaminergic signaling enhances synaptic transmission from Gr5a GRNs to the central taste center, the subesophageal ganglion (SOG), to promote PER.