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Modulation of Olfactory Receptor Neuron Sensitivity by Hunger in *Drosophila*

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Abstract

Hunger drives animals to search for food, a behavior that is heavily dependent on the olfactory system. The neuronal mechanism by which hunger modulates the behavioral response towards food odor, however, is not well understood. In this study, using a single-fly behavioral assay and single-unit recording, we have demonstrated that starved flies exhibit enhanced attraction towards an attractive odor, ethyl acetate, via increased sensitization of the olfactory receptor neurons (ORNs). These results suggest that the increased attraction behavior caused by hunger is due to enhanced sensitivity in the ORNs.

Keywords: *Drosophila,* olfaction, olfactory receptor neurons, starvation

Introduction

Behavioral plasticity dependent on the presence or absence of specific resources, like food, is frequently observed in animals. For most animals including insects, food search behavior depends on olfactory cues. Sense of smell plays an indispensable role in detection and localization of food. Important discoveries were made in mammals towards identifying the neuropeptides that regulate feeding behavior. It has been well established that the hypothalamus in mammals controls food uptake and energy metabolism through various appetite sensing hormones, such as insulin, leptin, amylin, and alpha-melanin stimulating hormone (Berthoud, 2002). Subsequent activation of neurons containing neuropeptide Y (NPY) and agouti-related peptide (AgRP), expressed in a subset of neurons in the arcuate nucleus of the hypothalamus, increase food intake and reduce energy expenditure (Barsh and Schwartz, 2002; Morton *et al*., 2006). Injection of NPY into the hypothalamus enhances food intake (Stanley and Leibowitz, 1985; Stanley *et al*., 1985). In *Drosophila*, short neuropeptide F (sNPF) and neuropeptide F (NPF), which are homologs of NPY, regulate feeding behavior (Wu *et al*., 2003; Lee *et al*., 2004; Lee *et al*., 2008). Despite much knowledge about various neuropeptides that control behavior associated with feeding, less is known about how hunger alters the olfactory responses and how these alterations lead to behavioral changes.

We have investigated whether hunger modulates the olfactory sensitivity that mediates food search behavior. We found that flies when starved exhibit enhanced attraction towards food odors. This apparent state dependence implies that, when starved, flies release signals that probably interact with the neural circuitry to regulate behavioral expression. While most research has focused on central modulation for such behavior, few studies have been done on the peripheral olfactory system. Blood feeding insects like mosquitoes are a good example of antennal sensitivity to host cues being reduced after a blood meal. This decrease in peripheral

sensitivity prevents female mosquitoes from looking for a host when blood-fed (Takken *et al*., 2001). Results obtained from these studies similarly suggest that receptor sensitivity is modulated by the animal's motivational state.

In the present study, we have investigated the effect of starvation at the level of olfactory receptor neurons (ORNs). In *Drosophila*, ORNs are housed in the olfactory sensilla on the third antennal segment and in the maxillary palp, and serve a distinct chemosensory function. The olfactory sensilla fall into three different morphological types known as basiconic, coeloconic and trichoid. Whereas basiconic sensilla are found on both the antennal and maxillary palp, trichoid and coeloconic sensilla are located exclusively on the antennal surface. Electrophysiological recording has been carried out from AB II sensilla basiconica containing two neurons. We have shown that the starved flies exhibit an enhanced sensitivity at the level of olfactory receptor neurons (ORNs). The increment in response is pronounced at lower attractive odor concentrations, but not significant at higher concentrations. In addition, we have further shown that when the fly is deprived of food, it exhibits an enhanced response to the attractive odor.

Materials and Methods

Fly rearing and maintenance

Flies were grown in glass vials with standard cornmeal medium at 21°C in the incubator with circadian cycle control (12 hour light: 12 hour dark). The Canton-S (CS) strain was used in all experiments.

Single Fly Behavior

Age-matched female flies (3 days old) were separated and starved for 30 hours in a vial containing wet tissue paper. For the single fly behavior test, five flies were tested in one set. Five glass tubes were taken with one fly in each. Each glass tube was closed on both ends with traps prepared with a micropipette tip and microcentrifuge tube (200µl). An overhead camera (Watec Monochrome Video Camera) was used to record the movement of flies during the test. A schematic representation of the behavioral setup is shown in Fig. 1.

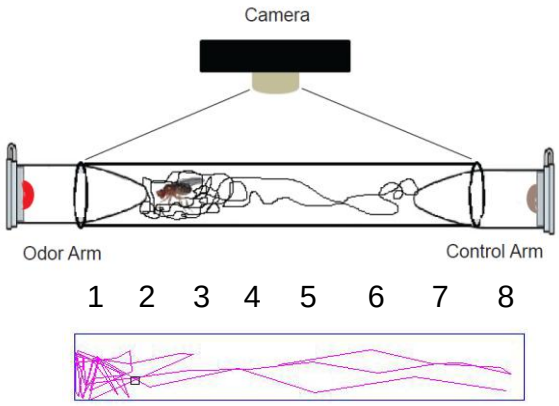


Figure 1. The schematic representation of the single fly behavior assay. Each glass tube was taken with one fly and the tube was closed on both ends with traps prepared with a micropipette tip and microcentrifuge tube (200µl). An overhead camera (Watec Monochrome Video Camera) was used to record the movement of flies during the test. For analysis, the tube was divided into 8 zones and zone 1 was designed as the odor zone, while zone 8 was designed as the control zone.

Analysis was made in a custom written program in MATLAB (R2007b) (Mathworks Inc. USA) which tracks the path of the individual fly in each tube. The flies spend 2 minutes (for attractive odors) or 5 minutes (for aversive odors) in the tube. The tube was divided into 8 zones (zone 1 to zone 8) and zone 1 was designated as the odor zone. The flies display exploratory behavior by making frequent visits to each end of the tube. In the presence of an attractive odor, a fly spends more time around the odor zone (zone 1), whereas in the presence of an aversive odor, it spends more time away from the odor zone (zone 8). The

response index was calculated as: time spent in zone1 – time spent in zone 8)/ Total time of the assay.

Single unit recording from olfactory receptor neurons (ORNs)

3 day -old female flies were cold anesthetized on ice and mounted in a 1.6 mm diameter glass capillary. The protruding head was immobilized with low melting myristic acid. The mounted fly was kept for 45 mins in a moist chamber before recording. Large sensillum basiconica on the third antennal segment were identified by their position on a standard map of the antennal surface. The ground electrode was inserted into the third antennal segment and the recording electrode was placed in the base of the basiconic sensillum. Signals were amplified 1000X (Model 750, World Precision Instruments, New Haven CONN. USA), digitized with a digitizer (NI-DAQ software) and stored in the computer for further analysis with LabVIEW 7.1 software (National Instruments Software, USA). AC signals (100–10,000 Hz) were recorded for 12 s, starting 10 s before stimulation. Action potentials were counted off-line in a 500 ms period before stimulation and during the 500 ms stimulation. The response of individual neurons was then calculated as the increase (or decrease) in action potential frequency (spikes/500 ms). Odor pulses of 500 ms durations separated with an inter-stimulus interval of 10 sec were given using a six port custom-built olfactometer.

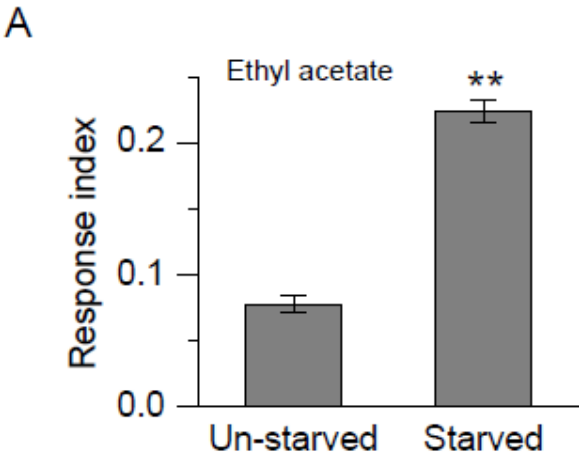
Statistical analysis

Data are shown as mean ± SEM (Standard Error of the Mean). Student's t-test was performed for statistical comparisons and the significance level was set at $P < 0.05$.

Results

Enhanced attraction behavior in starved flies

Behavioral expression of food-associated memory in the fruit fly is constrained by satiety and promoted by hunger, suggesting an influence by the motivational state (Krashes *et al.*, 2009). To determine whether hunger has an effect on food search behavior, we measured single fly behavioral response to ethyl acetate (EA). EA constitutes 33% of the volatiles in pineapple (Umano *et al.*, 1992). Flies were starved for 30 hours in a vial with only moist tissue paper, thereby allowing flies access to water but not food. Starved flies spent more time in the odor zone and exhibited robust increases in attraction behavior to EA, as compared to the un-starved flies (Fig.2A), indicating that hunger promotes food search behavior via the olfactory system. To confirm whether this behavior change is common for attractive odors in general, we measured the single fly behavior in response to another attractive odorant, geranyl acetate (GA). As shown in Fig.2B, starved flies exhibited remarkable increases in attraction behavior to GA as compared to un-starved flies. To test whether this behavioral change by starvation is specific to the attractive odor, we measured the single fly behavioral response to an aversive odor, benzaldehyde. The behavioral response to benzaldehyde was unaffected by starvation (Fig. 2C). Taken together these results suggest that starvation enhances the behavioral response to the attractive odor, but not the aversive odor.



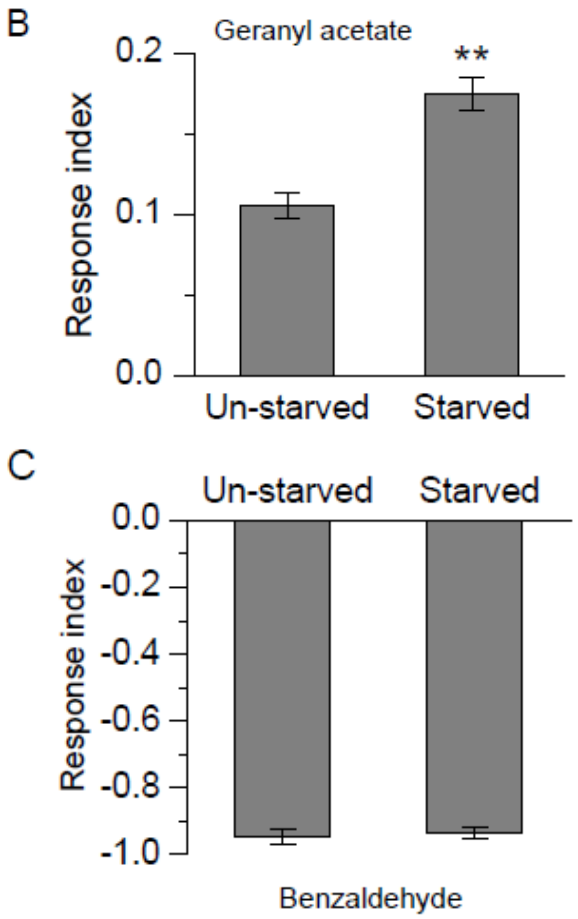


Figure 2: Effect of starvation on single fly behavior. A. Starved flies have a higher response index as compared to un-starved flies when tested with ethyl acetate (EA) at 10^{-6}. Error bars indicate SEM. ** indicates $P < 0.01$, $n = 20$. B. Starved flies have a higher response index as compared to un-starved flies when tested with geranyl acetate (GA) at 10^{-5}. Error bars indicate SEM. ** indicates $P < 0.01$, $n = 20$. C. No significant difference in the aversion behavior in flies measured with benzaldehyde. Error bars indicate SEM. $P > 0.05$, $n = 20$.

Starvation increases the sensitivity of ORNs

We next examined the effect of starvation at the level of olfactory receptor neurons (ORNs). Electrophysiological recordings were performed from the AB II sensillum which responds to a broad spectrum of odorants (mainly esters and ketones). AB II sensilla basiconica have two neurons and their spikes are well identified based on their amplitudes. The neuron with a larger spike amplitude is referred to as the 'A' neuron and the one with a smaller amplitude is referred to as the 'B' neuron (Fig. 3A). The 'A' neuron in AB II sensillum responds strongly to EA, represented by an increase in the firing frequency of the spikes, while the firing frequency in the 'B' neuron is unaffected by EA. Female flies were starved for 30 hrs in a vial with a moist tissue paper bed. We found that the starved flies showed a remarkable increase in the firing frequency of the spikes in the AB IIA neuron when stimulated with low concentrations of EA compared to the un-starved flies, suggesting that hunger enhances ORN sensitivity (Fig. 3B). However, no significant increase was observed in the spike firing rate at high concentrations of EA in the starved flies compared to the un-starved flies (Fig. 3B), indicating that the response at high concentrations has most likely been saturated.

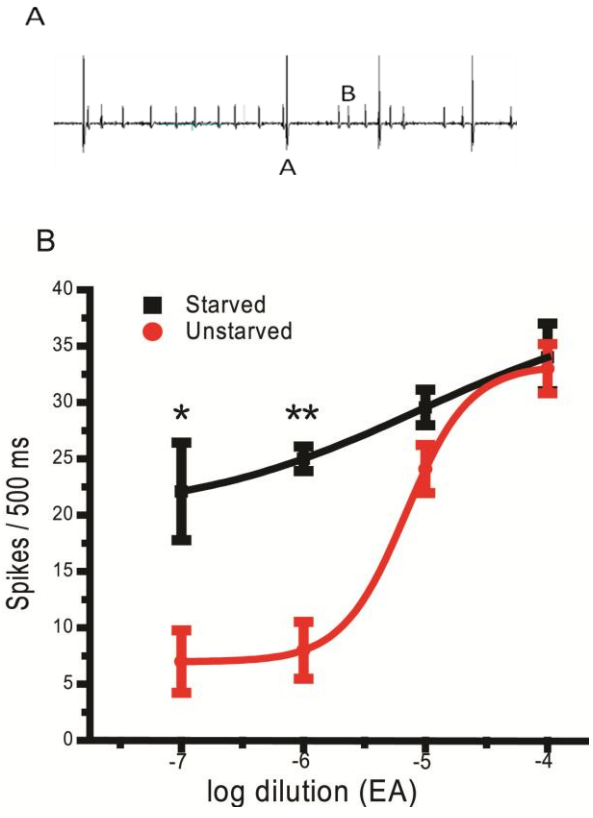


Figure 3: Sensitization of ORNs by starvation. A. Spontaneous spikes recorded in AB II sensillum of ORNs (showing A neuron and B neuron). B. Dose response curves of EA-evoked spikes in the AB IIA neurons of starved and un-starved flies. Wild type (CsBz) female flies were starved for 30 hours. Error bars indicate SEM. * indicates $P < 0.05$; ** indicates $P < 0.01$; $n = 10$.

Discussion

We report here that starvation modulates the sensitivity of the olfactory receptor neurons housed in AB II sensillae basiconica. Starvation increases behavioral attraction to food odors such as EA and GA. Enhanced peripheral sensitization due to starvation is in line with the observation that in Diptera, EAG amplitude increases with starvation (Den Otter *et al*., 1991). The starvation effect is more pronounced at lower concentrations of EA. Increased responsiveness at lower concentrations is found in both electrophysiological and behavioral experiments. The internal state of the fly during starvation acts on sensory neurons. Starvation modulates the peripheral sensitivity in order to match the changing physiological needs of the animal. Our study demonstrates that the firing frequency of AB IIA increases with starvation, indicating enhanced sensitivity of the AB IIA neuron. It is likely that starvation modulates some intracellular pathways to regulate hunger and this is related to the increase in ORN sensitivity. The exact mechanism underlying starvation induced sensitization is still poorly understood. Recently it has been shown that fluctuating metabolic cues control sNPFR1 levels in the AB IB neuron that houses Or42b receptors, which in turn modulate feeding behavior (Root *et al.*, 2011). However, it has yet to be determined whether the enhanced sensitization we measured in AB IIA neuron is also subject to sNPF-dependent modulation.

In mammals, when animals are starved due to lack of a food supply, metabolic changes occur in stored energy metabolites, including carbohydrates, lipids and proteins, which occurs sequentially in that order (Barsh & Schwartz, 2002). Animals are forced to look for food due to starvation-induced changes in the levels of hormones and neuropeptides, such as insulin, ghrelin, leptin and NPY, and these modulators act on the hypothalamus, the appetite controlling center in mammals (Brown et al., 1999; Hewes & Taghert, 2001; Mayer & Belsham, 2009; Hong et al., 2012). This raises the possibility that a central mechanism controlling feeding behavior is also important in *Drosophila*. A recent study demonstrates that appetitive memory requires the NPF receptor in the dopaminergic neurons that innervate specific lobes of the mushroom body (Krashes, et al., 2009). This raises the question of whether multiple neural substrates are required for starvation-dependent modulation. Enhanced peripheral sensitization due to starvation may serve as a switch for animals to detect specific food odors. Features of the peripheral olfactory system are very similar in *Drosophila* and mammals. It is very likely that in mammals hunger also reshapes the sensitivity of the olfactory system. The anatomical simplicity of *Drosophila* makes this organism a particularly amenable system for identifying the neuronal changes that result from starvation and linking starvation's role to behavior. Results from such experimentation could then provide direction for conducting mammalian studies.

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