

Feeding Behavior Regulation in the Fly: Effect of a Noxious Substance through the Taste and Olfactory Neurons

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Introduction

The contact chemosensilla on the labellum of the fly are in the form of a hair housing five sensory neurons, i.e. four contact chemoreceptor cells and one mechanoreceptor neuron (Ozaki and Tominaga, 1999). Three of the four chemoreceptor cells are the sugar, salt and water receptor cells, respectively. The remaining chemoreceptor cell, traditionally called the 'fifth cell', has recently been proposed to function as a deterrent cell (Liscia and Solari, 2000; Ozaki *et al.*, 2003). Thus, the balance of response between the sugar receptor cell and the deterrent cell could determine feeding behavior of the fly.

Previously, Ozaki *et al.* (1995) found an odorant-binding protein (chemical sense-related lipophilic ligand-binding protein, CRLBP) common to the taste and olfactory sensilla of the blowfly, *Phormia regina*. This acidic, small molecular protein binds lipophilic noxious compounds like D-limonene, and since aversive behavior is triggered when the fly touches such a noxious compound, this sensory cell may be related to aversive behavior via CRLBP.

On the other hand, during food searching by flies, a noxious compound such as D-limonene, which has strong oral toxicity (Ozaki *et al.*, 2003), should carefully be avoided. *Phormia regina* is not counted as a phytophagous insect but the adult flies are nectar feeders. Hence they could encounter monoterpenes, which are abundantly present in citrus rinds, by chance. The flies may exhibit more effective aversive behavior toward the D-limonene when both the taste and the olfactory receptors are stimulated spontaneously. Thus, the olfactory inputs from the antennae and/or the maxillary palps may be expected to influence the feeding or the aversive behavior in the flies.

Gustatory cue of D-limonene induces aversive reaction

To evaluate the oral toxicity of monoterpenes, we measured the electrophysiological response to them. Of all the monoterpenes examined, D-limonene exhibited the strongest oral toxicity (Ozaki *et al.*, 2003). When the flies were forced to ingest 0.3 μ l of D-limonene, L-limonene, cineol, citral or β -myrcene, 87, 40, 30, 0 or 0% of number of flies were killed within 30 min, respectively. Contact of a chemosensillum with D-limonene induced the severest aversive behavior with vomiting and/or excretion in the fly. D-Limonene, when dispersed in an aqueous solution including CRLBP, evoked impulses from the 'fifth cell'. Considering the relationship between the aversive effects of D-limonene and the response of the 'fifth cell' to D-limonene, we suggested that in the insect contact-chemosensillum, the CRLBP carries lipophilic members of the noxious taste substances to the 'fifth cell' through the aqueous sensillum lymph, and that the 'fifth cell' is indispensable receptor neuron for the toxin detection system in the fly. Thus, our work on *P. regina* is the first electrophysiological study of the role of an odorant-binding protein in an insect taste system.

Olfactory stimulation with D-limonene decreases the fly's appetite

The relationship between the feeding response and sugar concentration was investigated using the proboscis extension reflex (PER) test, and the feeding sensitivity of a group of flies was indicated as a mean value of the feeding threshold concentration of sucrose. The mean value of the feeding threshold was taken as an indicator of appetite and was determined by half of the maximum concentration of the PER-concentration curve. When the flies increased their appetite, the mean value of the feeding threshold decreased, and vice versa. We carried out the PER test at various concentrations of sucrose in the absence or presence of D-limonene odor. In the presence of D-limonene, the mean value of the feeding threshold increased threefold. The appetite reducing effect was also observed after the dietary experience with the D-limonene odor. The flies, which were fed on sucrose in the presence of D-limonene for 5 days after eclosion, exhibited obvious appetite reduction to sucrose even in the absence of this compound. Thus, dietary experience with the odor of a toxic substance suppresses the feeding motivation in the flies, reducing the probability of ingesting toxic substances.

Effect of mushroom body ablation

Considering the experiential effect of the diet in the presence of D-limonene, one may expect that the mushroom body, a neural structure involved in learning in the insect brain, may contribute to the effect. We succeeded in ablating the mushroom body of *P. regina* by hydroxy urea treatment of the larvae (de Belle and Heisenberg, 1994). When we carried out the same PER test in the mushroom body-ablated flies, they showed appetite reduction in the presence of D-limonene both before and after the dietary experience with that odor. Thus, the fly can integrate olfactory information about D-limonene with the taste information about sucrose without the mushroom body. However, the mushroom body-ablated fly showed normal appetite to sucrose even after the dietary experience with the odor of D-limonene. This suggested that the mushroom body-ablated fly could not learn or remember the dietary experience, through which aversive conditioning between the taste of sucrose and the odor of D-limonene should occur.

Effect of antennae or maxillary palps

We also carried out the same PER tests with the flies whose antennae or maxillary palps were removed. Removal of antennae influenced the memory of dietary experience with the odor of D-limonene, but removal of maxillary palps did not. Thus, the neural routes from the maxillary palps may not be involved in formation of associative memory between the taste of sucrose and the noxious odor of D-limonene.

Fluorescence labeling of maxillary afferents revealed a distinct fiber bundles that projected into the subesophageal ganglion (SOG) and ascended further into the glomeruli in the ipsilateral and contra-

lateral antennal lobes. The antennal afferents innervated into all the remaining glomeruli in the ipsilateral and contralateral antennal lobes, and some fiber bundles projected into SOG. Thus, the projection patterns from the antennae and the maxillary palps may not overlap, suggesting that olfactory inputs from the antennae are processed independently of those from the maxillary palps.

Conclusion

The blowfly, *P. regina*, has taste sensilla with four contact-chemoreceptor cells. The sugar receptor cell activity induces feeding response of the fly. We measured the electrophysiological response of the 'fifth cell' to monoterpenes having oral toxicity for the flies. D-limonene, which exhibited the strongest oral toxicity of all the monoterpenes examined, evoked impulses of the 'fifth cell' with the help of an odorant-binding protein in the taste sensillum, and induced strong aversive behavior (vomiting or excretion). The 'fifth cell' may be a warning cell that functions as a taste system for detecting and avoiding dangerous foods.

Moreover, the odor of D-limonene inhibited the feeding behavior, which was induced by excitation of the sugar receptor cell. The dietary experience with the odor caused appetite reduction lasting for a month or longer (The life time of *Phormia* is up to 2 months). Thus, one substance, D-limonene, when detected as a taste and an odor

through two different modalities of chemical senses, respectively, strongly deterred the flies from feeding.

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