

## Circuitry of Second-order Gustatory Neurons

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11:00 ~ 12:00

**Seminar Room A7F**

With a relatively simple nervous system and a plethora of genetic tools, *Drosophila* affords an excellent model for mapping neural circuits. Previous studies identified a number of gustatory sensory neurons (GSNs) that relay taste information to the gnathal (subesophageal) ganglia of the brain. However, little is known about the identities of the neurons that follow the GSNs, or how these followers process taste information. Here we used a combination of established and novel genetic tools to identify candidate second-order gustatory neurons. After screening ~5,000 GAL4 lines -anatomically, we identified 32 lines that label neurons whose dendrites arborize in the gnathal ganglia. As a secondary screen, we used the GRASP (GFP reconstitution across synaptic partners) technique to visualize potential contacts between the candidate neurons' dendrites and the axonal terminals of Gr5a-expressing GSNs, which have been shown to respond to sucrose. To differentiate mere membrane contacts from true synapses, we incorporated an active-zone marker (Brp-mCherry) to label presynaptic sites of Gr5a-expressing GSNs and checked whether GRASP signal is colocalized with presynaptic sites. Finally, by expressing a genetically-encoded calcium indicator (G-CaMP6m) in the candidate neurons and by using a novel tastant-delivery system, we determined whether the neurons showed changes in activity upon delivering sucrose to the proboscis. Two types of candidate second-order neurons met these criteria. Together, these results suggest our candidate neurons receive excitatory input from sucrose-responsive Gr5a-expressing GSNs. Further, we analyzed distributions of input and output sites of one of the candidate neurons using GFP/RFP-tagged acetylcholine receptor subunit (Dα7) and active-zone marker (Brp), respectively. Whereas postsynaptic sites were almost coincident with the synaptic contacts to Gr5a-expressing neurons, presynaptic sites were distributed in distinct regions, suggesting that the labeled neurons transmit information to distinct third-order neurons.

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