Rapid degeneration of *Drosophila* olfactory neurons in *Orco* mutant maxillary palps

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Abstract

*Drosophila melanogaster* vinegar flies have two olfactory organs: the antenna and maxillary palp. Olfactory neurons in these tissues respond to odorants via odorant receptors. Insect odorant receptors are heterotetramers of two proteins: an odorant binding OrX subunit and an Odorant Receptor Co-Receptor (*Orco*). Mutation of *Orco* disrupts odorant receptor formation, and abolishes olfactory responses. Some antennal olfactory neurons in *Orco* mutants have been previously shown to degenerate. Here, we examine if maxillary palp olfactory neurons also degenerate in *Orco* mutants. We find degeneration occurs both more broadly and more rapidly in *Orco* mutant maxillary palp olfactory neurons than reported for antennae, with ~60% of all mutant olfactory neurons absent in maxillary palps by 7 days post eclosion. Interestingly, the subset of *Orco* mutant olfactory neurons that express the Or42a receptor appear resistant to degeneration. These results suggest the maxillary palp might be a suitable model for examining the molecular mechanisms underlying neurodegeneration in sensory neurons.

Figure 1. Degeneration of olfactory neurons in *Orco* mutant maxillary palps: A. *Orco* mutant olfactory neuron cell bodies in the maxillary palps are lost by 7 days post eclosion. An anti-elav antibody was used to visualize all neuronal cell bodies in the palps. This includes all olfactory neurons (~120), as well as a small population of presumed mechanosensory neurons (~20). Wildtype (top row) and *Orco* mutant (bottom row) palps compared at three timepoints: 3 (left column), 7 (middle column), and 15 (right column) days post eclosion (DPE). B. Quantification of elav+ cells in wildtype and *Orco* mutant palps at the three timepoints (N = 3 per condition). Wildtype flies have consistent numbers of neurons throughout. *Orco* mutant palps appear normal at 3 DPE but lose approximately 60% of their neurons by day 7. At 15 DPE the *Orco* mutant palps appear normal at 3 DPE but lose approximately 60% of their neurons by day 7.
The odorant receptor co-receptor Orco forms ligand-gated ion channels with odorant receptors (ORs) tuned to specific odors; Orco is required for trafficking of these complexes to the dendrites of olfactory neurons (Larsson et. al., 2004). Mutation of the Orco gene not only abolishes olfactory neuron activity but has also revealed potential new roles for this gene in the development and maintenance of olfactory systems in a variety of insects. In ants and honeybees, Orco mutation leads to a loss of olfactory sensory neurons in the periphery, as well as severe defects in the morphology of the antennal lobe, the first olfactory processing center in the insect brain (Trible et al., 2017; Yan et al., 2017; Chen et al., 2021). These deficits are likely developmental as they are apparent at adult eclosion. Orco mutant hawkmoth males show more modest losses in antennal lobe volume associated with pheromone-sensing olfactory neurons (Fandino et al., 2019). In contrast, in Anopheles coluzzii and Aedes aegypti mosquitoes Orco mutation does not appear to lead to olfactory neuron loss or gross deficits in antennal lobe anatomy (though the latter has not been examined in depth) (Sun et al., 2020; DeGennaro et al., 2013).

In the vinegar fly Drosophila melanogaster, development of the adult olfactory system in Orco mutants appears normal (Larsson et. al., 2004). However, over time some populations of olfactory neurons in the antennae degenerate. This degeneration is initially evident in the olfactory neuron axons innervating the antennal lobe, which start to show signs of blebbing and retraction four to six days post eclosion (DPE) (Chiang et al., 2009). By 14 DPE, some but not all antennal olfactory neurons lose their cell bodies (Hueston et al., 2016). There is evidence that the cause of antennal olfactory neuron degeneration in the Orco mutant is due to a lack of neuronal activity (Chiang et al., 2009). Here, we investigated if neurodegeneration in Orco mutants also occurs in the Drosophila maxillary palp, a simpler olfactory organ with ~120 olfactory neurons.

To examine the effects of the loss of Orco on maxillary palp olfactory neurons, we performed whole-mount antibody staining on maxillary pals using the pan-neuronal marker, elav (Robinow and White, 1991). This marker should label all ~120 olfactory neurons in the pals, as well as a small population (~20) of presumed mechanosensory neurons (Singh and Nayak, 1985). Neuronal cell death should result in a reduction in the number of elav+ cells. Potential alternative approaches could be to use DAPI staining or the TUNEL assay to visualize cell loss and DNA fragmentation associated with cell death, among others (Meehan et al., 2015). We compared anti-elav antibody staining in wildtype and Orco2 mutant flies at three timepoints: 3, 7, and 15 days post eclosion (DPE) (Fig 1A). In the wildtype, we found consistent neuronal cell counts across timepoints. The Orco2 mutant palsps of 3-day-old flies were indistinguishable from wildtype (Fig 1A). However, by 15 DPE, extensive loss of cell bodies was detectable in the Orco2 mutants (Fig 1A). This neurodegeneration was evident at 7 DPE (middle column), suggesting that cell body loss in the maxillary palp occurs more quickly and potentially more broadly than in the antennae. Overall, Orco2 mutants lose 60 – 70% of their maxillary palp neurons. These results are quantified in Fig 1B. Both 7 and 15 DPE Orco2 mutants had significantly fewer elav+ cells compared to all wildtype timepoints as well as 3 DPE Orco2 mutants (p ≤ 0.0002) but were not statistically significantly different from each other (p = 0.4055) (one-way ANOVA with Tukey’s HSD; see figure legend for F statistic and exact p values for all pairwise comparisons). There was no difference between any of the wildtype conditions (p > 0.99), nor between the 3 DPE Orco2 mutant and any of the wildtype timepoints (p > 0.79).

We further examined if sub-populations of olfactory neurons in the maxillary palp might be resistant to degeneration. There are six different populations of olfactory neurons in the maxillary palp as defined by the odor-responsive OrX they express. Using available genetic reagents, we examined neuron degeneration in two of these populations in the Orco mutant background. We used the GAL4/UAS system (Brand and Perrimon, 1993) to GFP label the pb1A (Or42a-
Fly husbandry and Drosophila genetics

Fly stocks were maintained at 20 – 25°C on standard cornmeal-agar food. Male and female flies used for experiments were 3 – 15 days old. Exact age for each experimental condition indicated in Figure 1. Experimental flies were homozygous for Orco<sup>2</sup>, while control flies were either w<sup>1118</sup> wildtypes (Figure 1A-B), or heterozygous for Orco<sup>2</sup> (Orco<sup>2</sup>/TM6b; Figure 1C-D). Full genotypes of stocks used in Figure 1C-D: Or42a-Gal4, 10XUAS-IVS-mCD8::GFP / CyO ; Orco<sup>2</sup> / TM6b and Or46a-Gal4, 10XUAS-IVS-mCD8::GFP / CyO ; Orco<sup>2</sup> / TM6b.

Immunohistochemistry

All immunostaining steps were done while rotating. Fly proboscises (labela and palps) were dissected in 1XPBS and fixed in 4% paraformaldehyde in PBT (1XPBS + 0.3% Triton X-100) for 15 minutes at room temperature. Tissue was washed three times for 15 minutes each at room temperature in PBT, then blocked for at least 30 minutes at room temperature in blocking solution (PBT + 5% normal goat serum). Primary antibodies were added to fresh blocking solution, and tissue was incubated in this solution overnight at 4°C. On day two, tissue was washed three times for 15 minutes each at room temperature in PBT, then incubated in secondary antibodies in fresh block overnight at 4°C in the dark. On day three, tissue was washed three times for 15 minutes each at room temperature in the dark and mounted in SlowFade Gold (ThermoFisher S36936). Palps were dissected from labela on the slide before mounting. Primary antibodies were used at 1:100 concentration, secondary antibodies were used at 1:200 concentration. See Reagents for antibodies used.

Confocal imaging and analysis

Palps were imaged on a Zeiss LSM 700 confocal microscope equipped with a C-Apochromat 63x/1.2 water Korr M27 objective. Images were acquired at 512 x 512-pixel resolution with 0.58 μm z-step. For illustration purposes, confocal images were processed in Fiji/ImageJ to collapse Z-stacks into a single image using maximum intensity projection. Fiji was also used to adjust the gain in separate channels; no other image processing was performed on the confocal data. Elav<sup>+</sup> and GFP<sup>+</sup> cells were counted manually in Fiji using the Cell Counter plugin.

Statistics

All statistical analyses and plots were done in GraphPad Prism (version 8). For all analyses, significance level α = 0.05. In Figure 1A-B, one-way ANOVA with Tukey’s HSD post-hoc test was used to compare elav<sup>+</sup> cell counts across both
genotypes at the three timepoints. In Figure 1C-D, Mann Whitney U tests were used to compare GFP+ cell counts in the two genotypes within each neuron type.

Reagents

**Drosophila melanogaster** stocks used:

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<td>Orco(^2) mutant: w[<em>]; T(1)[w+[m</em>]=T1]Orco[2]</td>
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Antibodies used:

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References


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