Candidate pheromone receptors of codling moth *Cydia pomonella* respond to pheromones and kairomones

Cydia pomonella L.

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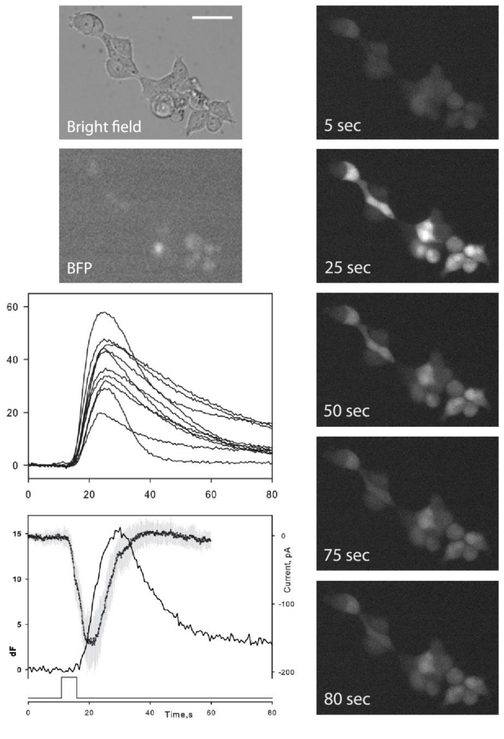
Background

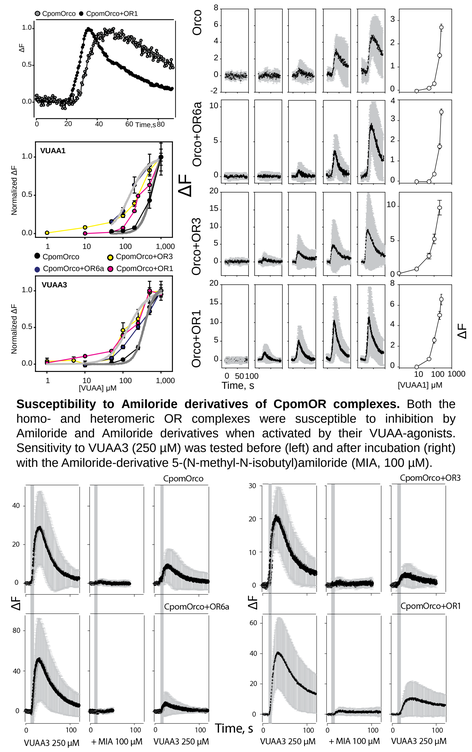
Olfaction plays a dominant role in the mate-finding and host selection behaviors of the codling moth (*Cydia pomonella*), an important pest of apple, pear and walnut orchards. Antennal transcriptome analysis (Bengtsson et al. 2012, Walker et al. 2016) revealed a number of abundantly expressed genes related to the moth olfactory system, including those encoding the olfactory receptors (ORs) CpomOR1, CpomOR3 and CpomOR6a, which belong to the putative pheromone receptor (PR) lineage, and the co-receptor (CpomOrco). Using heterologous expression, in both human embryonic kidney (HEK293T) cells and in *Drosophila* olfactory sensory neurons, coupled with calcium imaging and electrophysiological recording, respectively, we characterize the basic physiological and pharmacological properties of these receptors and demonstrate that they form functional ionotropic receptor channels.

Functional expression of CpomORs in Human Embryonic Kidney (HEK293T) cells

Functional expression of homomeric CpomOrco in HEK293T cells. Functional expression of CpomOrco was verified by Calcium imaging and single-cell patch clamp recording experiments (stimulus - 250 µM VUAA1, scale bar - 20 µm).

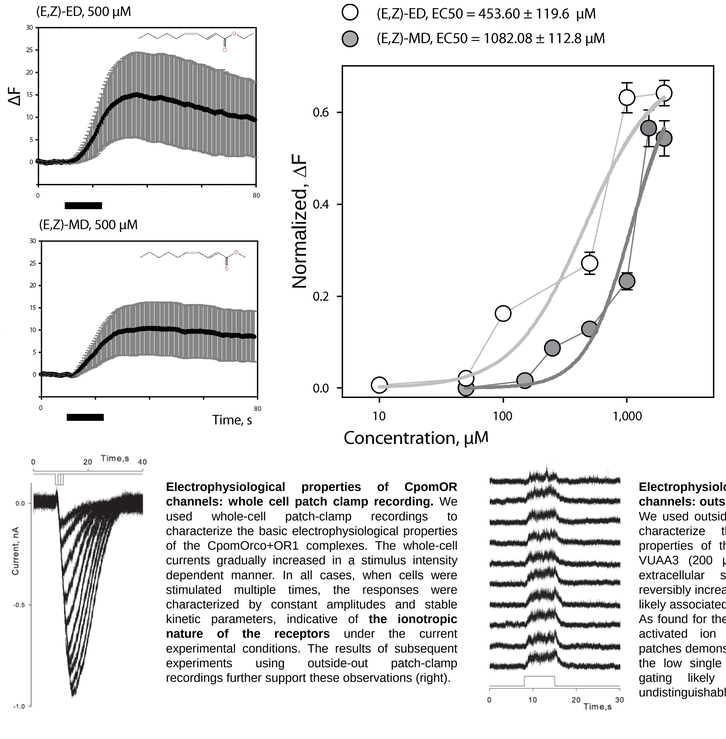


Activation of homomeric CpomOrco and heteromeric CpomOrco+OR complexes by VUAA-agonists. Different OR complexes showed different sensitivity to VUAA1 and VUAA3 agonists and demonstrated different activation/inactivation kinetics.

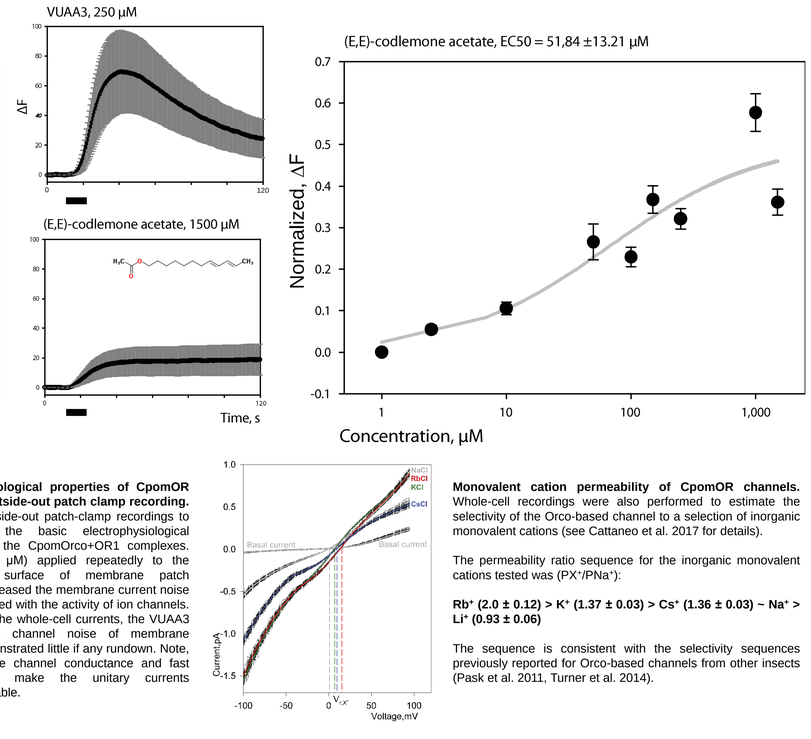


Susceptibility to Amiloride derivatives of CpomOR complexes. Both the homo- and heteromeric OR complexes were susceptible to inhibition by Amiloride and Amiloride derivatives when activated by their VUAA-agonists. Sensitivity to VUAA3 (250 µM) was tested before (left) and after incubation (right) with the Amiloride-derivative 5-(N-methyl-N-isobutyl)amiloride (MIA, 100 µM).

Functional characterization of CpomOR3. Left: comparison of CpomOrco+OR3 amplitudes of the Calcium responses (mean of the maximum response ± SEM) to 500 µM **ethyl-(E,Z)-2,4-decadienoate** (pear ester, (E,Z)-ED, 15.07 ± 9.48, dF; up) and to 500 µM **methyl-(E,Z)-2,4-decadienoate** (methyl ester, (E,Z)-MD, 10.40 ± 5.91, dF; down); n = 151. Black bar: stimulus. Right: normalized dose-response of pear ester (white) and methyl ester (grey).



Functional characterization of CpomOR6a. Left: comparison of CpomOrco+OR6a amplitudes of the Calcium responses (mean of the maximum response ± SEM) to 250 µM VUAA3 (69.71 ± 27.29, dF; up) and to 1500 µM (E,E)-8,10-dodecadien-1-yl acetate [(E,E)-codlemone acetate, 18.91 ± 10.31, dF; down]; n = 68. Black bar: stimulus. Right: normalized dose-response to (E,E)-codlemone acetate.



Electrophysiological properties of CpomOR channels: whole cell patch clamp recording. We used whole-cell patch-clamp recordings to characterize the basic electrophysiological properties of the CpomOrco+OR1 complexes. The whole-cell currents gradually increased in a stimulus intensity dependent manner. In all cases, when cells were stimulated multiple times, the responses were characterized by constant amplitudes and stable kinetic parameters, indicative of **the ionotropic nature of the receptors** under the current experimental conditions. The results of subsequent experiments using outside-out patch-clamp recordings further support these observations (right).

Electrophysiological properties of CpomOR channels: outside-out patch clamp recording. We used outside-out patch-clamp recordings to characterize the basic electrophysiological properties of the CpomOrco+OR1 complexes. VUAA3 (200 µM) applied repeatedly to the extracellular surface of membrane patch reversibly increased the membrane current noise likely associated with the activity of ion channels. As found for the whole-cell currents, the VUAA3 activated ion channel noise of membrane patches demonstrated little if any rundown. Note, the low single channel conductance and fast gating likely make the unitary currents undistinguishable.

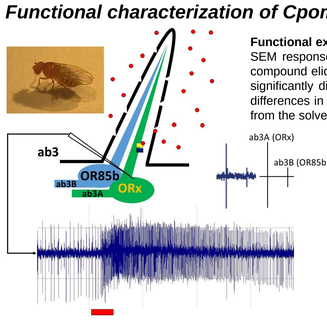
Monovalent cation permeability of CpomOR channels. Whole-cell recordings were also performed to estimate the selectivity of the Orco-based channel to a selection of inorganic monovalent cations (see Cattaneo et al. 2017 for details).

The permeability ratio sequence for the inorganic monovalent cations tested was (PX^+/PNa^+):

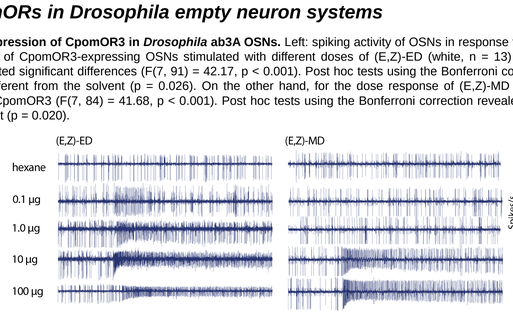
Rb^+ (2.0 ± 0.12) > K^+ (1.37 ± 0.03) > Cs^+ (1.36 ± 0.03) ~ Na^+ > Li^+ (0.93 ± 0.06)

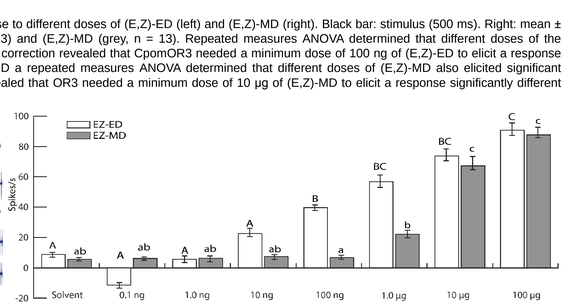
The sequence is consistent with the selectivity sequences previously reported for Orco-based channels from other insects (Pask et al. 2011, Turner et al. 2014).

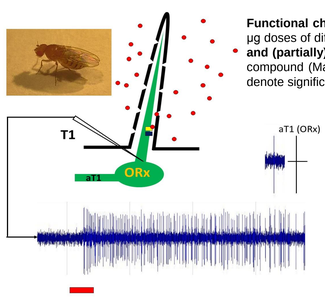
Functional characterization of CpomORs in Drosophila empty neuron systems



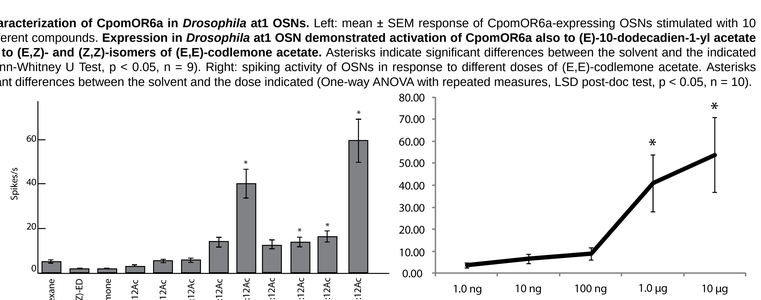
Functional expression of CpomOR3 in *Drosophila* ab3A OSNs. Left: spiking activity of OSNs in response to different doses of (E,Z)-ED (left) and (E,Z)-MD (right). Black bar: stimulus (500 ms). Right: mean ± SEM response of CpomOR3-expressing OSNs stimulated with different doses of (E,Z)-ED (white, n = 13) and (E,Z)-MD (grey, n = 13). Repeated measures ANOVA determined that different doses of the compound elicited significant differences ($F(7, 91) = 42.17$, $p < 0.001$). Post hoc tests using the Bonferroni correction revealed that CpomOR3 needed a minimum dose of 100 ng of (E,Z)-ED to elicit a response significantly different from the solvent ($p = 0.026$). On the other hand, for the dose response of (E,Z)-MD a repeated measures ANOVA determined that different doses of (E,Z)-MD also elicited significant differences in CpomOR3 ($F(7, 84) = 41.68$, $p < 0.001$). Post hoc tests using the Bonferroni correction revealed that OR3 needed a minimum dose of 10 µg of (E,Z)-MD to elicit a response significantly different from the solvent ($p = 0.020$).







Functional characterization of CpomOR6a in *Drosophila* at1 OSNs. Left: mean ± SEM response of CpomOR6a-expressing OSNs stimulated with 10 µg doses of different compounds. **Expression in *Drosophila* at1 OSN demonstrated activation of CpomOR6a also to (E)-10-dodecadien-1-yl acetate and (partially) to (E,Z)- and (Z,Z)-isomers of (E,E)-codlemone acetate.** Asterisks indicate significant differences between the solvent and the indicated compound (Mann-Whitney U Test, $p < 0.05$, $n = 9$). Right: spiking activity of OSNs in response to different doses of (E,E)-codlemone acetate. Asterisks denote significant differences between the solvent and the dose indicated (One-way ANOVA with repeated measures, LSD post-doc test, $p < 0.05$, $n = 10$).



Conclusions

- We characterized recombinant Codling Moth ORs transiently expressed in HEK293T cells.
- Using Calcium imaging and whole-cell/outside-out patch clamp recordings, we demonstrated that both the homomeric CpomOrco channel forming-subunit and heteromeric CpomOR complexes have different kinetics of activation by the Orco agonists VUAA1 and VUAA3 and they are also susceptible to inhibition by amiloride derivatives.
- Whole cell and outside-out patch clamp recordings demonstrated CpomOR complexes forming functional ionotropic receptor channels.
- Functional expression of CpomOR3 confirmed sensitivity to pear ester (Bengtsson et al. 2014) and to the analogous methyl ester.
- Functional expression of CpomOR6a demonstrated sensitivity to (E,E)-codlemone acetate.
- Heterologous methods in *Drosophila* empty neuron systems demonstrated activation to the same ligands and identified partial agonists for CpomOR6a.

Functional expression of CpomORs therefore represents a valuable tool that can be utilized to further investigate mechanisms of insect OR function and develop novel means to intervene and control the pest's behavior (Cattaneo 2018).

References

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