

Supplementary Materials for

Activation State of the M3 Muscarinic Acetylcholine Receptor Modulates Mammalian Odorant Receptor Signaling

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The PDF file includes:

- Fig. S1. The M3-R increases the activation of untagged ORs in Hana3A cells.
- Fig. S2. The muscarinic acetylcholine receptor family members modulate OR signaling.
- Fig. S3. The M2-R is not detectable in the olfactory cilia.
- Fig. S4. Effects of carbachol, forskolin, and ionomycin on OR signaling.
- Fig. S5. Detailed response curves and response values of OSNs.
- Fig. S6. The M3-R does not affect the ligand specificity of ORs.
- Fig. S7. FLAG–OR-S6 is present at the cell surface when coexpressed with the M3-R and RTP1S.
- Fig. S8. FLAG–OR-S6 responds as robustly as Rho–OR-S6 and more robustly than untagged OR-S6.
- Fig. S9. Graphical explanation of the trapezoidal method.
- Fig. S10. The M3-R enhances the function of various mammalian ORs.
- Table S1. GPCRs tested for potentiation of OR signaling.

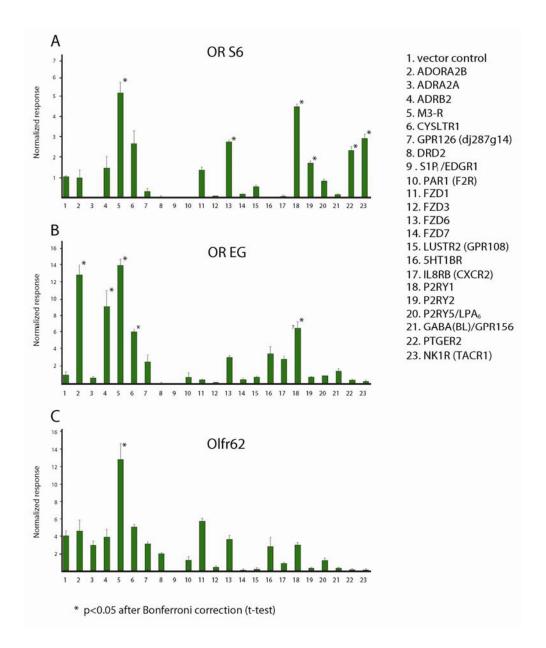


Fig. S1. The M3-R increases the activation of untagged ORs in Hana3A cells. Hana3A cells were cotransfected with plasmids encoding one of 22 GPCRs, as described for Fig. 1. The M3-R was the only candidate to significantly potentiate the activation of all three untagged ORs.

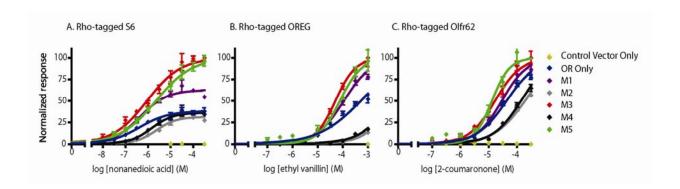


Fig. S2. The muscarinic acetylcholine receptor family members modulate OR signaling. Although the M3-R was the most potent receptor for potentiating OR signaling and was the only receptor to do so for a broad range of ORs, other Gq-coupled (odd-numbered) muscarinic family members also potentiated OR signaling. In contrast, the Gi-coupled (even-numbered) muscarinic receptors inhibited the activation of (**A**) OR S6, (**B**) OR-EG, and (**C**) Olfr62.

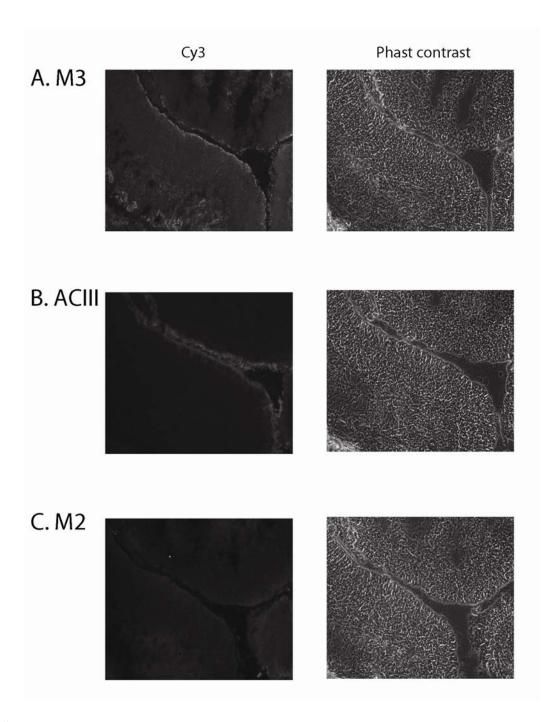


Fig. S3. The M2-R is not detectable in the olfactory cilia. In comparison to the M3-R and ACIII, the M2-R was not present in olfactory cilia, suggesting that, in contrast to M3-R, the M2-R does not play a substantial role in olfaction in vivo.

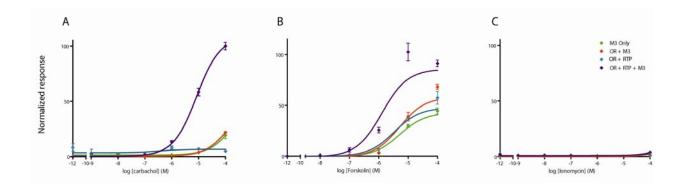


Fig. S4. Effects of carbachol, forskolin, and ionomycin on OR signaling. (**A**) Stimulation of cells containing the M3-R, an OR, and RTP1S with carbachol did not substantially alter the amount of cAMP generated at concentrations lower than 10^{-6} M; however, at higher concentrations, carbachol induced increased responses, apparently through transactivation of OR signaling. This response was not observed in the absence of the M3-R. (**B**) A small increase in response at 1mM carbachol may be attributed to the low abundance of endogenous M3-R in HEK 293T cells. Administration of forskolin showed that in the presence of the M3-R, the extent of OR activation was increased in the absence of odorant, suggesting that the M3-R–OR interaction potentiated OR signaling downstream of odorant binding. (**C**) Ionomycin had no effect on OR activation in the presence or absence of the M3-R, confirming that the M3-R–OR interaction was not as a result of crosstalk of the cAMP and Ca²⁺ signaling pathways in the activation of the CRE-luc reporter.

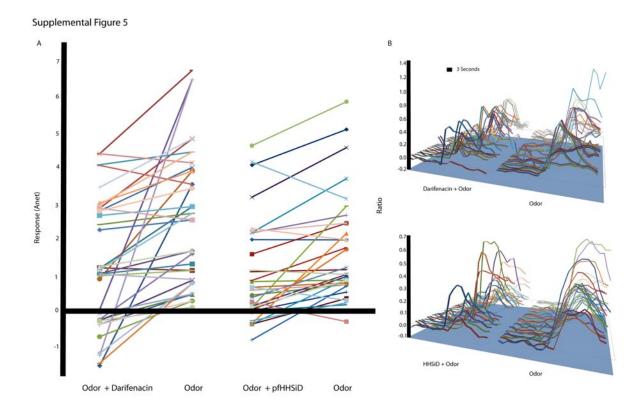


Fig. S5. Detailed response curves and response values of OSNs. Paired recordings of OSN responses that were used for statistical analysis in Fig. 5 are shown. (**A**) single-neuron response profiles; (**B**) two-point analysis of net response area (A_{net}) comparing the responses to either antagonist with odor and odor alone.

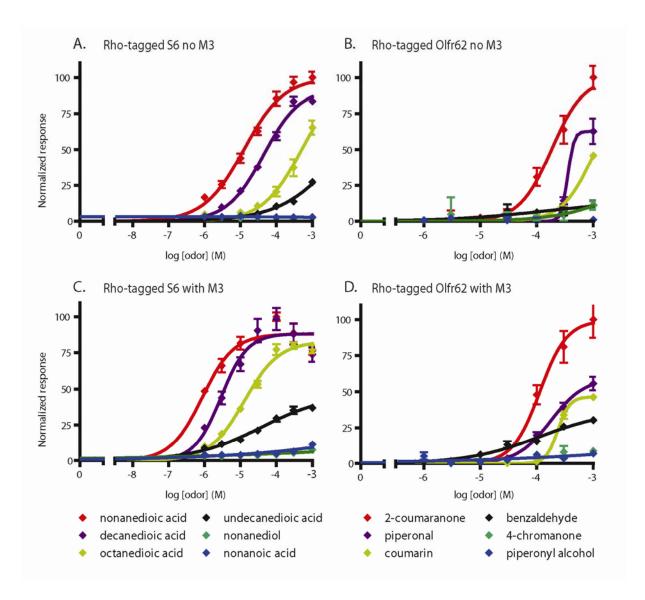


Fig. S6. The M3-R does not affect the ligand specificity of ORs. Dose-response curves of luciferase assays performed in HEK 293T cells transfected with plasmids encoding (**A**) Rhotagged OR-S6 and the M3-R, (**B**) Rhotagged OR-S6 alone, (**C**) Rhotagged Olfr62 and the M3-R, and (**D**) Rhotagged Olfr62 alone. Error bars indicate the SEM, and assays were completed in triplicate.

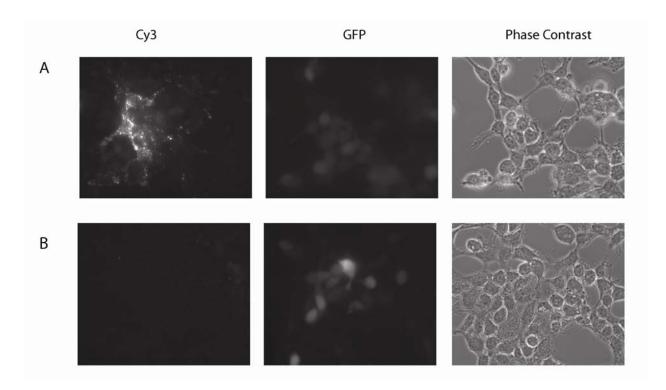


Fig. S7. FLAG-OR-S6 is present at the cell surface when coexpressed with the M3-R and RTP1S. (**A**) To ensure that FLAG-OR-S6 was present at the cell surface and could therefore interact with the M3-R localized at the cell surface, we used antibodies against the FLAG tag to detect ORs at the surface of HEK 293T cells cotransfected with plasmids encoding the M3-R and RTP1S. (**B**) In contrast, when RTP1S and the M3-R were not present, the abundance of FLAG-OR-S6 at the cell surface was low.

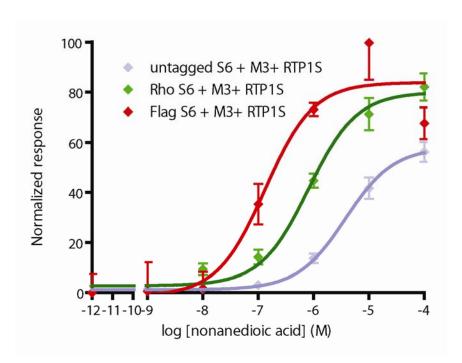


Fig. S8. FLAG-OR-S6 responds as robustly as Rho-OR-S6 and more robustly than untagged OR-S6. To ensure that FLAG-tagged OR-S6 proteins functioned similarly to Rho-tagged OR-S6 proteins in transfected cells, we performed a cAMP-mediated gene assay to assess the functional response of FLAG-OR-S6 in comparison to that of Rho-tagged OR-S6. We found that FLAG-tagged OR-S6 responded similarly, if not more robustly, than did Rho-tagged OR-S6, indicating that FLAG-tagged OR-S6 was functionally active on the cell surface. Results were obtained in triplicate; each experiment was repeated three times.

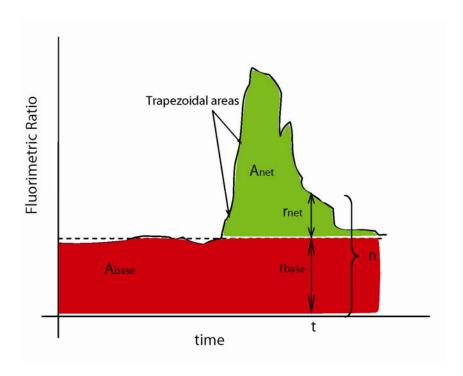


Fig. S9. Graphical explanation of the trapezoidal method. The trapezoidal method was used to calculate the net responses of individual OSNs presented in Fig. 5. Briefly, the green region marks the area of response as calculated by totaling the average response (r_t) over the a specified time interval multiplied by a set increment of time, t, to produce trapezoidal approximations of the area underneath the response curve. The total area under the curve was then normalized by subtracting the area underneath the r_{base} , which is A_{base} , to obtain the net response, A_{net} , that represents the net response r_{net} over the entire response time. For detailed explanations, see the Materials and Methods.

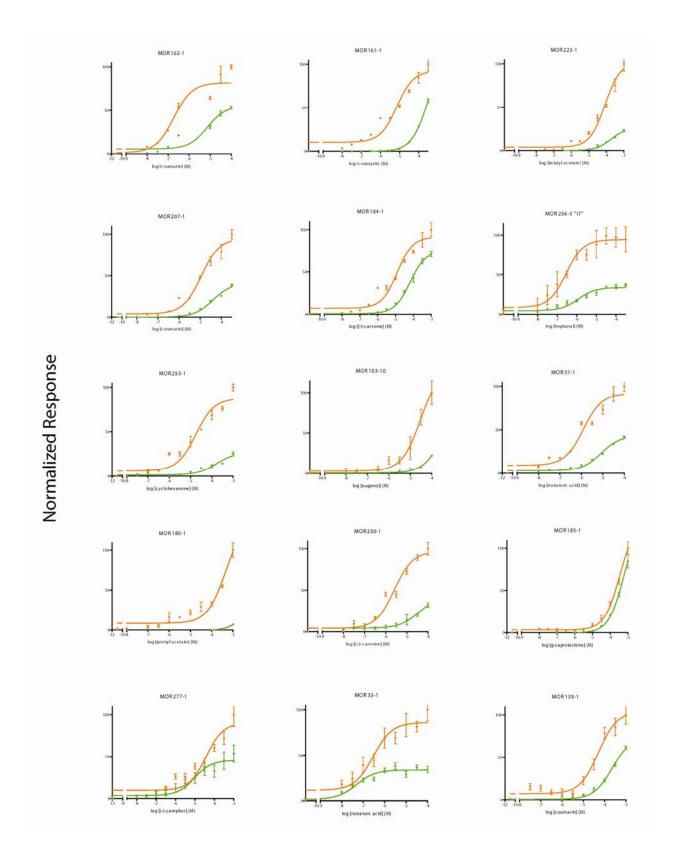


Fig. S10.

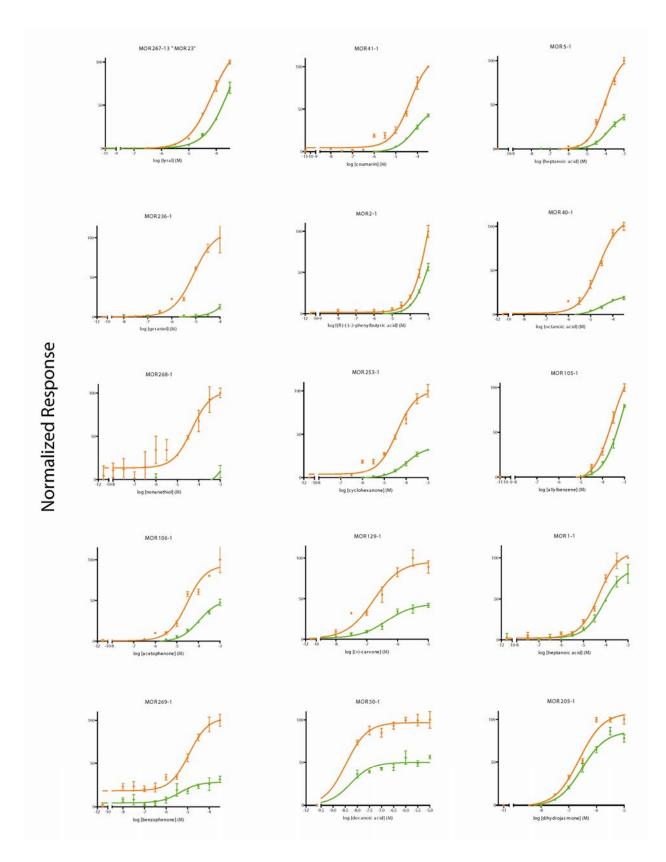


Fig. S10. Continued.

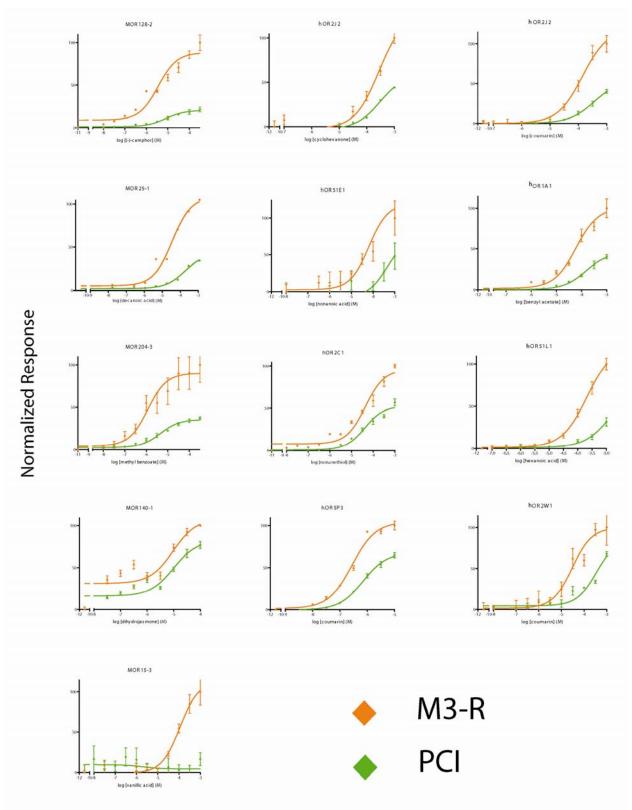


Fig. S10. The M3-R enhances the function of various mammalian ORs. Dose-response curves of luciferase assays for over 30 mammalian ORs in the presence and absence of the M3-R are shown. All of the ORs tested were tagged with Rho unless otherwise noted. Average values were taken from quadruplicate samples and the experiment was replicated at least twice.

Table S1. GPCRs tested for potentiation of OR signaling. Listed in the order as presented in Fig. 1 are the full names of the 22 GPCRs that were tested in the luciferase assay screen for their ability to potentiate OR signaling. The numbered items correspond to the x-axis labels in Fig. 1.

- 1. mammalian expression vector PCI
- 2. adenosine A_{2B} receptor
- 3. alpha-2A adrenergic receptor
- 4. beta-2 adrenergic receptor
- 5. muscarinic acetylcholine receptor 3
- 6. cysteinyl leukotriene receptor 1
- 7. GPR126
- 8. dopamine receptor D2
- 9. sphingosine-1-phosphate receptor 1
- 10. protease-activated receptor
- 11. frizzled receptor 1
- 12. frizzled receptor 3
- 13. frizzled receptor 6
- 14. frizzled receptor 7
- 15. lung seven transmembrane receptor 2
- 16. 5-hydroxytryptamine receptors
- 17. interleukin 8 receptor, beta
- 18. purinergic P2Y receptor, G-Protein Coupled 1
- 19. purinergic P2Y receptor, G-Protein Coupled 2
- 20. lysophosphatidic acid receptor 6/purinergic P2Y receptor, G-Protein Coupled 5
- 21. GABA B-like receptor
- 22. prostaglandin E2 receptor
- 23. tachikinin receptor 1