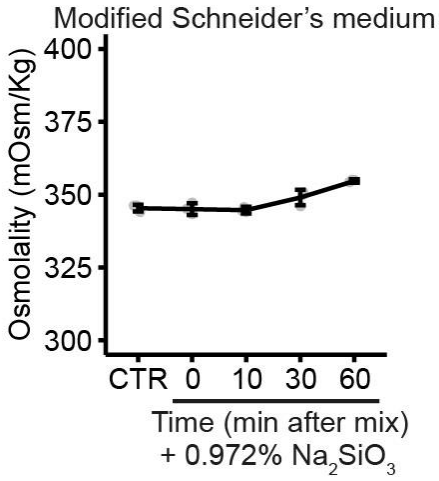
Tissue embedding in a silica hydrogel for functional investigations

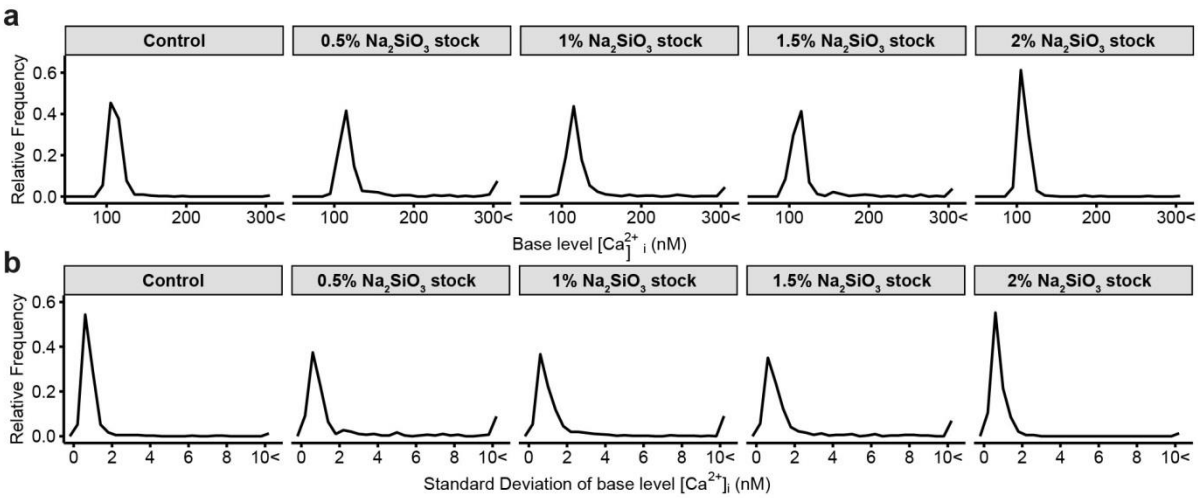
Fabio Miazzi, Sabine Kaltofen, Jan E. Bello, Bill S. Hansson and Dieter Wicher

Supplementary Information



Supplementary Figure 1 | Osmolality changes in the modified Schneider's medium-based sodium metasilicate gel during polymerization.

Changes in osmolality during polymerization of the modified Schneider's medium used to embed *Drosophila* olfactory sensory neurons (as shown in Fig. 2-3) (CTR, buffer alone) with 0.972% of the Na_2SiO_3 stock solution (≥ 27% SiO_2 basis), measured immediately after mixing the components (0 min) or after 10, 30 and 60 min. 0 min vs. 10 min: p =1, 0 min vs. 30 min: p = 1, 0 min vs. 60 min: 0.3714. Two-tailed Welch's t-tests with Holm's multiple test correction. Graphs show mean ± SD and individual data points. n = 3 for each treatment.



Supplementary Figure 2 | Distributions of analyzed parameters for HEK293 cells ROIs.

Relative frequency distribution of $[Ca^{2+}]_i$ base levels at t = 0 s (a) and standard deviation of $[Ca^{2+}]_i$ base levels over the 50 s time course (b) of all pooled HEK293 cells (as ROIs) for each treatment. Control: 403 ROIs from 8 independent replicates; 0.5% Na_2SiO_3: 294 ROIs from 7 independent replicates; 1% Na_2SiO_3: 467 ROIs from 10 independent replicates; 1.5% Na_2SiO_3: 319 ROIs from 8 independent replicates; 2% Na_2SiO_3: 384 ROIs from 9 independent replicates.

Treatments		t statistics (Welch's t-test)	95% Conficence Interval	df	Uncorrected p value
Alginate vs. Na_2SiO_3	0%	1.732	-1.484, 3.484	2.000	0.2254
	0.5%	12.075	6.601, 11.399	2.941	0.001343
	1.0%	22.000	17.697, 26.303	2.000	0.00206
	1.5%	29.445	30.452, 37.548	3.200	5.264e-05
	2.0%	67.529	47.934, 52.732	2.941	8.694e-06
Agarose vs. Na_2SiO_3	0%	-1.000	-3.535, 2.202	2.000	0.4226
	0.5%	4.810	1.261, 10.739	2.941	0.001343
	1.0%	6.003	5.765, 17.569	3.275	0.00718
	1.5%	8.699	12.357, 26.976	2.919	0.003553
	2.0%	24.981	30.056, 38.610	3.124	0.0001057

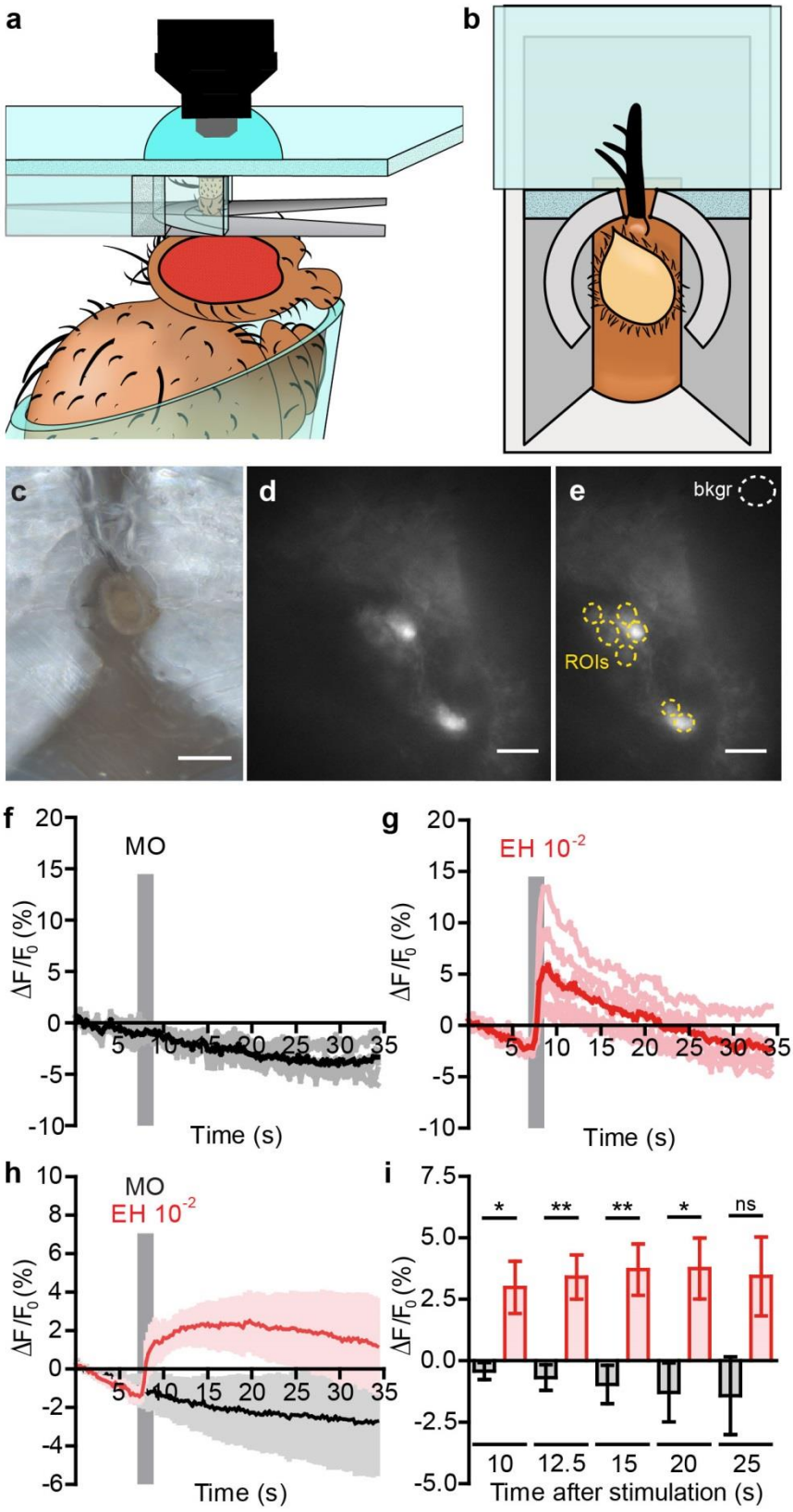
Supplementary Table 1| Statistical analysis of Figure 1b.

Treatment	t statistics (Welch's t-test)	95% Conficence Interval	df	Uncorrected p value
0 vs 10 min	1.265	-2.503, 5.170	2.439	0.3133
0 vs. 30 min	0.277	-3.208, 3.874	3.484	0.7972
0 vs. 60 min	0.000	-4.303, 4.303	2.000	1.000

Supplementary Table 2| Statistical analysis of Figure 1c.

Treatments		W statistics (Wilcoxon test)	95% Conficence Interval	Difference	Uncorrected p value
$[Ca^{2+}]_i$ base level	0 vs 0.5%	49	1.130, 11.320	7.078	0.01399
	0 vs. 1.0%	65	0.735, 10.985	5.703	0.02665
	0 vs. 1.5%	42	-5.000, 5.890	1.855	0.3282
	0 vs. 2.0%	26	-6.030, 2.040	-2.348	0.3704
S. D. of $[Ca^{2+}]_i$	0 vs 0.5%	44	-0.030, 0.330	0.104	0.07211
	0 vs. 1.0%	56	-0.056, 0.304	0.140	0.1728
	0 vs. 1.5%	51	0.026, 0.447	0.278	0.04988
	0 vs. 2.0%	28	-0.214, 0.182	-0.054	0.4807

Supplementary Table 3| Statistical analysis of Figure 1d, e

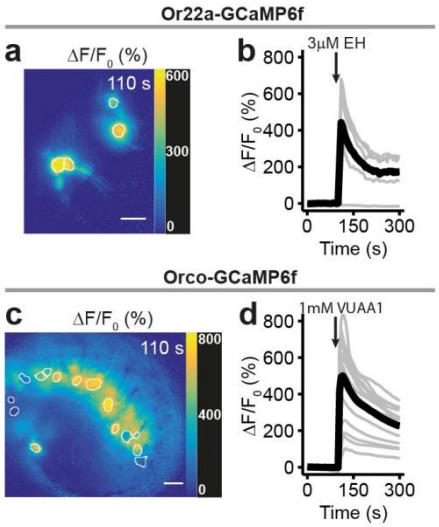


Supplementary Figure 3 | Functional calcium imaging from an *in vivo* antennal preparation.

(a) Schematic representation of the *in vivo* preparation from the side view. The fly is placed in a pipette tip and the antenna is held in vertical position on a custom holder placed within the thickness of a #0 glass coverslip; after the tip of the funiculus is cut with a scalpel blade, a thin #00 glass coverslip moistened with halocarbon 700 oil is placed on top in order to seal the open antenna without soaking the sensilla. (b) Schematic representation of the *in vivo* preparation from a top view. The open antenna is held within the thickness of a #0 glass coverslip thanks to an aluminum foil holder placed on its bottom and a plastic ring around the antenna. A posterior slit on the plastic ring allowed fixing the arista directly on the #0 coverslip with odor-free glue. (c) Picture of the *in vivo* preparation from a top view. It is possible to recognize the antenna in the middle, the aluminum foil holder and the plastic ring fixed with glue to the #0 plastic coverslip. Scale bar = 0.5 mm (d) Normalized fluorescence base level intensity from a fly preparation expressing GCaMP3.0 in Or22a olfactory neurons. It is possible to recognize several neurons on different focal planes. Scale bar = 10 µm. (e) same picture as in (d), here regions of interest (ROIs) used for analysis are marked in yellow and the area used for the background subtraction (bkgr) is marked in white. Scale bar = 10 µm. (f, g) Example of the recorded fluorescence intensity (expressed in $\Delta F/F_0$) over time from the same preparation as shown in (d). (f) The fly was first stimulated with mineral oil (MO, negative control); the stimulus duration is marked with a vertical grey bar, each ROI as in (e) is represented in gray and the mean value in black. (g) The fly was then stimulated with ethyl hexanoate (EH) at a 10^{-2} dilution in mineral oil; the stimulus duration is marked with a vertical grey bar, each ROI as in (e) is represented in light red and the mean value in red. (h) Pooled responses from n = 5 antennae to MO (black) and EH 10^{-2} (red). Traces represents mean ± SEM. (i) Intensity of the responses to MO (black) and EH 10^{-2} (red) calculated subtracting the fluorescence value at the moment of stimulation (7 s) from the fluorescence intensity at a given time expressed in seconds after stimulation. The response to EH is long lasting and is statistically significant until 20 seconds after stimulation (correspondent to time = 27 s plotted in panel g). Paired t-tests, without multiple comparison correction, * $p < 0.05$, ** $p < 0.01$, ns = not significant. Graphs represent mean ± SEM. Statistics for each test is reported in the Supplementary Table 4.

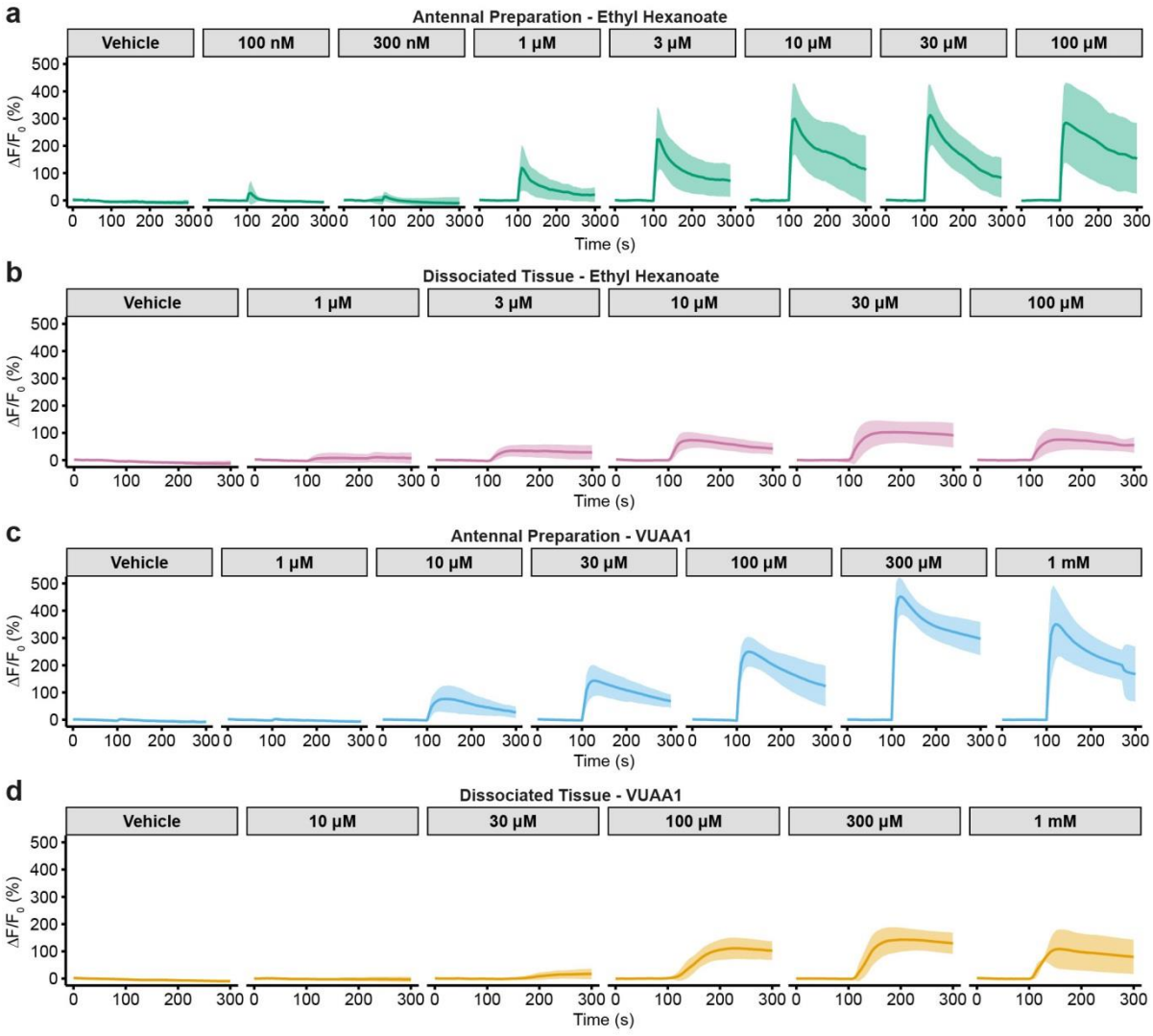
Time after stimulation (s)	MO (mean ± SEM)	EH (mean ± SEM)	t	df	p value
10	-0.4224 ± 0.3465	2.982 ± 1.064	3.281	4	0.0305
12.5	-0.6814 ± 0.5194	3.404 ± 0.901	5.11	4	0.0069
15	-0.9627 ± 0.7789	3.712 ± 1.048	4.661	4	0.0096
20	-1.285 ± 1.197	3.756 ± 1.244	3.399	4	0.0273
25	-1.421 ± 1.582	3.429 ± 1.605	2.131	4	0.1

Supplementary Table 4| Statistical analysis of Supplementary Figure 3i



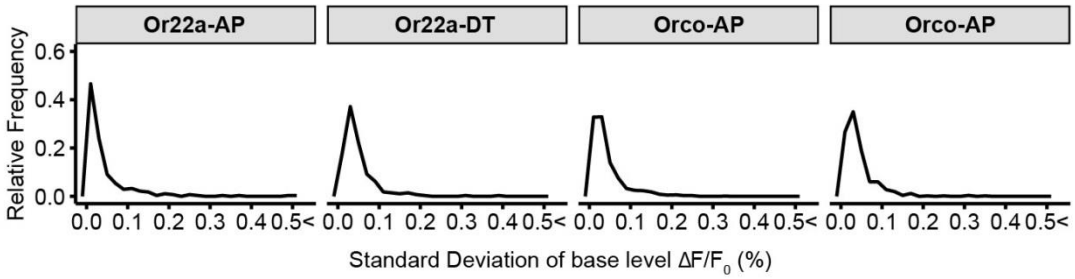
Supplementary Figure 4 | Calcium imaging from *D. melanogaster* undissociated antennal tissue

Examples of Ca^{2+} imaging from Or22a (a-b) and Orco-expressing (c-d) OSNs from undissociated antennal tissue. (a, c) antennal samples under 475 nm light with the ROIs highlighted in white. (b, d) $\Delta F/F_0$ (%) of the ROIs shown in (a, c) with the average intensity (black line) after the application of 3 μM ethyl hexanoate (EH) or 1 mM VUAA1 respectively. Scale bar = 10 μm



Supplementary Figure 5 | Response profile of *D. melanogaster* OSNs from undigested antennal preparations and dissociated antennal tissues stimulated with OR agonists.

Ethyl hexanoate and VUAA1 elicited concentration-dependent responses in both the antennal preparation and dissociated tissue, although response profiles including the maximum response intensity and response decay time are different between these two conditions. All graphs show mean ± SD; (a) $5 \leq n \leq 11$, (b) $8 \leq n \leq 10$, (c) $5 \leq n \leq 11$, (d) $4 \leq n \leq 11$ for each concentration.



Supplementary Figure 6 | Distribution of standard deviation $\Delta F/F_0$ basal values for analyzed OSN ROIs.

Relative frequency distribution of standard deviation of $\Delta F/F_0$ basal levels before OR agonist stimulation. Antennal preparation from Or22a OSNs (Or22a-AP): 270 ROIs from 71 independent experiments; dissociate tissue from Or22a OSNs (Or22a-DT): 272 ROIs from 60 independent experiments; antennal preparation from all Orco-expressing OSNs (Orco-AP): 924 ROIs from 60 independent experiments; dissociated tissue from Orco OSNs (Orco-DT): 499 ROIs from 49 independent experiments.

Preparation	Parameter	Estimate	St. error	t value	p (>|t|)
Or22a-AP	Upper limit	300.98	15.58	19.32	< 2.2e-16
	Slope	-3.86	0.93	-4.17	5.56e-05
	EC_{50} (log)	-5.84	0.08	-73.31	< 2.2e-16
Or22a-DT	Upper limit	88.07	17.43	5.05	1.50e-6
	Slope	-4.24	3.50	-1.21	0.228
	EC_{50} (log)	-5.41	0.25	-21.71	< 2.2e-16
Orco-AP	Upper limit	399.62	20.81	19.21	< 2.2e-16
	Slope	-2.80	0.43	-6.55	2.32e-09
	EC_{50} (log)	-4.30	0.07	-60.88	< 2.2e-16
Orco-DT	Upper limit	103.21	24.76	4.17	6.39e-05
	Slope	-8.78	26.19	-0.335	0.738
	EC_{50} (log)	-3.97	0.15	-26.82	< 2.2e-16

Supplementary Table 5 | Curve fitting parameters for ethyl hexanoate and VUAA1 dose-response curves.

Curve fitting of concentration-dependent responses in Figure 3c-f was performed using three-parameter logistic models (lower limit = 0), after logarithmic transformation of concentration values, with the R drc package (see Methods section in the main text)

Treatment	t statistics (Welch's t-test)	95% Conficence Interval	df	p value
Or22a DT vs. AP	-0.1214	-0.0120, 0.0106	118.80	0.9036
Orco DT vs. AP	-0.0231	-0.0080, 0.0079	105.61	0.9816

Supplementary Table 6| Statistical analysis of Figure 3g.

Treatment	Estimate	Standard error	t value	p value
Or22a DT vs. AP	1.0787	0.0518	1.5189	0.1313
Orco DT vs. AP	1.0817	0.0441	1.8529	0.06676

Supplementary Table 7| Statistical analysis of Figure 3h.

Supplementary Video 1 | Functional calcium imaging on Or22a olfactory sensory neurons from *D. melanogaster* dissociated antennal tissue.

Calcium imaging from the same preparation shown in Figure 2c and Figure 3a. The left panel shows the raw fluorescence intensity expressed in arbitrary units (counts), the right panel shows the variation of fluorescence intensity respect to base level expressed in percentage (% $\Delta F/F_0$). The stimulus consisted of 100 μM ethyl hexanoate.