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"Processing of odor mixtures in the *Drosophila* antennal lobe reveals both global inhibition and glomerulus specific interactions"

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Captions for the Supplemental Material

Supplemental Figure 1

(A) Schematic (lateral) view of the preparation. The antennae are pulled forward to allow optical access to the antennal lobes. The frontal view of the antennal lobes obtained with this preparation is tilted with respect to the neural axis. Inset: AN: antennal nerve; AL: antennal lobe; MB: mushroom body; V* and D* stand for the ventral and dorsal directions used here and that are tilted with respect to the neural axis. **(B)** Null hypothesis for the analysis of mixture interactions when all components elicit positive responses. The responses to the best odor (odor A, in yellow) at single and double concentration (R[A] and R[2A]) are considered the boundaries of the no-interaction interval. Responses to the mixture of AB (green) above or below these boundaries are indicative of mixture interactions. **(C)** Analysis of mixture interactions when positive and negative components are mixed. Since such responses likely originate from different mechanisms (e.g.: for OSNs, different binding sites) any response between the responses to the positive and the negative components might occur without mixture interaction. Responses to the mixture outside of this range would be indicative of mixture interactions.

Supplemental Figure 2:

Calcium responses in OSNs (A) and PNs (B) to the odors 2-heptanol (2hep - left) and 1-hexanol (1hex, right) in different concentrations measured in glomeruli DM2, X1, DM5 and DM3. Concentrations 1x and 2x correspond to those shown in Figures 1, 2 and 6 and were

presented with the olfactometer described in Materials and Methods. Concentrations -5 to -2 indicate the $\log_{10}$ of the concentration (vol/vol) diluted in mineral oil (e.g.: -2 = 1/100), and were presented with a multisampler which injected 1ml (speed = 1ml/s) headspace from a 20 ml vial containing 5 ml of the corresponding dilution into a permanent air stream (5ml/s) (see Pelz et al., 2006). Values are the median of 9-10 animals (OSNs) and 10-12 animals (PNs). Error bars indicate the Q25 and Q75. The concentrations used in this paper are within the dynamic range in all glomeruli.

Supplemental Figure 3:

PN responses to 2-heptanone (2hep) and 1-hexanol (1hex) before and during application of PTX 5µM. Color coded pictures show mean ΔF/F amplitude during stimulation. Compare with Figure 6A, where the corresponding pictures for the mixture are shown.

Supplemental Figure 4:

(A) PN responses to 2-heptanone, 1-hexanol and their mixture before, during and after application of DMSO 0,005%. Mean values and standard deviations of 6 animals are shown. Application of DMSO alone does not significantly change odor response amplitude (Two-way RM ANOVA showed no significant effect of the factor treatment and no interaction between factor treatment and factor odor).

(B) Median time traces of odor responses before and during DMSO application. DMSO application alone does not change the time course of odor responses.

Table S1 - Responses to the mixtures and lower bound of the no-interaction interval

Median and interquartile interval (median $[Q_{25};Q_{75}]$) for all mixture responses and the corresponding lower bound values. b.o.: best odor. Values are expressed as ΔF/F (%) and are the median values over all measured animals (n = 9 for OSN and 10-15 for PN). Grey cells:

response to the mixture significantly different from the lower bound (Wilcoxon Signed Rank Test, $p>0.05$)

Movie 1 (additional material for the reviewers):

Real time absolute fluorescence change in PNs upon stimulation with 1-hexanol (before pharmacological treatment). The white square on the top-left corner indicates the time of stimulation with 1-hexanol.

Movie 2 (additional material for the reviewers):

Real time absolute fluorescence change in PNs upon stimulation with 1-hexanol during application of Picrotoxin 100 μM. Notice a strong fluorescence change that originates on the bottom left of the picture (probably at the γ/β lobe) before odor stimulation and scatters to the antennal lobes and the contra lateral side.