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Supplemental Data

Activity-Dependent Plasticity

in an Olfactory Circuit

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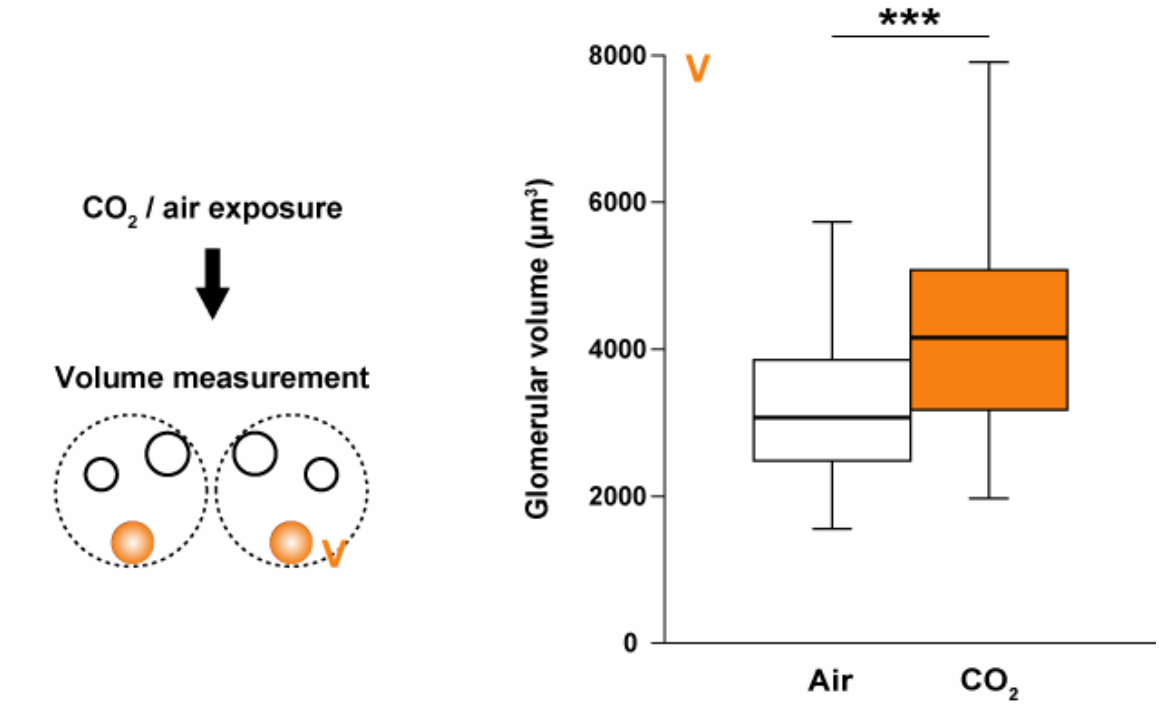
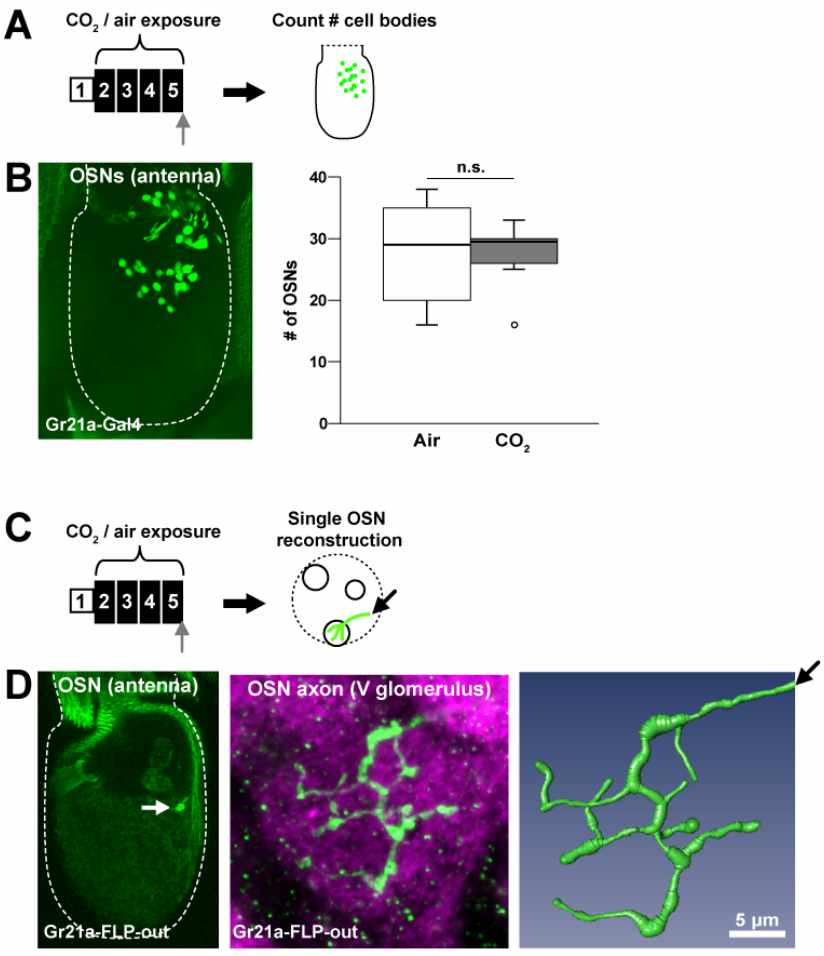


Figure S1. Stimulus-Dependent Volume Increase Is Higher Than Between-Experiment Variability
Volume measurements of the V glomerulus of experiments shown in Figs. 1B, 1C and 2B were pooled (n=61-70) and are presented as a box plot (see legend to Fig. 1 for box plot conventions). The volume increase due to 5% CO_2 exposure is highly significant in CO_2-exposed flies versus air-exposed flies (***p<0.001, unpaired, two-tailed t-test).



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		# branches	Total length [µm]	Surface [µm²]	Volume [µm³]
n.s.	CO_2	13.7 ± 4.9	123 ± 26	360 ± 86	110 ± 35
	air	16.4 ± 3.7	117 ± 16	374 ± 48	109 ± 20

Figure S2. OSN Number and Morphology Are Not Modulated by CO_2 Exposure
(A) Schematic of cell counting experiment, using genetic markers for V-glomerulus OSNs (*Gr21a*-Gal4;UAS-NLS-GFP).
(B) Antennal whole-mount with GFP-labeled cell bodies of *Gr21a* neurons (left). Box plots show quantification of the number of *Gr21a* OSNs of flies that have been exposed to either CO_2 or air (right). The number of cell bodies is not significantly different after CO_2 treatment (n=8-9).
(C) Morphological change due to CO_2 exposure was studied for single OSNs expressing *Gr21a*.
(D) Single *Gr21a*-expressing OSNs were labeled with the FLP-out technique, exposed to CO_2 (5%) or air, and their axonal termini reconstructed from 3-D confocal scans. A single GFP-labeled *Gr21a* neuron on the antenna is indicated with the white arrow (left). Axonal aborization of a single OSN in the V glomerulus (middle) and its 3D-reconstruction (right) are shown. Staining: nc82 (magenta); anti-GFP (green). Black arrow marks entry of the antennal nerve to the V glomerulus in (C) and (D).
(E) Table quantifies morphological aspects of OSN axons, expressed as mean±SEM; n=5-11 animals per category. n.s.: not significantly different at $p<0.05$; unpaired, two-tailed t-test. CO_2 concentration: 5% CO_2 compared to ambient air (0.04%).

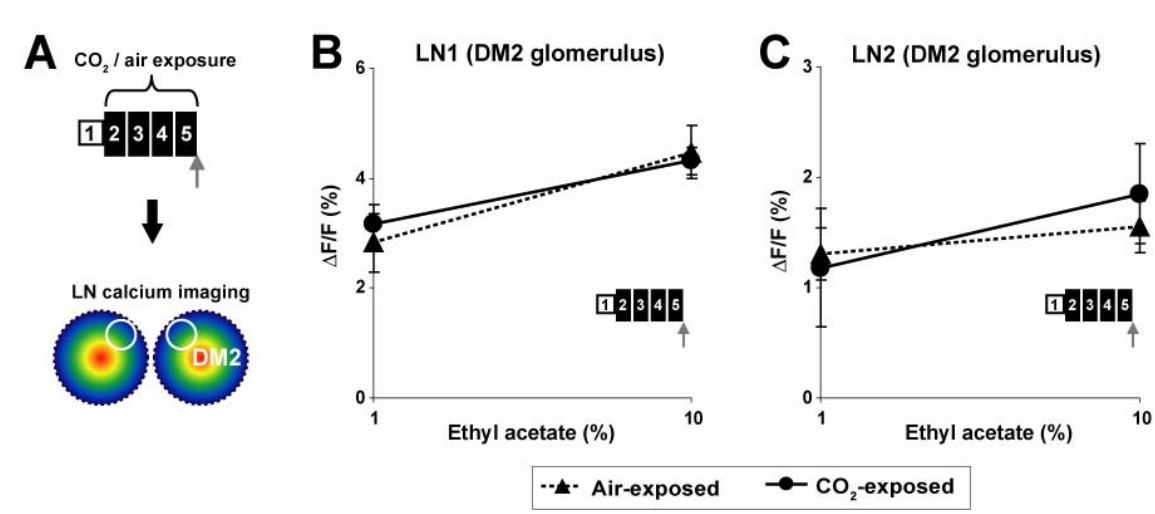

Figure S3. CO_2 Exposure Does Not Modify the Functional Activity of LNs in Glomerulus DM2 to Ethyl Acetate

(A) Schematic for measuring calcium activity to ethyl acetate in two different populations of LNs after CO_2 versus air exposure.

(B, C) Calcium responses of LN1 and LN2 in the DM2 glomerulus in CO_2- or air-exposed flies to two different concentrations of ethyl acetate are shown. CO_2 exposure does not affect the odor responses in the DM2 glomerulus, which is responsive to ethyl acetate but not to CO_2. Mean±SEM, n=3-4 flies per manipulation.

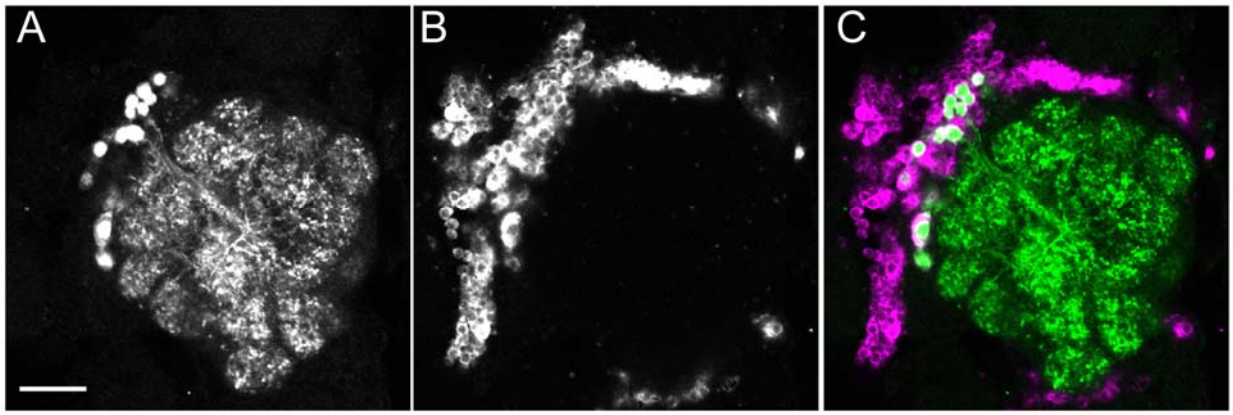

Figure S4. Expression of Gad1 in LN2 Neurons
(A) Cell bodies and neural fibers of the LN2 neurons, visualized with UAS-GFP.
(B) Distribution of the mRNA encoding GABA synthesis enzyme *Gad1*, visualized with *in situ* mRNA hybridization.
(C) Montage (green: LN2, magenta: *Gad1*). *Gad1* mRNA is observed in all the cell bodies of the LN2 neurons. Confocal single optical section of the antennal lobe of the right hemisphere. Frontal view, lateral to the left. Scale bar = 20 µm. *Gad1*-expressing cells were visualized by fluorescent *in situ* hybridization with Tyramide Signal Amplification biotin indirect system (Perkin Elmer, Boston, USA). The DNA probe was generated from EST clone GH27947 provided by the Berkeley *Drosophila* Genome Project.