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Supplemental Information

Neural processing of the reward value of pleasant odorants

Maëllie Midroit, Laura Chalençon, Nicolas Renier, Adrianna Milton, Marc Thevenet, Joëlle Sacquet, Marine Breton, Jérémy Forest, Norbert Noury, Marion Richard, Olivier Raineteau, Camille Ferdenzi, Arnaud Fournel, Daniel W. Wesson, Moustafa Bensafi, Anne Didier, and Nathalie Mandairon

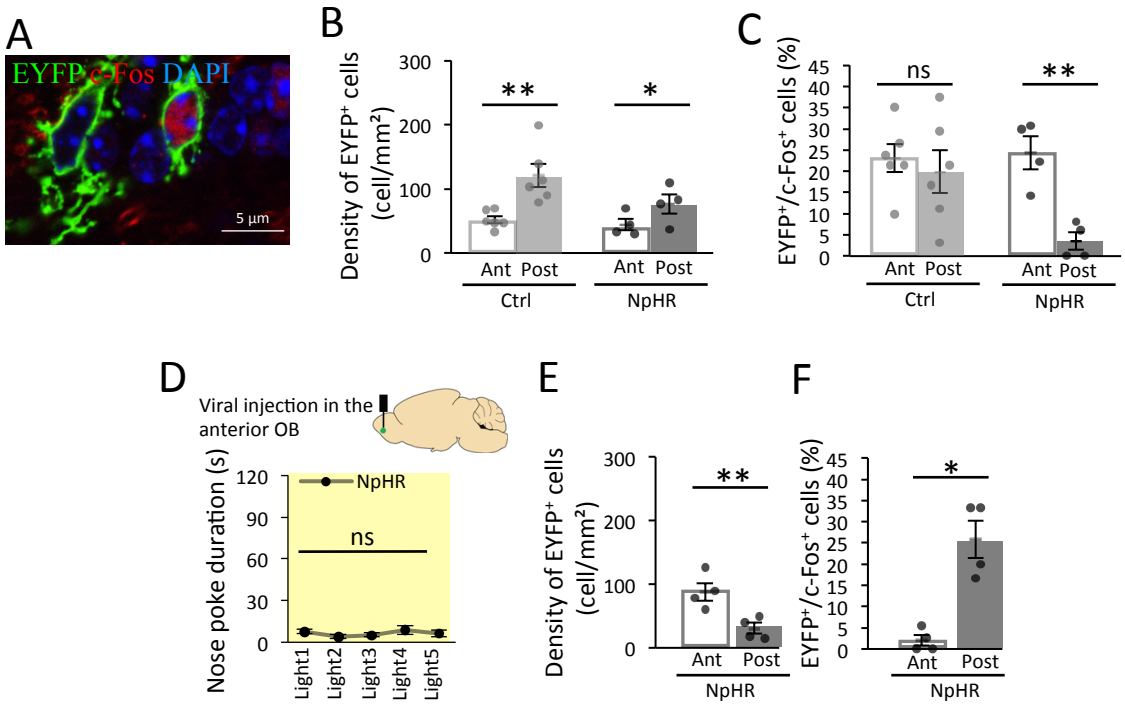

Figure S1. Optogenetic experiment, related to Figure 1. A. Representative image of double labelled EYFP+/c-Fos + cells in the OB. **B.** Density of EYFP+ neurons is higher in pOB (the injection site) compared to aOB for each group (NpHR n=4, Ctrl n=6) (Bonferroni corrected One-Tailed Paired T-Test, NpHR t=-3.759 p=0.033 Cohen's d=-1.879 and Ctrl t=-4.578 p=0.006 Cohen's d=-1.869). Bars represent means of individual data points ± sem. **C.** A lower percentage of EYFP+ cells expressing c-Fos was observed in pOB of NpHR mice compared to aOB (Bonferroni corrected One-Tailed Paired T-Test, t=4.570 p=0.009, Cohen's d=2.285). This difference was not observed in control mice (Bonferroni corrected One-Tailed Paired T-Test, t=0.418 p=0.693, Cohen's d=0.347). Bars represent means of individual data points ± sem. **D. Top.** Site of viral injection in the anterior granule cell layer of the OB (n=9). **Bottom.** Nose poke duration remained stable across trials in which light was available (Friedman trial effect $F_{(4,35)}$=5.90 p=0.207). Values represent means ± sem. **E.** Density of EYFP+ neurons is higher in aOB compared to pOB (n=4; One-Tailed Paired T-Test, t=8.044 p=0.002, Cohen's d=4.022) in NpHR mice injected in the aOB. Bars represent means of individual data points ± sem. **F.** In NpHR mice, the percentage of EYFP+ cells expressing c-Fos is lower in the aOB compared to pOB (One-Tailed Paired T-Test, t=-5.842 p=0.005, Cohen's d=-2.921) in mice injected in the aOB. Bars represent means of individual data points ± sem. *:p<0.05 **:p<0.01

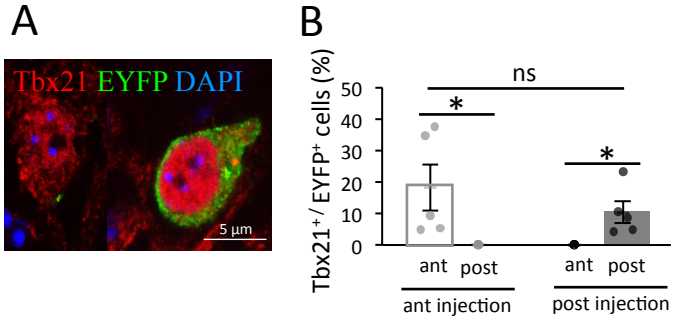

Figure S2. Pathway between OB and OT, related to Figure 2. **A.** Representative image of double labeled Tbx21+/EYFP+ cells in the OB. **B.** The percentage of Tbx21+/EYFP+ cells is higher in the aOB for mice injected in the aOB compared to pOB (n=5) and higher in pOB for mice injected in pOB compared to aOB (n=5) (Bonferroni corrected One-Tailed Wilcoxon, ant injection p=0.043, post injection p=0.043). The percentage of Tbx21+/EYFP + cells is similar between the injection sites of the two groups (Two-Tailed Mann-Whitney, W=17.000 p=0.403, Rank-Biserial Correlation=0.360). Bars represent means of individual data points ± sem. * p<0.05.

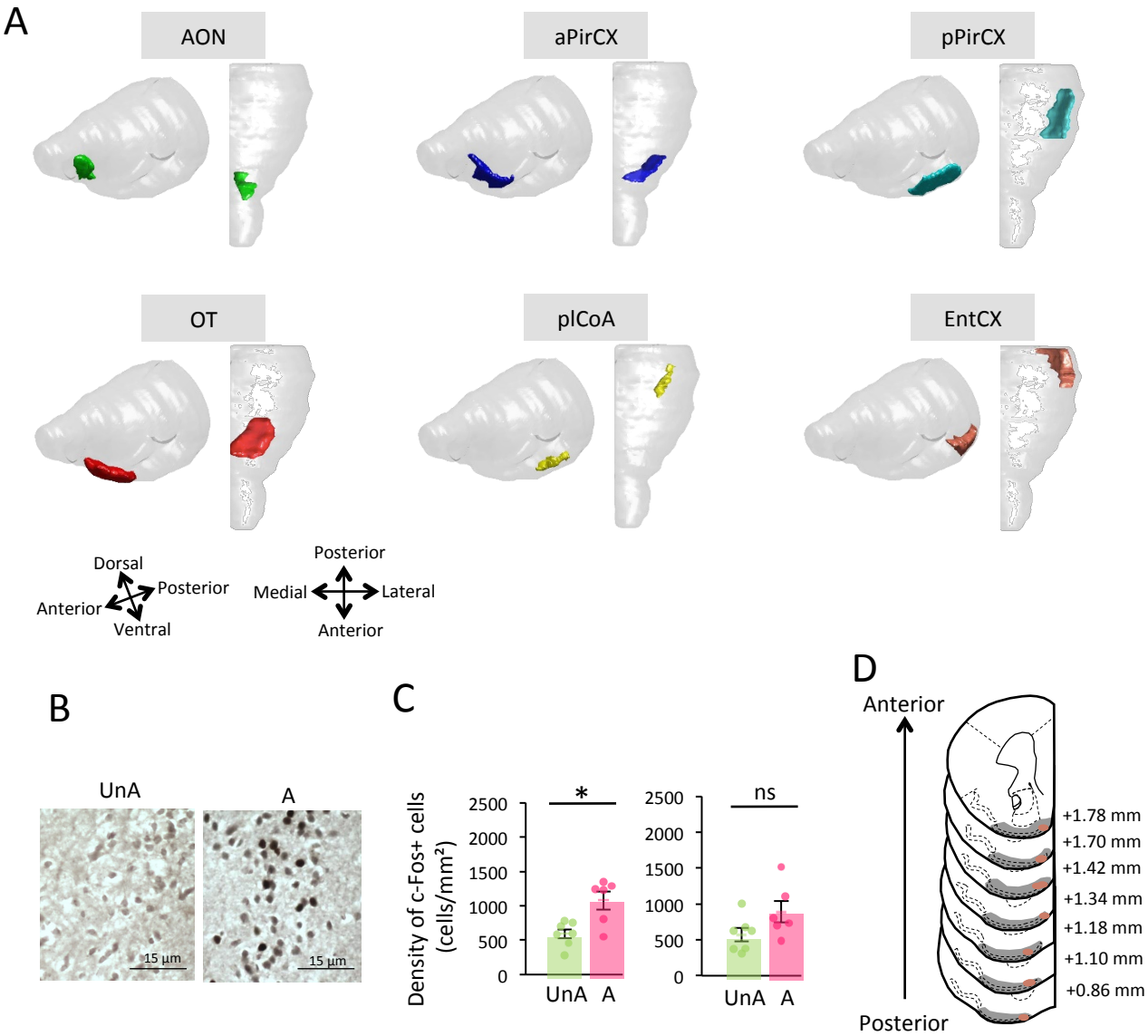

Figure S3. Cell mapping, related to Figure 3. A. Secondary olfactory structures ROI. **B.** Representative images of c-Fos+ cells in the OT in response to an unattractive odorant (UnA, left) and to an attractive one (A, right). **C.** In the mOT, c-Fos density was higher in response to attractive (A, n=6) compared to unattractive odorants (UnA, n=7). This is not true in the lOT (Right, Bonferroni corrected Two-Tailed Unpaired T-Test; mOT t=-3.402 p=0.012 Cohen's d=-1.893, lOT t=-1.899 p=0.168 Cohen's d=-1.057). Bars represent means of individual data points ± sem. **D.** Electrode tip locations within the right mOT. The red ellipses show on the coronal paxinos atlas panels the approximate location of electrode placement following histologic verification (n=10 mice, 1 electrode/animal). All recordings had electrode tips confirmed within the mOT. The extent of the OT is indicated by gray shading. Sections span from 0.86 to 1.78 mm anterior of bregma. * p<0.05.

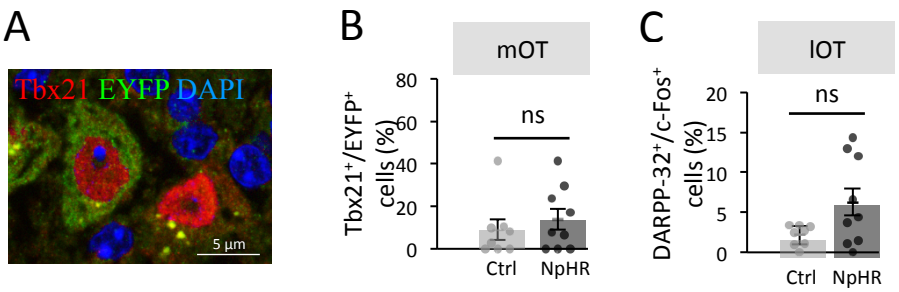

Figure S4. Cellular controls of the functional involvement of the pOB-mOT pathway, related to Figure 4. **A.** Representative image of Tbx21/EYFP-positive cell. **B.** Similar percentage of double-labelled Tbx21/EYFP-positive cells was observed between Ctrl and NpHR groups indicating that similar percentage for mitral cells was transduced between control and experimental group (Two-Tailed Mann-Whitney, W=30.500 p=0.630, Rank-Biserial Correlation=-0.153). Bars represent means of individual data points ± sem. **C.** The percentage of DARPP-32+ cells expressing c-Fos was similar in NpHR mice compared to Ctrl in in lOT (Bonferroni corrected One-Tailed Mann-Whitney; W=17.500 p=0.083 Rank-Biserial Correlation=-0.514). Bars represent means of individual data points ± sem. * p<0.05.

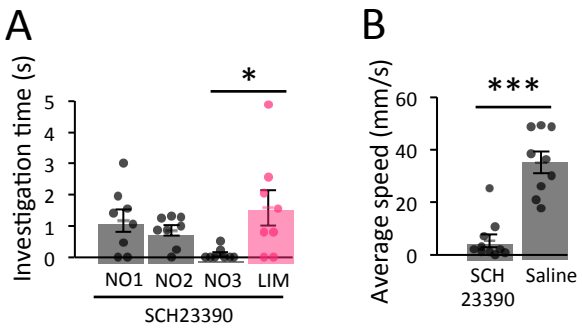

Figure S5. Olfactory and motor controls of the conditioned place preference experiment, related to Figure 5. A. Using habituation/dishabituation test, we showed that SCH23390 injection did not alter LIM detection (n=8). First, a decrease in investigation time was observed during the 3 habituation trials with no odor (NO) (Anova for repeated measure between NO1 and NO3, $F(2, 14)=5.14$, $p=0.025$). At the fourth trial, LIM was presented and a significant increase of investigation time was observed compared to the last habituation trial (NO3) (Two-Tailed Paired T-Test for difference between NO3 and LIM, $t=-2.576$ $p=0.037$, Cohen's $d=-0.911$). Bars represent means of individual data points ± sem. **B.** The average locomotion speed is altered in SCH compared to Saline group (SCH 23390 n=10, Saline n=9; Two-Tailed Unpaired T-Test, $t=-6.414$ $p<0.001$ Cohen's $d=-2.947$). Bars represent means of individual data points ± sem. *$p<0.05$. ***$p<0.001$.

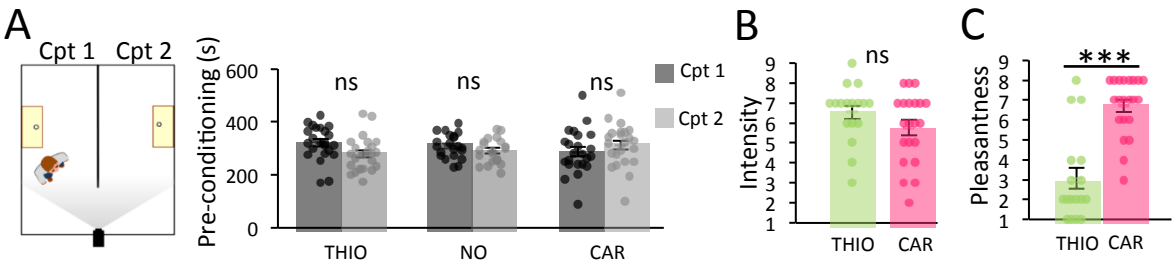

Figure S6. A. Conditioned place preference experiment in humans, related to Figure 6. A. Assessment of the time spent in each compartment for the 3 experimental groups during the pre-conditioning phase. **Left.** Drawing of the living lab (Cpt 1 and Cpt 2). **Right.** Subjects explored equivalently the two compartments before conditioning (Two-Tailed Paired T-Tests for comparisons between Cpt 1 and Cpt 2; THIO n=23, t=1.532 p=0.140 Cohen's d=0.320; NO n=21, t=0.916 p=0.371 Cohen's d=0.200; CAR n=23, t=-0.716 p=0.482 Cohen's d=-0.149). Bars represent means of individual data points ± sem. **B.** After post-conditioning test, subjects were asked whether they detected an odorant in the living lab during conditioning phase and if yes, what was its intensity? (Ratio of participants who smelled the odorant: THIO n=18/23, CAR n=21/23). No difference of intensity was found between THIO (n=23) and CAR (n=23) (Two-Tailed Mann-Whitney W=222.500 p=0,188, Rank-Biserial Correlation=0.246). Bars represent means of individual data points ± sem. **C.** After post-conditioning test, subjects were asked to rate whether they liked the odorant in the living lab if they smelled one. A higher level of pleasantness rating was found in CAR than THIO group (Two-Tailed Mann-Whitney W=41.000 p<0.001, Rank-Biserial Correlation=-0.770). Bars represent means of individual data points ± sem. ***p<0.001.

Experiment	Odorant name	Abbreviation	CID	Dilution in mineral oil (vol/vol)
Experiment 3-4	+limonene	LIM	1133	0.20%
	citronellol	CITRO	8842	17.80%
	camphor	CAM	159055	0.46%
	pyridine	PYR	1049	0.01%
	p-cresol	CRE	2879	1.84%
	guaiacol	GUA	460	2.09%
Experiment 5	+limonene	LIM	1133	1.99%
	citronellol	CITRO	8842	68.42%
	pyridine	PYR	1049	0.10%
	p-cresol	CRE	2879	15.77%
Experiment 6	L-carvone	CAR	439570	16.40%
	thioglycolic acid	THIO	1133	13.62%
Experiment 7	isoamyl acetate	ISO	31276	0.32%
	+limonene	LIM	1133	100%
	terpinen-4-ol	TER	7462	100%
	cis-3-hexenol	CIS3	5281167	0.24%
	3-methyl-3-sulfanylhexan-1-ol	MSH	10130039	1%
	3-hydroxy-3-methylhexanoic acid	HMHA	16666688	1%
	butanoic acid	BUT	264	0.1%

Table S1. Related to Figures 3, 4, 5, 6 and 7. Odorant names, abbreviations, CID and dilution for the different experiments.

Structures	Volume
AON	1.14
aPirCX	0.52
pPirCX	0.47
OT	1.55
plCoA	0.41
EntCX	1.60

Table S2. Related to Figures 3 and S3. Volumes in mm3 of ROIs of structures of interest after matching procedure to atlas (right hemisphere). Abbreviations: anterior olfactory nucleus, AON; anterior piriform cortex, aPirCx; posterior piriform cortex, pPirCx; olfactory tubercle, OT; postero-lateral cortical amygdala, plCoA; entorhinal cortex, EntCx.