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Supplemental Information

Human Olfaction without Apparent Olfactory Bulbs

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Supplementary Materials for:

Human Olfaction Without Apparent Olfactory Bulbs

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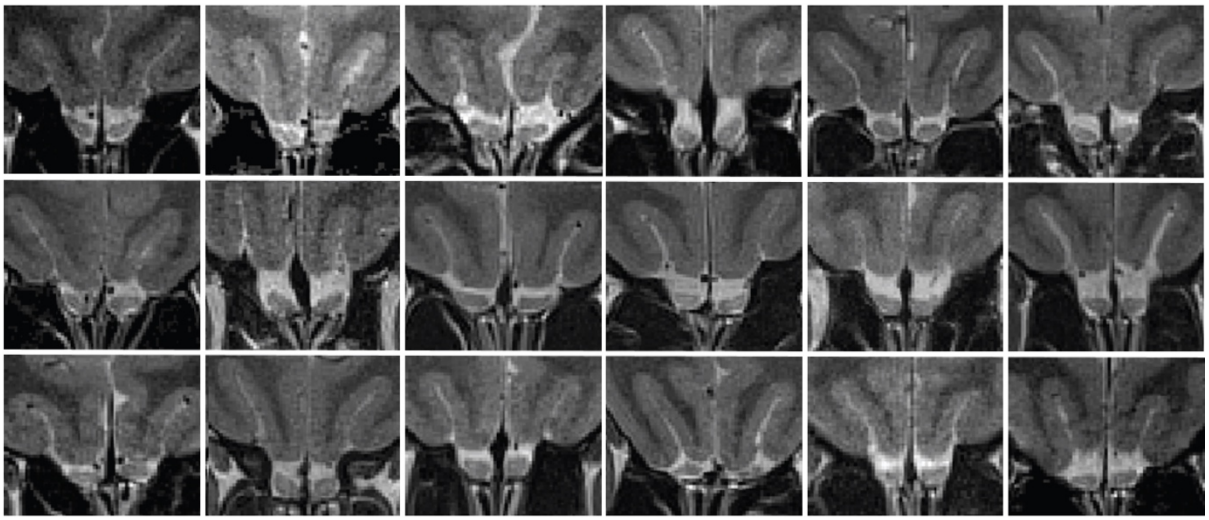
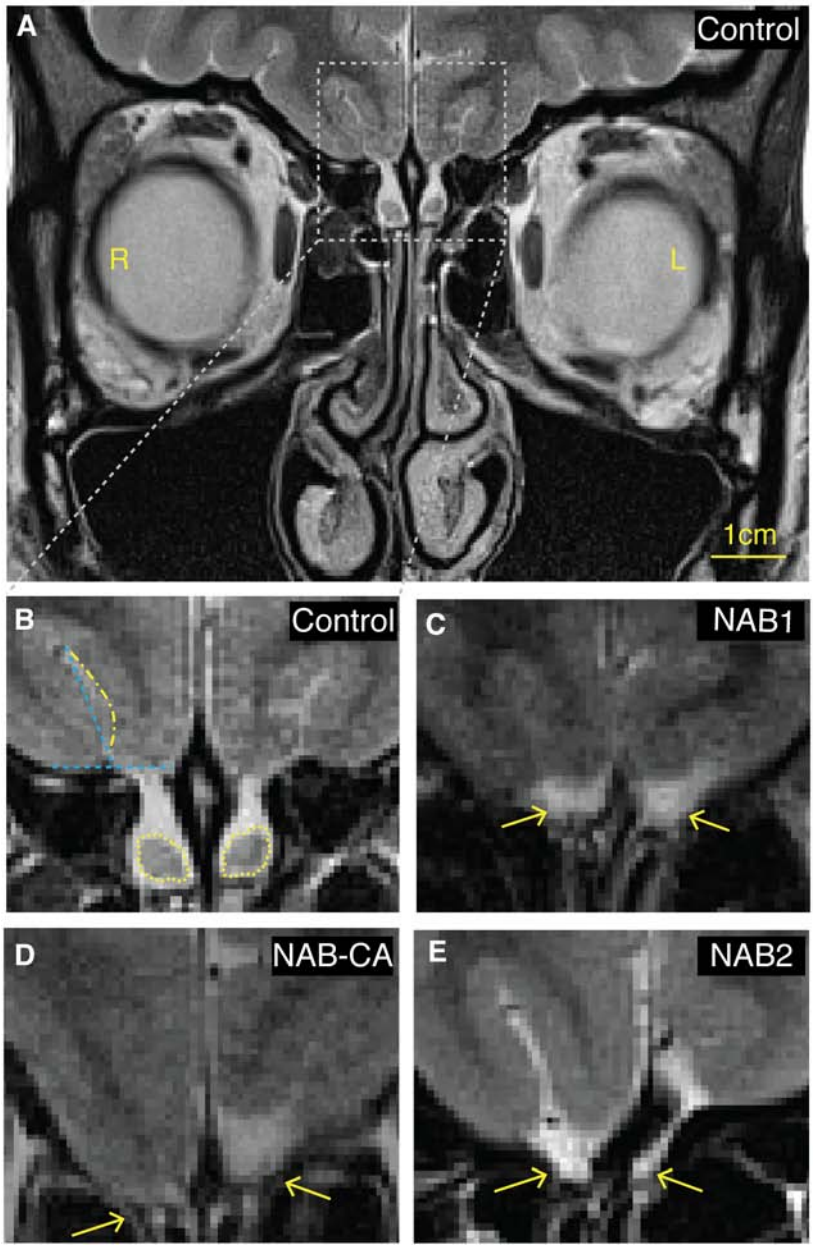


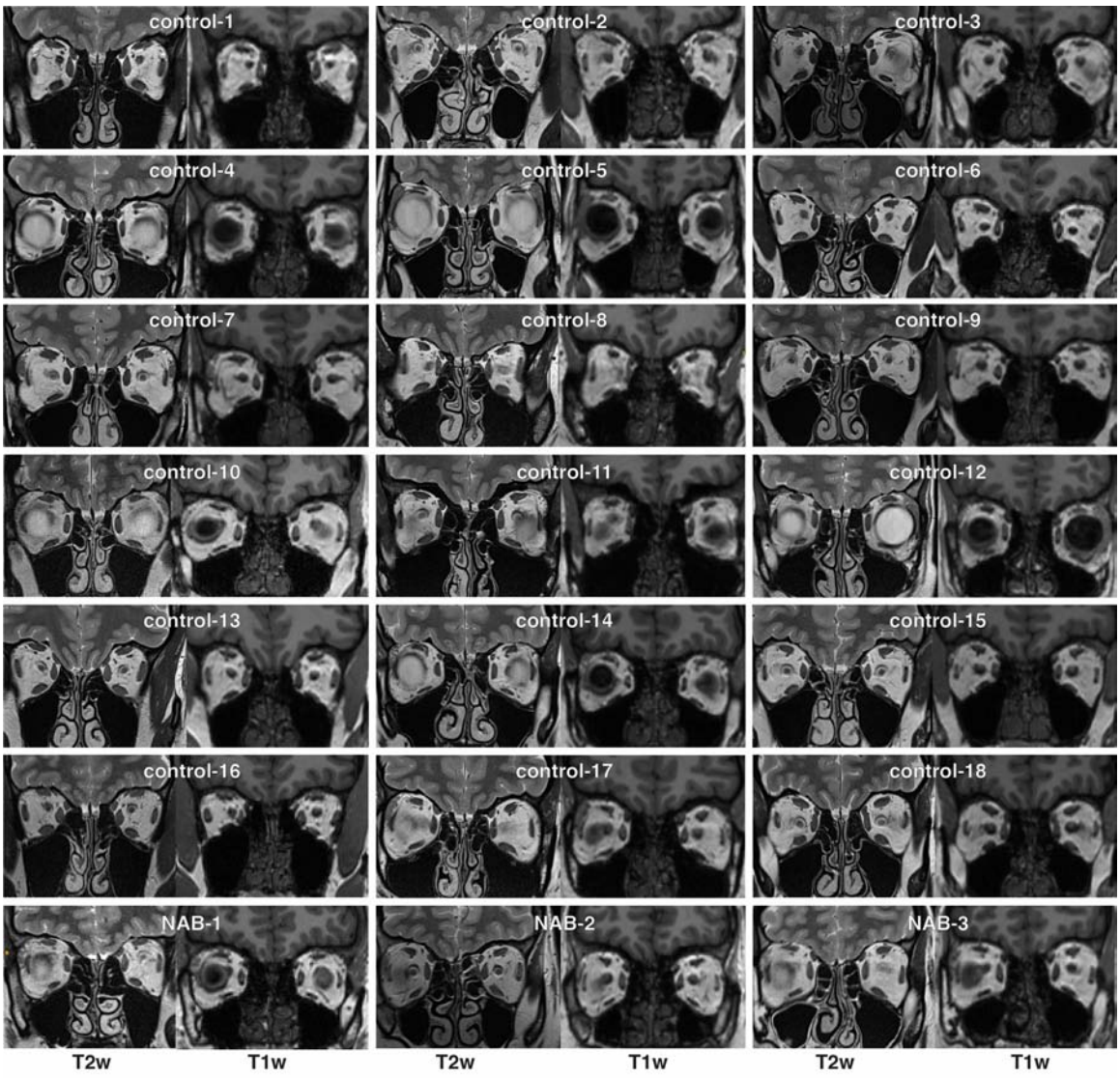
Supplementary Figure 1: Olfactory bulbs in 18 control left-handed women. Related to Figure 1.

Bulbs and olfactory sulci are very clearly seen in the coronal T2-weighted image of each participant.



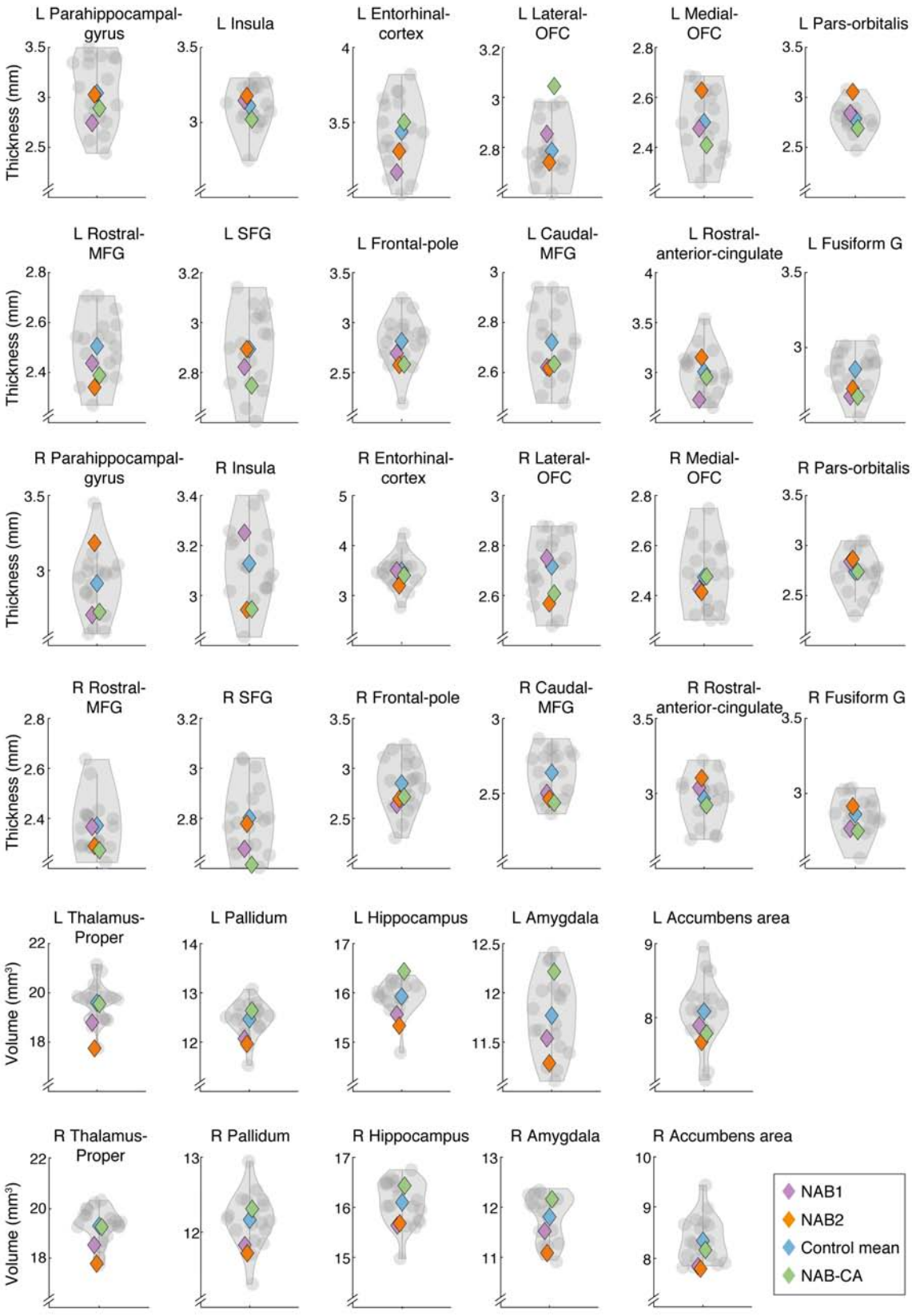
Supplementary Figure 2: Zoomed structural images of humans without apparent olfactory bulbs. Related to Figure 1.

A. A coronal T2-weighted MR image of a control participant. **B.** Zoom into the area of the OBs at the typical zoom we use to examine these images. The OBs outlined in yellow, and the right olfactory sulcus marked with a dashed yellow line. Blue lines indicate measurement technique for olfactory sulcus depth. **C.** Image of NAB1, expected location of OBs marked with yellow arrow. **D.** Image of NAB-CA, expected location of OBs marked with yellow arrow. **E.** Image of NAB2, expected location of OBs marked with yellow arrow.



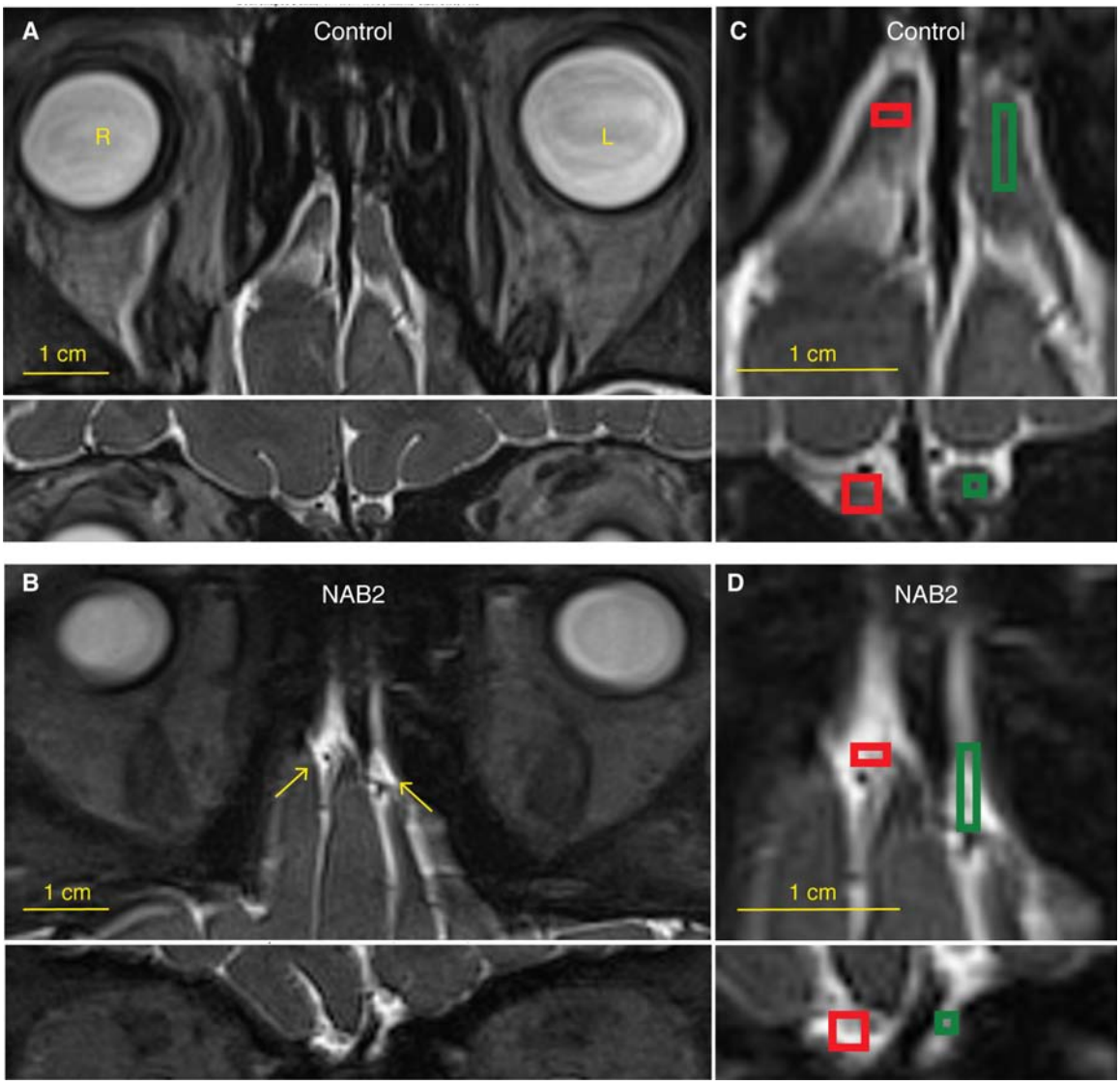
Supplementary Figure 3: Comparison of T1 and T2-weighted images of the OB. Related to Figure 1.

For each participant, the T2 image is on the left and corresponding T1 on the right. In our hands, we obtained far better separation at T2. That said, we acknowledge that this is a question of optimization efforts, and with greater efforts, the T1-weighted images may have also achieved better resolution (Held et al., 2000).


L Parahippocampal-gyrus
L Insula
L Entorhinal-cortex
L Lateral-OFC
L Medial-OFC
L Pars-orbitalis
L Rostral-MFG
L SFG
L Frontal-pole
L Caudal-MFG
L Rostral-anterior-cingulate
L Fusiform G
R Parahippocampal-gyrus
R Insula
R Entorhinal-cortex
R Lateral-OFC
R Medial-OFC
R Pars-orbitalis
R Rostral-MFG
R SFG
R Frontal-pole
R Caudal-MFG
R Rostral-anterior-cingulate
R Fusiform G
L Thalamus-Proper
L Pallidum
L Hippocampus
L Amygdala
L Accumbens area
R Thalamus-Proper
R Pallidum
R Hippocampus
R Amygdala
R Accumbens area
Thickness (mm)
Volume (mm³)
NAB1
NAB2
Control mean
NAB-CA

Supplementary Figure 4: Cortical volume and thickness in various brain areas. Related to Figure 2.

Congenital anosmia, which is associated with no OBs, is also associated with changes in brain anatomy beyond the olfactory system alone (Frasnelli et al., 2013). To ask whether NAB1 and NAB2 had any structural brain changes beyond the OBs and its associated structures, we conducted a volume-based morphometry (VBM) study, comparing them to the 18 bulbar controls. The VBM failed to uncover any significant differences. Given the limited sensitivity of VBM when comparing an individual to a group, we further measured subcortical volumes (thalamus, pallidum, hippocampus, amygdala, nucleus accumbens), and cortical thickness (parahippocampal gyrus, insula, entorhinal cortex, medial and lateral orbitofrontal cortex (OFC), caudal and rostral middle-frontal gyrus (MFG), superior-frontal gyrus (SFG), caudal and rostral anterior-cingulate gyrus, frontal pole, pars orbitalis, fusiform gyrus), but observed no differences between NAB vs the bulbar control group (all $p > 0.1$). Comparing NAB-CA to the group, the left lateral-orbito-frontal cortex was the only region that was significantly different (uncorrected) in NAB-CA (control = 2.79 ± 0.11, NAB-CA = 3.045, $t(17) = 2.32$, $p = 0.033$).

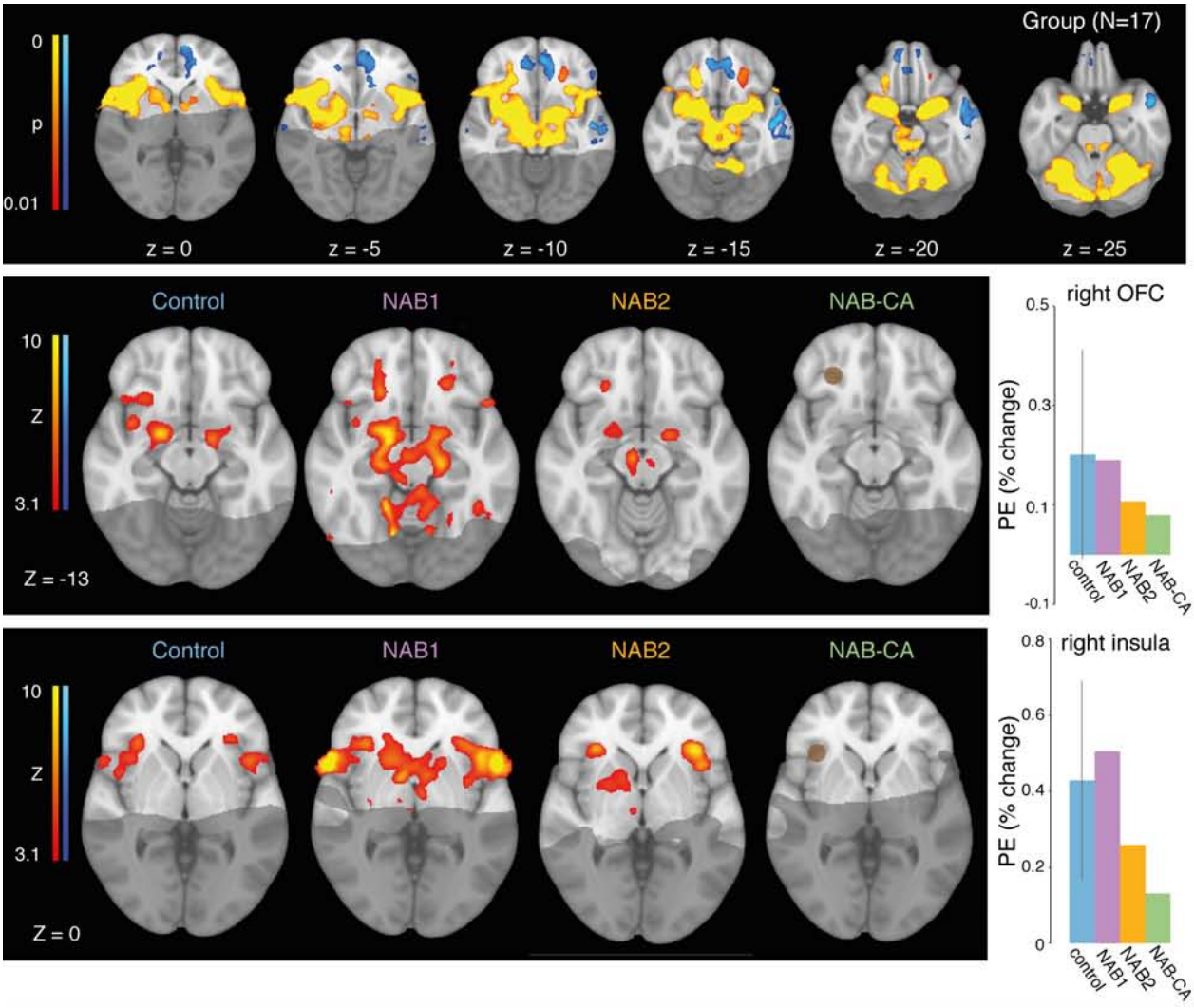


Supplementary Figure 5: High-resolution scans of the olfactory bulbs. Related to Figure 1.

A. An axial and coronal T2-weighted MR image of a control participant at ultra-high resolution (3D SPACE). OBs outlined in yellow **B.** An axial and coronal T2-weighted MR image of NAB2 at ultra-high resolution (3D SPACE). Expected OBs denoted by yellow arrows. Only CSF is evident; **C, D.** Illustration of voxel-counts in our high-resolution scans in the area of missing OBs in control and NAB2. An OB could be "mushroom" shaped, showing up mostly in a coronal image, or "cucumber" shaped, showing up mostly in an axial image. The top image is a coronal slice, with a 1x1 voxel "cucumber OB" in green, and a 3x3 voxel "mushroom OB" in red. The bottom image is an axial slice, with a 1x3 voxel "mushroom OB" in red, and a 1x9 voxel "cucumber OB" in green. We reiterate that it is unlikely we would miss such OBs in our very careful review of these participants. We further reiterate that even a 9 voxel OB at this resolution reflects an OB that is ~1.6% of a typical OB.

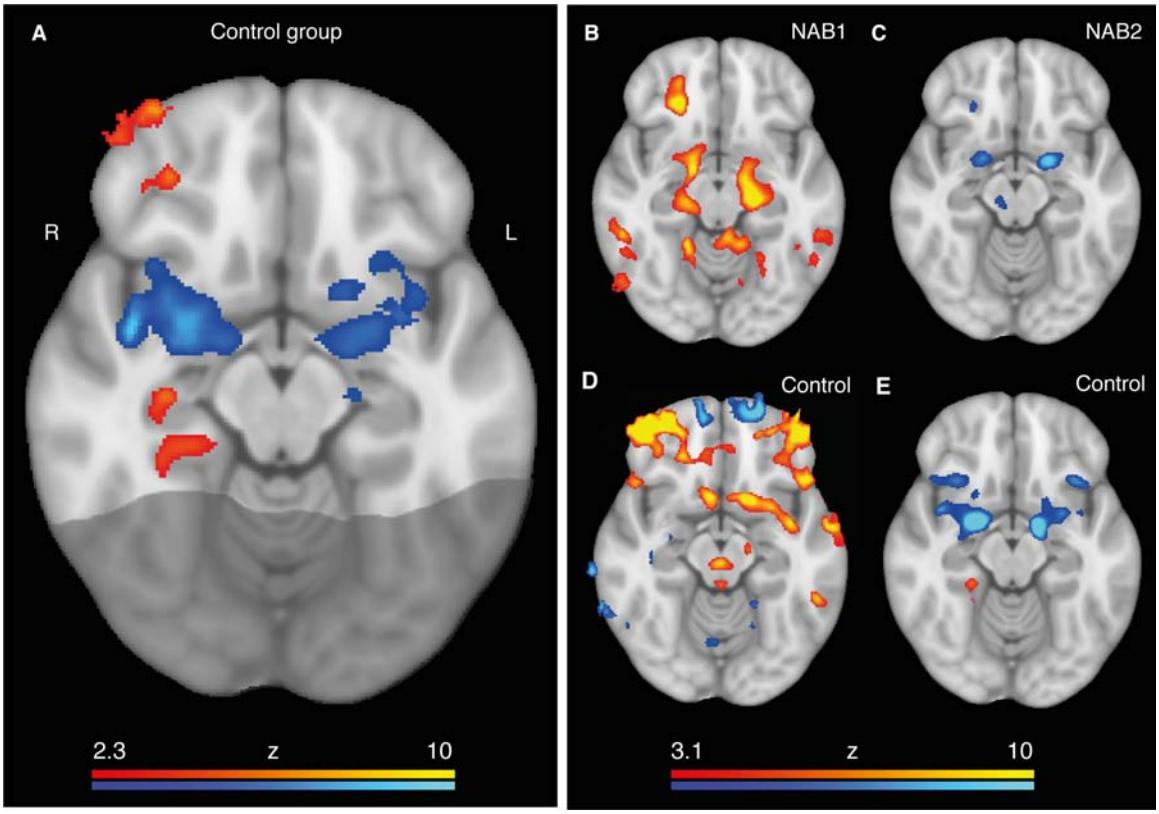
Supplementary Figure 6: Application of verbal descriptors across odorants. Related to Figure 4.

Histograms of descriptor ratings applied by NAB1 (purple), NAB2 (orange), and 140 control participants, for 10 odorants (y-axis) along 11 descriptors (x-axis). NAB1 and NAB2 occasionally stood out (e.g., NAB1's ratings of strawberry), but on average they were with the group, and not even in one of the 110 histograms did they differ beyond corrected p value.



Supplementary Figure 7: Odorant induced activity in insula and OFC. Related to Figure 5.

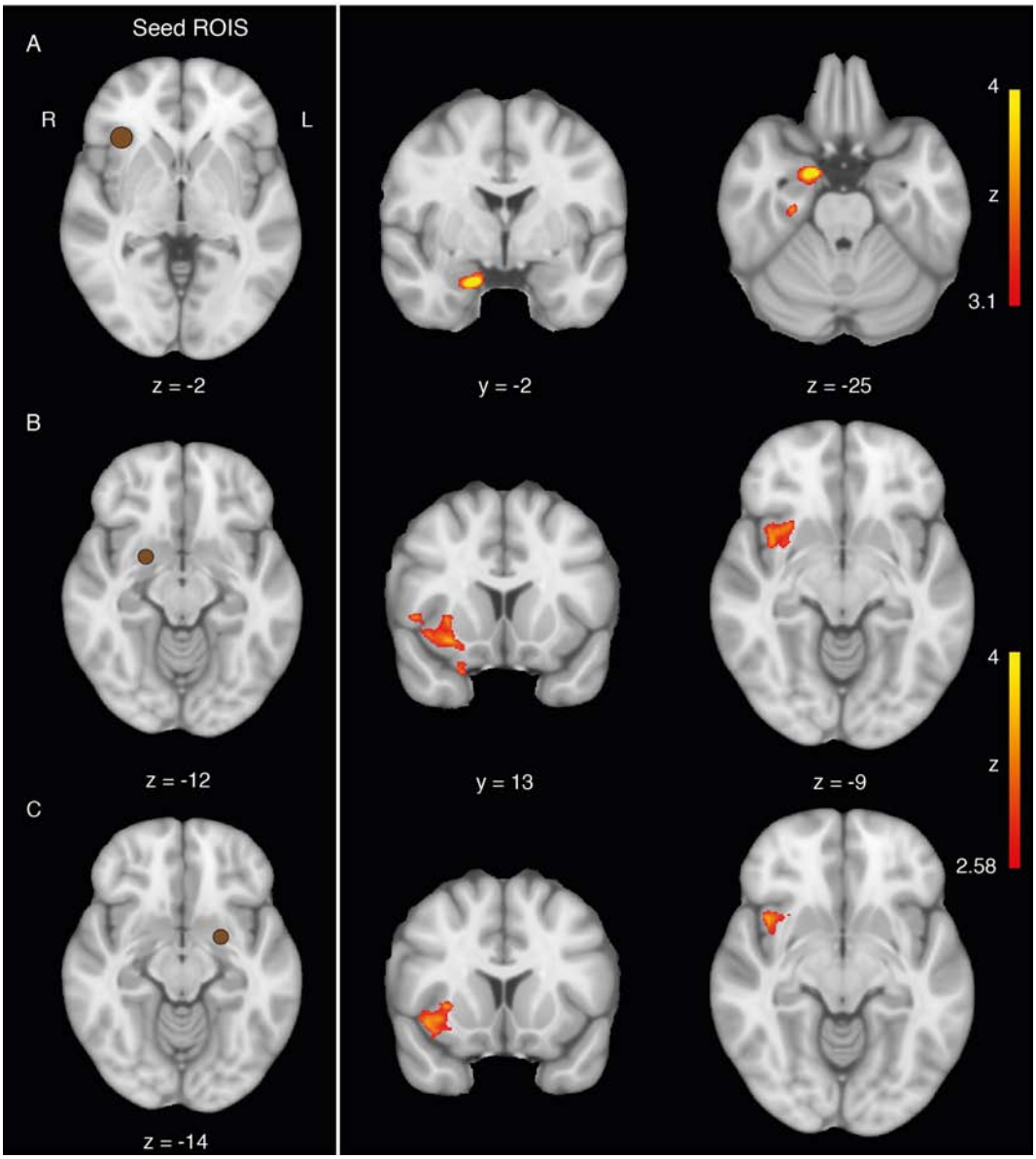
A. Control group-image (n = 17) contrast of increased activity during odorant presence (hot colors) vs. increased activity during odorant absence (cold colors), TFCE corrected. The shaded posterior section reflects the area that was not acquired in the functional scans. Ensuing panels reflect same contrast as in A, but in: **B.** Area of the OFC **C.** Area of the insula. In each case we depict activity in a typical control, and the NAB group. Activation patterns are followed by ROI-based parameter estimates.



Supplementary Figure 8: Variability in odorant-pleasantness related activity. Related to Figure 5.

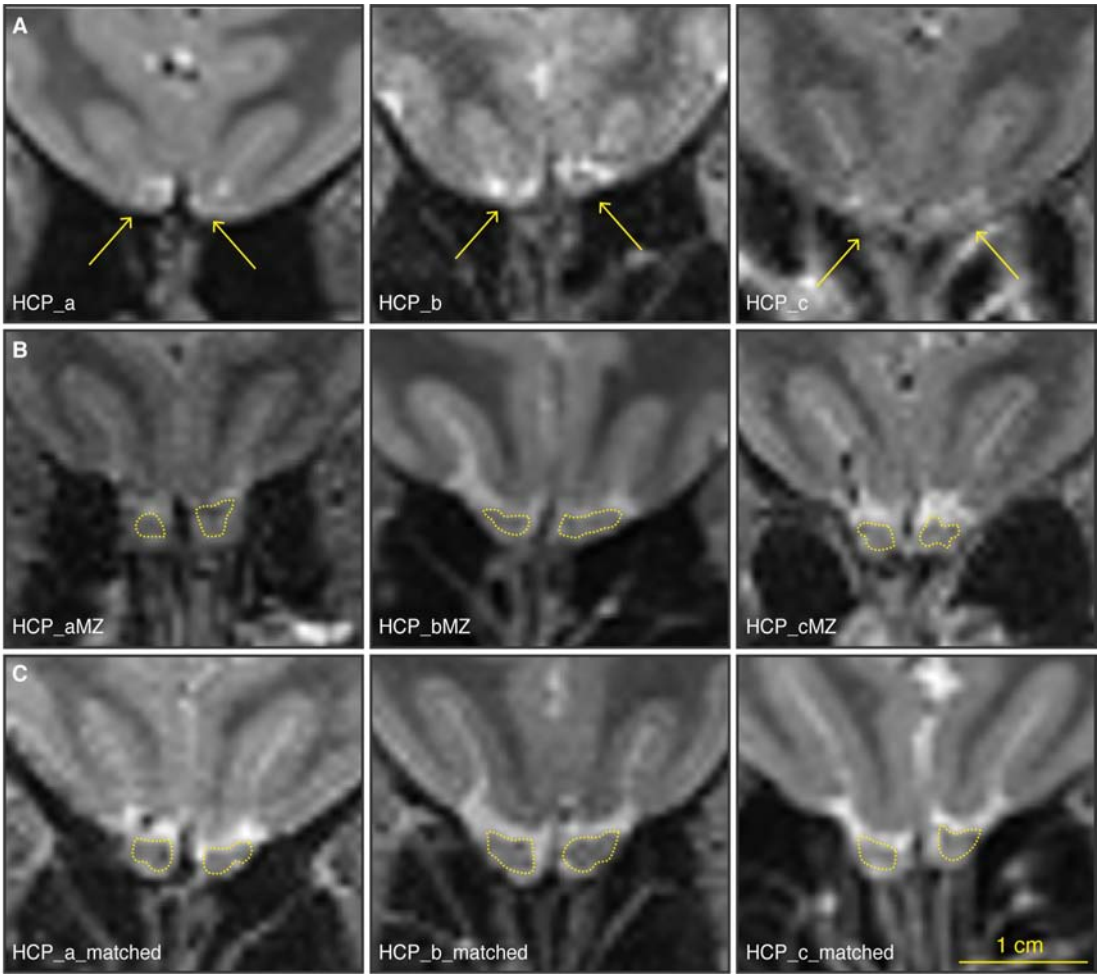
A. The parametric group image (n=17), activity associated with increased pleasantness in hot colors, and increased unpleasantness in cold colors. **B.** The same analysis in NAB1. **C.** The same analysis in NAB2. **D-E.** The same analysis in two different control individuals. **B-E.** Z statistic images are thresholded using clusters determined by $Z > 3.1$ and a cluster significance threshold of $p = 0.05$.

The group image (A) provides for a depiction of valence-related activity, consistent with the published literature (Gottfried and Zald, 2005). At first glance, NAB1 and NAB2 differ from the group. More specifically, NAB1 is consistent with the group in secondary orbitofrontal cortex, but opposite the group in primary piriform cortex. In turn, NAB2 is opposite the group in secondary orbitofrontal cortex, but consistent with the group in primary piriform cortex. However, the variability in these patterns was such that we could find control participants that looked like NAB1 (D), and control participants that looked like NAB2 (E). In other words, the valence-response is just too noisy for making single-subject statements. We see a difference in NAB1 and NAB2, but we cannot determine if and in what way this is meaningful.



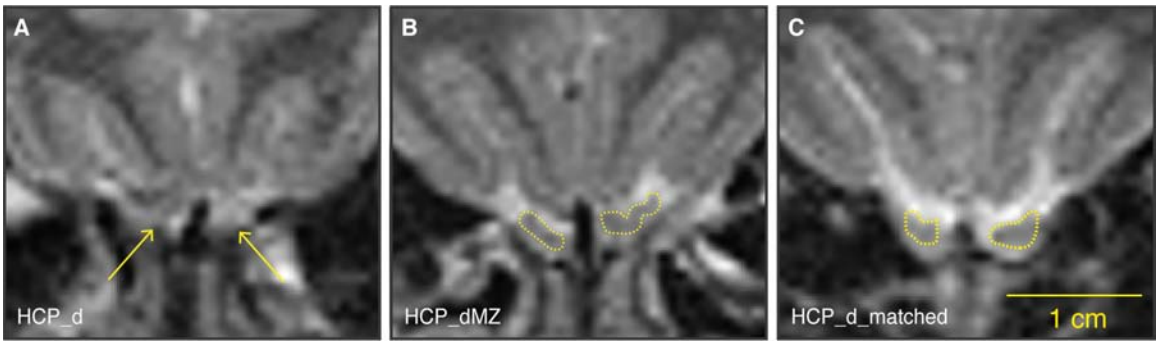
Supplementary Figure 9: Control group Image of Functional Connectivity Related to Figure 5.

Whole-brain PPI tests, reflecting greater correlation with seed ROI time series (physiological regressor) for odor presentation (odors>clean-air) (psychological regressor). Separate analyses were conducted for three seed ROIs: A. right insula, B. right piriform, C. left piriform (these ROIs are from a published meta-analysis (Seubert et al., 2013), and we note that we would consider this the superior tip of the piriform, and not its typical body) In the right insula (seed ROI) we observed significant functional connectivity during odor presentation with the anterior part of parahippocampal gyrus. In the right and left piriform we observed significant connectivity with the right insula. Only in the right piriform did we observe significant connectivity with the left cerebellum (VI).



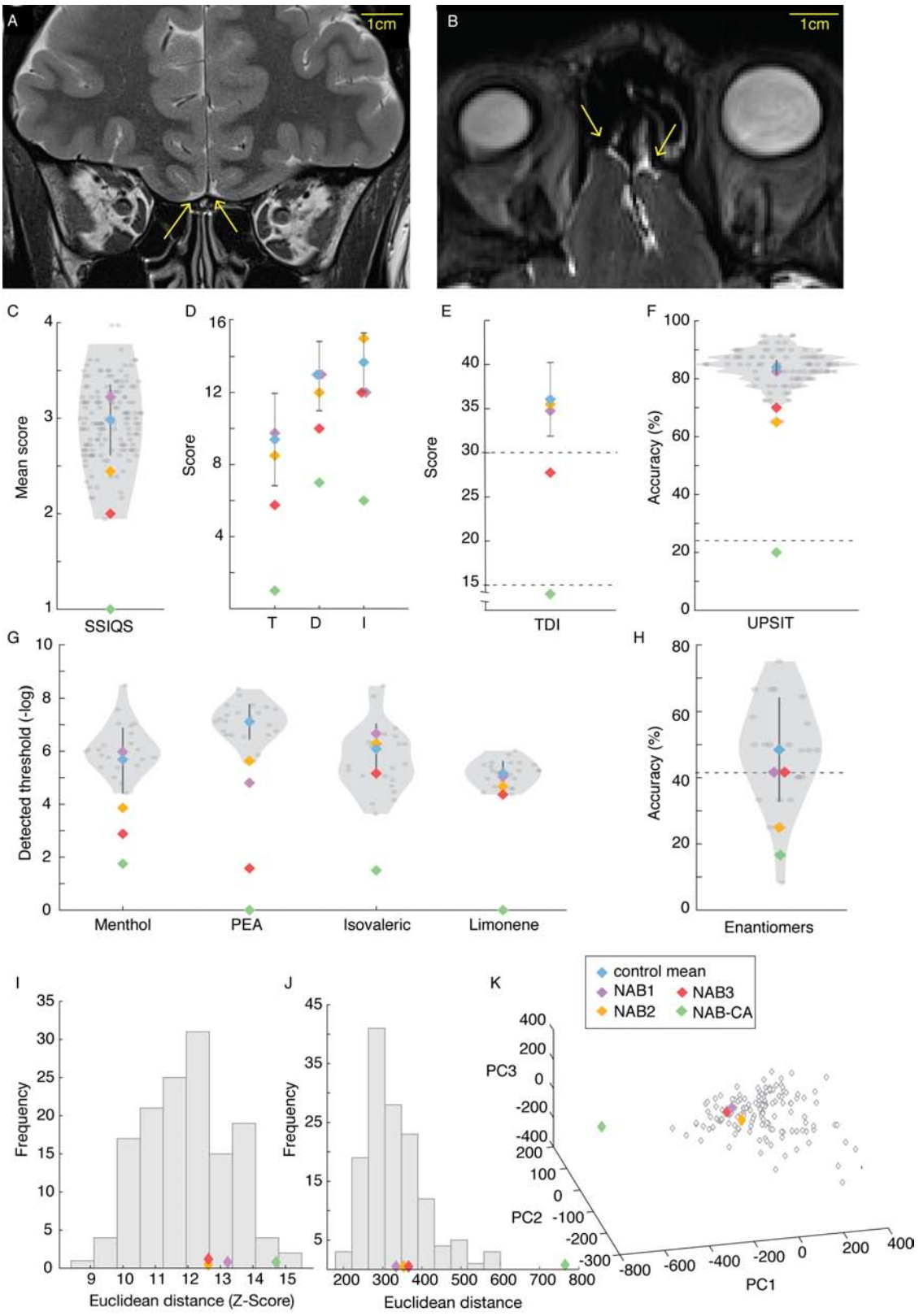
Supplementary Figure 10: Zoomed structural images of HCP participants without apparent olfactory bulbs. Related to Figure 6.

A. Coronal images from three women without OBs identified in the HCP data. Yellow arrows denote expected location of OBs. The HCP personal identifiers cannot be reported because we report restricted data (e.g., handedness), contact the corresponding authors for details. **B.** Coronal images of the mono zygotic twins of the three women in A. OBs outlined in yellow. **C.** Coronal images of age and gender matched controls from the HCP. OBs outlined in yellow. All images after bicubic interpolation by a factor of 4.



Supplementary Figure 11: A fourth participant in the HCP potentially without olfactory bulbs. Related to Figure 6.

A. Coronal image with yellow arrow pointing at what might be an OB. **B**. The OBs of this participant's twin. These OBs are also small, but clearly visible. **C.** The OBs in an age matched HCP control. The image in A is in fact the only image in the acquisition of this participant that hints at an OB, and it was enough for us to not include this participant in the main manuscript. We estimate, however, that most clinicians would determine that this participant as well is without OBs. The HCP personal identifiers cannot be reported because we report restricted data (e.g., handedness), contact the corresponding authors for details.


A
1cm
B
1cm
C
Mean score
SSIQS
D
Score
T
D
I
E
Score
TDI
F
Accuracy (%)
UPSIT
G
Detected threshold (-log)
Menthol
PEA
Isovaleric
Limonene
H
Accuracy (%)
Enantiomers
I
Frequency
Euclidean distance (Z-Score)
J
Frequency
Euclidean distance
K
control mean
NAB1
NAB2
NAB3
NAB-CA
PC3
PC2
PC1

Supplementary Figure 12: NAB3 - A third participant in lab without olfactory bulbs. Related to Figures 1, 3, 4.

A. Coronal plane, T2-w TSE sequence (400 x 400 μm, slice thickness: 1.6 mm), **B.** Axial plane, 3D T2-w SPACE sequence (470×470×470 μm^3) with expansion of the area of expected OBs, with absolutely no sign of OBs. **C-H.** Performance at olfactory tasks as in Figure 3 of the main manuscript, with NAB3 here added in red (NAB1 in purple, NAB2 in orange, NAB-CA in green). E. TDI score- NAAB3 is significantly lower than the norm, but orders of magnitude above chance and above NAB-CA. In other words, she is not normosmic like NAB1 and NAB2, but rather hyposmic. Nevertheless, she has a sense of smell (albeit poor) with no OBs. G. Concentrations detection thresholds: Menthol score (-log 10) = 2.88, two-tailed $t(22) = -2.28$, $p = 0.03$; PEA score (-log 10) = 1.58, two-tailed $t(21) = -8.04$, $p = 7.6\times10^{-8}$; Isovaleric score (-log 10) = 5.16, two-tailed $t(22) = -0.99$, $p = 0.33$; Limonene score (-log 10) = 4.36, two-tailed $t(22) = -1.78$, $p = 0.09$. H. Enantiomer discrimination: 5 correct discriminations out of 12 (41.7%), two-tailed $t(21) = -0.42$, $p = 0.68$.

I-K. The world smells similarly with and without apparent olfactory bulbs as in Figure 4 of the main manuscript, with NAB3 here added in red. I. Raw ratings across odorants and descriptors: The Euclidean distance between 110-value vectors (Z-score): similarly to NAB1 and NAB2, NAB3 do not stand out in this measure (mean Euclidean distance across participants = 12 ± 1.3, NAB3 = 12.6, two-tailed $t(139) = 0.5$, $p = 0.6$). J. A histogram of the distances for each participant versus all others, using the 45-value pairwise odorant similarity vector: mean Euclidean distance across 140 control participants: 331.3±77.2, NAB3 = 366.13, two-tailed $t(139) = 0.45$, $p = 0.65$. K. The 140 participants projected into a PCA space of olfactory perception. First 3 PCs shown. Perceptual fingerprints of NAB3 was interspersed with the perceptual fingerprints of the group., and the difference between NAB group was significantly different from the distance across all 9730 pairwise comparisons of the control group (average distance: 288.1 ± 110.4, NAB1-NAB2 distance: 130.85 ($p = 0.04$), NAB1-NAB3 distance: 112 ($p = 0.02$), NAB2-NAB3 distance: 142.4 ($p=0.064$). Taken together, these analyses imply that NAB1, NAB2 and NAB3 smell the world as does an average woman of their age, yet they are more similar to each other than expected by chance.

Subject index	Open Neuro	Age	Laterality index	Handedness	UPSIT	R OS (mm)	L OS (mm)	R OB volume (mm^3)	L OB volume (mm^3)
Control-01	sub-01	22	-80	L	36	6.8	8.7	48.2	38.1
Control-02	sub-02	29	-100	L	32	9.8	8.1	58.9	53.8
Control-03	sub-03	24	-100	L	26	6.5	7.8	37.5	44.9
Control-04	sub-04	23	-60	L	38	9.1	7.7	72.5	84.1
Control-05	sub-05	24	-70	L	34	8.7	10.1	60.7	51.5
Control-06	sub-06	24	-90	L	34	12.0	10.6	62.0	67.9
Control-07	sub-07	21	-90	L	36	9.9	10.4	57.4	67.3
Control-08	sub-08	29	-100	L	35	11.2	8.5	56.3	62.9
Control-09	sub-09	32	-100	L	37	8.0	8.5	74.9	78.0
Control-10	sub-10	28	-70	L	35	9.0	8.3	74.1	75.6
Control-11	sub-11	28	-100	L	35	8.3	9.0	49.1	51.3
Control-12	sub-12	26	-80	L	31	8.9	9.0	48.7	53.7
Control-13	sub-13	27	-70	L	36	10.0	9.2	21.7	37.3
Control-14	sub-14	22	-40	L	35	8.1	8.1	64.0	63.4
Control-15	sub-15	24	-40	L	26	8.4	8.1	74.9	51.0
Control-16	sub-16	29	30	L	34	9.1	6.2	57.0	64.9
Control-17	sub-17	25	-100	L	37	9.1	8.6	48.9	47.7
Control-18	sub-18	29	-100	L	38	10.1	9.4	60.9	57.6
NAB-01	sub-19	29	-80	L	33	6.4	2.5	0.0	0.0
NAB-02	sub-20	26	-10	L	26	9.6	1.7	0.0	0.0
NAB-03	sub-21	24	-40	L	28	4.8	5.3	0.0	0.0
NAB-CA	sub-22	33	100	R	8	0.0	3.7	0.0	0.0

Supplementary Table 1, Related to Figure 1.

Olfactory sulcus (OS) and olfactory bulb (OB) measurements across participants. Laterality index based on Edinburgh Handedness Inventory.