The Laryngoscope

Brain Volume Changes in Hyposmic Patients Before and After Olfactory Training

Janine Gellrich, MD; Pengfei Han, PhD; Cedric Manesse, PhD; Amelie Betz; Anne Junghanns; Claudia Raue, MD; Valentin A. Schriever, MD; Thomas Hummel, MD

Objectives/Hypothesis: Olfactory dysfunction is thought to be associated with reduced gray matter (GM) volume in olfactory-related brain areas. The aim of this study was to determine GM structural changes within olfactory-related regions of the brain in patients with smell loss due to upper respiratory tract infection (URTI) before and after olfactory rehabilitation.

Study Design: Prospective intervention case-control study.

Methods: Magnetic resonance imaging structural brain images were collected from 30 patients with smell loss due to URTI and 31 controls. Patients exposed themselves to odors (olfactory training [OT]) over 12 weeks and then were rescanned. Olfactory testing was performed using the validated Sniffin' Sticks test. GM was investigated with voxel-based morphometry.

Results: GM volumes were found to be reduced in the limbic system and thalamus among pretraining patients compared to controls; in patients, OT was associated with a significant increase of GM volume in these two regions. The GM volume within other olfactory-related regions was not different between patients and controls. In addition, no relevant difference between the GM volume pre- and post-OT was observed in primary olfactory-related regions.

Conclusions: OT was associated with an increase in GM volume of the hippocampus and the thalamus, possibly pointing toward a strategy for more effective exploitation of olfactory signals based on a higher degree of attention toward odors and association of memories with olfactory input.

Key Words: Upper respiratory tract infection, olfactory disorders, gray matter, voxel-based morphometry, olfactory bulb, olfactory training.

Level of Evidence: 3b.

***Laryngoscope*, 128:1531–1536, 2018**

INTRODUCTION

Upper respiratory tract infections (URTI) are one of the major causes of olfactory disorders. Approximately 11% of olfactory disorders treated in German, Austrian, and Swiss university hospitals are caused by URTI.[1] Even if the olfactory system has high plasticity,[2] only about one-third of the postinfectious patients exhibit spontaneous recovery.[3,4] Olfactory training (OT) is a method to stimulate recovery.[3,4]

It has already been shown there is a correlation between olfactory bulb (OB) volume and odor identification.[5,6] Patients with olfactory loss showed lower identification scores and smaller OB than healthy controls. Less is known about higher cerebral structural changes that would accompany olfactory recovery.

Voxel-based morphometry (VBM) is a mass-univariate technique to detect differences in the volume of gray (GM) and white matter (WM).[7] Studies have reported hyposmia is correlated with a decreased volume in olfactory-related brain regions.[8–11] Whereas Bitter and colleagues were the first to describe the GM alteration in anosmic and hyposmic patients,[8,9] Peng and colleagues showed an association between the duration of smell loss and amount and size of atrophies.[11] Yao and colleagues investigated GM volume in patients with idiopathic olfactory loss and found results similar to the previous studies.[8–11]

The aim of this study was to determine OB volume and GM structural changes in patients with hyposmia following URTI before and after OT in comparison to healthy controls.

MATERIALS AND METHODS

The study received approval of the ethics committee of the Medical Faculty of the Technical University of Dresden (EK96032015) and was conducted in accordance with the Declaration of Helsinki on Biomedical Studies Involving Human Subjects. The study was explained to all participants, both verbally and in writing; written consent was obtained prior to inclusion.

From the Department of Otorhinolaryngology (J.G., P.H., C.M., A.B., A.J., T.H.), Department of Neuropediatrics (J.G., V.A.S), and Department of Neuroradiology (C.R.), Technical University of Dresden, Dresden, Germany.

Editor's Note: This Manuscript was accepted for publication November 10, 2017.

This study was supported by a grant from the Else Kröner-Fresenius-Stiftung to V.A.S., J.G., and P.H., and from the Deutsche Forschungsgemeinschaft to T.H. (DFG HU441-18-1).

The authors have no other funding, financial relationships, or conflicts of interest to disclose.

Send correspondence to Janine Gellrich, Department of Neuropediatrics, Carl Gustav Carus Hospital, Technical University of Dresden, Fetscherstrasse 74, 01307 Dresden, Germany. E-mail: janine.gellrich@uniklinikum-dresden.de

DOI: 10.1002/lary.27045

Participants

Sixty-one subjects took part; there were 31 controls (age range 45–69 years, average age 53.5 ± 6.7 years [mean ± standard deviation], 17 female, 14 male) and 30 hyposmic patients (age range 38–80 years, average age 60.7 ± 10.3 years, 16 female, 14 male). The diagnosis of smell loss due to URTI was confirmed by medical history[12] and nasal examination including endoscopy and testing with the Sniffin' Sticks battery.[13] The average of self-reported duration of olfactory loss was 2.8 ± 5.1 years (4 months–20 years).

Olfactory Testing

TDI score (threshold, discrimination, identification) was assessed using the Sniffin' Sticks,[14] which are pen-like odor dispensers. Threshold was measured in blindfolded participants using 16 stepwise dilutions of phenylethyl alcohol in a three-alternative forced-choice (3AFC) procedure. For discrimination, a nonfitting suprathreshold odorant was identified in a 3AFC procedure. The participant's task for identification was to label 16 suprathreshold odors, each from a list of four descriptors, presented as both pictures and words.[15,16] Overall results were combined to TDI score. Whereas control subjects were only measured once, hyposmic participants were measured before and after 12 weeks of OT. Some of the participants underwent the extended identification test, which is twice the length (32 items) of the normal identification.[17]

Olfactory Training

Thirty patients underwent OT for 12 weeks twice a day. Controls performed no OT. All patients received four labeled glasses with odorants (phenylethyl alcohol: rose, eucalyptol: eucalyptus, citronella: lemon, and eugenol: cloves [all Sigma-Aldrich, Deisenhofen, Germany]). Patients were instructed to sniff odors for 10 seconds and to focus attention on the current odor.[4]

Structural Image Acquisition

Magnetic resonance imaging (MRI) scans were performed on a 3T Siemens Verio scanner (Siemens, Erlangen, Germany) with a 12-channel phased-array head coil. Using a three-dimensional (3D) magnetization prepared gradient rapid-acquisition gradient echo sequence, the T1-weighted images were acquired with the following parameters: time repetition 2,530 ms; time echo 2.34 ms; inversion time 1,100 ms; field of view 256 mm; voxel size 1 × 1 × 1 mm; flip angle 7°, 192 contiguous slices of 1 mm thickness. Images were acquired in the axial plane oriented parallel to the planum sphenoidale to minimize artifacts. OB sequence included acquisition of 2-mm-thick T2-weighted fast spin-echo images, with 2 × 2-mm voxel dimension, without interslice gap in the coronal plane covering the anterior and middle segments of skull base.

For this study, VBM analyses were performed using the toolbox of Cat12 (available at: http://dbm.neuro.uni-jena.de/vbm) implemented through SPM12 software (available at: http://www.fil.ion.ucl.ac.uk/spm) and MATLAB (MathWorks, Natick, MA). The analysis was performed as described in previous publications.[7] First, segmentation of the T1 images into GM, WM, and cerebrospinal fluid (CSF) was done. The classification of voxels was done depending on the grey steps and the classification of the surrounding voxels. Second, segmented GM images were spatially normalized in the customized template in standardized anatomical space using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL).[18] The goal of the normalization was to balance the global differences in brain shape between individual MRI brain scans of subjects. Third, images were smoothed with a Gaussian kernel (full width at half-maximum 8 mm) and normalized to Montreal Neurological Institute (MNI) space. Finally, all separated volumes (GM, WM, CSF) were summed to the total intracranial volume (TIV). All voxels with values less than 0.2 (corresponding to absolute threshold masking) were excluded to avoid possible edge effects between different tissue types.[7]

OB Volume Measurement

Segmentation of the OB was performed using AMIRA 3D (Visage Imaging, Carlsbad, CA) to process T2-weighted images.[19] Two researchers obtained OB volumes by manual bordering and following addition of all slices, which were multiplied by the slice thickness to yield volume. Whenever the two measurements of a OB volume differed less than 10% between observers, an average of the two was used. In cases of more than a 10% difference, a third observer measured.[20] For analyses, the sum of right and left OB was used.

Statistical Analyses

Analyses were performed using SPSS 23.0 (IBM, Armonk, NY) software, with the level of significance set at $P < .05$. For psychophysiological testing and olfactory bulb comparison, one-way analysis of variance was used to investigate differences between the patient and control group. For analyzing the difference before and after OT in patients, paired t tests were applied. Whenever appropriate nonparametric tests were used, VBM analysis was done, using age, sex, and TIV as covariates in a multiple regression. An uncorrected threshold with $P < .001$ and a cluster size of 100 voxels was applied to investigate changes in the olfactory-related brain areas (piriform, entorhinal, orbitofrontal, insular cortex, anterior cingulate, hippocampus, and thalamus).[10,11] To label areas of volume changes, the automated anatomic labelling atlas was used.[21] The association between variables was analyzed using Pearson's correlation.

RESULTS

Psychophysical Measurement

In comparison to controls, the patients had significantly lower age- and sex-corrected threshold (T) scores, indicating decreased sensitivity (control T = 9.4 ± 2.9, patients T = 1.8 ± 1.4, $P < .001$). Based upon the threshold, nearly all of the controls were characterized as normosmic (n = 30 normosmic, n = 1 hyposmic).

After OT patients reached significantly higher scores for threshold (before T = 1.8 ± 1.4, after T = 4 ± 3, $P = .004$), discrimination (D) (before D = 7.3 ± 2.6, after D = 9.5 ± 2.7, $P = .005$), identification (I) (before I = 7.3 ± 2.4, after I = 9 ± 2.7, $P = .007$), and combined TDI score (before TDI = 16.4 ± 3.6, after TDI = 21.9 ± 5.6, $P < .001$). Sixteen patients (53%) improved 5.5 or better in TDI, indicating a clinically significant improvement.

GM Volume Results

GM volume was compared between controls and patients and between patients before and after OT. With a threshold of $P < .001$ and voxel size ≥ 100 voxels, significantly reduced GM volume was observed in patients in two clusters, mainly in the areas of the hippocampus

TABLE I.
Reduction in Gray Matter Density for Upper Respiratory Tract Infection Patients With Severe Olfactory Dysfunction Compared to Healthy Controls.

Cluster No.	Side	Regions	Share of Cluster	MNI Coordinates (mm)			Peak T	Cluster Size
				X	Y	Z		
1	R	Parahippocampus	73.49%	27	−30	−6	3.87	166
		Hippocampus	22.29%					
		Thalamus	2.41%					
2	L	Parahippocampus	85.83%	−21	−38	3	3.75	127
		Hippocampus	7.09%					

The result is the threshold at $P < .001$ (uncorrected), cluster size ≥ 100 voxels. All coordinates are given in MNI (Montreal Neurological Institute) space, labeled through automated anatomic labeling.
L = left; MNI = Montreal Neurological Institute; R = right.

(Table I). No significant cluster in primary olfactory regions could be found comparing controls with patients. We further compared the GM volume between patients with long-term olfactory loss (n = 8, smell loss $\geq$ 24 months) and controls with superior olfactory function (n = 8). Results showed no GM volume difference in olfactory-related areas.

Following OT, patients exhibited an increase of GM volume in hippocampus, thalamus, and cerebellum (Table II, Fig. 1). Patients with a longer duration of smell disability showed no additional results. We separately analyzed patients with a TDI-improvement over 5.5 (threshold $P < .001$, 10 voxels cluster size). For these patients, we saw similar results and additionally a cluster in areas of the left anterior and medial orbitofrontal cortex (OFC) (MNI scale X = −29, Y = 33, Z = −20, cluster size 15).

OB Volume

Although a trend for OB volume difference between controls and patients before and after OT was shown (controls = 27.03 ± 10.17, before = 23.73 ± 8.18, after = 28.31 ± 10.84), no significant group difference was found ($F = 1.747$, $P = .18$). In approximately 90% of cases, a third observer was necessary.

VBM showed no significant correlation between GM volume of olfactory-related areas and OB volume.

TABLE II.
Volume Increase in Gray Matter Density for Upper Respiratory Tract Infection Patients Before and After Olfactory Training

Cluster No.	Side	Regions	Share of Cluster	MNI Coordinates (mm)			Peak T	Cluster Size
				X	Y	Z		
1	L	Parahippocampus	44.91%	−21	−21	−12	11.51	953
		Hippocampus	23.40%					
		Lingual	23.29%					
		Cerebellum	1.36%					
		Putamen	1.36%					
		Fusiform cortex	0.31%					
		Thalamus	0.21%					
2	L/R	Thalamus L	93.14%	−6	−3	6	8.71	175
		Thalamus R	6.29%					
3	R	Parahippocampus	50.21%	20	−29	−8	8.47	480
		Lingual	25.83%					
		Hippocampus	17.92%					
		Thalamus	5.83%					
4	L	Pallidum	52.07%	−9	3	11	7.76	121
		Nucleus caudate	45.45%					
5	L	Cerebellum	70.91%	−32	−53	−62	6.08	322
6	R	Cerebellum	3.93%	27	−53	−62	5.05	178
7	R	Vermis	64.24%	18	−56	−27	4.86	151
		Cerebellum	33.11%					

The result is the threshold at $P < .001$ (uncorrected), cluster size ≥ 100 voxels. All coordinates are given in MNI (Montreal Neurological Institute) space, labeled through automated anatomic labeling.
L = left; MNI = Montreal Neurological Institute; R = right.

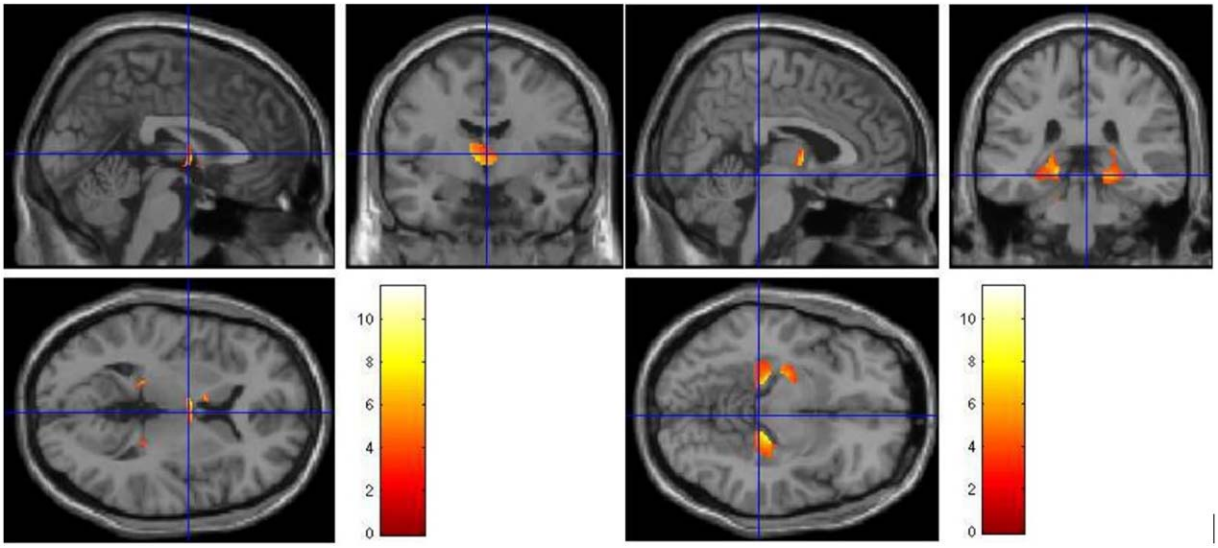

Fig. 1. Results from voxel-based morphometry analysis showing that patients after olfactory training have a higher volume in the hippocampus and thalamus. Color scale: 0 to 10 represents *Z* score. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

Analysis of the correlation between the changes in TIV, OB volume, and olfactory function showed no significant result for subgroups of pre- and post-OT patients.

Controls and patients pre-OT displayed no significant correlation between identification and OB, whereas patients post-OT showed a correlation of the extended identification test[17] and OB (extended I/OB $r = 0.41$, $P = .023$).

DISCUSSION

The Sniffin' Sticks test results indicated that URTI patients in this study were hyposmic or anosmic.[13] Even though patients did 12 weeks of OT instead of the now recommended 36 weeks,[22] patients' TDI score was significantly improved (before TDI = 16.4 ± 3.6, after TDI = 21.9 ± 5.6, $P < .001$). More than 50% of the patients improved on TDI with 5.5 points or more (n = 16 of 30). This confirmed the beneficial effect of OT for patients with smell loss caused by URTI as it had been shown previously.[3,4]

The olfactory system is known to have a high plasticity and the ability for spontaneous recovery after smell loss due to an URTI.[3] The association between olfaction and OB volume of patients with URTI smell loss has already been reported.[6] It could be shown that the OB is a highly plastic structure, with continuous remodeling of synaptic connections due to synaptogenesis and cell regeneration. If the olfactory input is decreased by URTI-caused epithelium destruction, these processes could lead to a reduction of OB volume.[23,24] Furthermore, a correlation between identification ability and OB volume has been reported.[5] Although we saw a trend of volume changes between the study groups from a higher OB volume in controls to a lower volume in patients before OT, and an increase of the volume after OT, we did not see significant results for this change and did not observe a significant correlation of OB volume and identification for controls or patients before OT. Only patients after OT showed such correlation. One possible explanation for these results could be the relatively small sample size or a nonlinear association between the development of smell loss and OB volume reduction in patients. Quite often a third observer for OB volume measurement was necessary, because the initial two observers differed more than 10%. Nevertheless, the reliability of the method is considered to be high. Two independent studies showed an inter- and intraobserver reliability over 90%.[25,26]

The current study did not yield significant differences in GM volume between controls and patients in primary olfactory related regions. Former studies showed a decrease of GM volume in primary and secondary olfactory regions for anosmic and hyposmic patients. However, these studies did not only focus on URTI, but included a wide spectrum of etiologies of olfactory dysfunction (e.g., head injuries and sinonasal disorders), with some of the participants having very long durations of olfactory loss.[8,9,11,27] One could speculate the maintained olfactory input in hyposmic URTI patients could be enough to prevent GM alteration.

Although there was no significant change of GM volume of primary olfactory-related regions in this study, an increase of GM volume has been seen in the hippocampus, thalamus, and cerebellum in patients following OT. This result was also found for a subgroup of patients with a TDI-improvement over 5.5. These patients showed an increase of GM volume in the medial and anterior OFC. Controls showed a higher GM volume in the hippocampus and thalamus than patients before OT.

Other studies also reported changes in regions of the hippocampus, thalamus, and cerebellum as well as the OFC. Bitter and colleagues showed in 2010, for anosmic and hyposmic patients, changes in GM volume of various olfactory-related regions including the OFC.

They reported a decreased volume in cerebellum and in regions of the hippocampal structure.[8,9] Peng and colleagues found for the primary olfactory cortex only changes for the right hemisphere. They assumed VBM analysis was not the right method to display changes in the relatively small structures of the primary olfactory-related regions. As in the present study, they reported changes in the cerebellum and parahippocampal structures, and discussed especially the role of the cerebellum in olfactory perception.[11] Yao and colleagues reported a case of a boy with congenital anosmia and performed a study with 20 healthy controls comparing GM volume. They also did a study with patients suffering from idiopathic smell loss. They saw changes in primary olfactory-related regions as well as in regions of the hippocampus, thalamus, and cerebellum.[10,28] None of these studies examined potential changes that could result from OT and a potential reversibility of GM volume changes.

The hippocampus, which plays a key role in the organization of memory, is also part of secondary olfactory-related brain regions[29]; it has also been shown to be influenced by physical exercise[30] and memory training in the elderly.[31] The hippocampus is involved in processing olfactory information.[32,33] Although the OT task does not involve an active memory component it can be assumed that exposure to odors (and eventually also the confrontation with odorous perception) also involves memory-related activations. It appears natural, although this was not controlled for and was not explicitly intended by the training, that patients would think more about smells and try to search for odor-related memories. Nevertheless, it is possible that the obtained differences in the hippocampus was based upon other influences that were not measured in this study.

The thalamus was the second structure, which in patients exhibited a loss of GM volume in comparison to controls, and showed an increase after OT. Previous work indicated functional relevance of the thalamic pathway as an active modulatory target of olfactory attention.[34] Thalamic volume is also known to correlate with cognitive speed of healthy people[35]; other work showed attention-induced activation in thalamus.[36] As the patients were asked to concentrate on the different odors during OT, attention played some role in this everyday exercise.

Besides the sniffing procedure during OT, which should be practiced twice a day, a motion sequence is implicated (take the jar, open it up, sniff, close) that may stimulate the cerebellum. This could be shown for the sniff of odors as well as for the motion of taking a breath through the nose without having an odor in front.[37] A second study reported that the cerebellum is not only involved in modulating the sniffing process but also in olfactory cognitive processing.[38] Furthermore, it is descripted that the cerebellum is activated during processing of odor intensity, quality, recognition, and episodic memory.[39]

The anterior and medial OFC, which showed a small area of significantly increased GM volume in the 16 patients with a clinically significant improvement, are part of the processing of olfactory information.[40,41] Although former VBM studies[8–11] showed a decrease of GM in the OFC, we were able to detect a recovery of this structure after OT. A study about the connectivity after OT also reports an increasing connectivity of the integrative network to which the OFC belongs.[42]

Limitations of the study should be acknowledged. Based on the recent literature, OT (executed over 12 weeks in this study) is now suggested for a longer duration, and various odors should be changed every 3 months to achieve higher rates of recovery. Although our patients received the classical OT, they achieved excellent results (16 patients over 5.5 points improvement in TDI score). A repetition of this study with updated OT might lead to an even higher level of improvement among patients and, accordingly, to different results in VBM analysis.

Another point that needs to be discussed is that participants were not asked to keep a diary about the OT to measure compliance. It would be interesting to use adherence as a covariate in the future. Based upon the results, we can assume that the patients implemented the training in their routine because we obtained an improvement of 53%. The self-recovery rate of patients suffering from olfactory impairment caused by URTI is approximately 30%.[43]

Because of a higher degree of spontaneous recovery in the first year, it might be worth restricting study to only patients with a longer duration of illness. Nevertheless, we decided not to implement such a restriction because spontaneous recovery is also possible after the first year, although the likelihood to recover decreases after 2 or 3 years.[44] Furthermore, OT could work as a promotor of recovery, so it appears reasonable to begin OT during the period of relatively high regenerative activity. Nevertheless, one could not deny that some effects may be due to spontaneous improvement itself and not to OT.

Controls were slightly younger than patients because they were not fully age matched. This could affect results of TDI and GM volume, with decreasing olfactory function and TIV in the elderly. The influence on this study seems to be low; patients already showed impairment caused by URTI. As the GM volume decreases with aging, especially in the neocortical, prefrontal, and parietal cortex, but to a much lesser degree in hippocampal and thalamic areas,[45] such an effect would not change the results here because we saw no difference in neocortical, prefrontal, and parietal cortex between controls and patients.

VBM is one of the many approaches to investigate human neural features.[11] Other studies addressed a change of neuronal connectivity as an effect of OT.[42,46] Although VBM analysis could only display information about volume changes, this method could make an assertion to functional changes.

CONCLUSION

This study showed that OT in URTI patients with decreased olfactory function is associated with an

increase of GM volume of the hippocampus, thalamus, and cerebellum, but not with a change in primary olfactory-related regions. This may indicate that exposure to odor predominantly influences the processing and evaluation of olfactory input, although this speculation awaits further research.

BIBLIOGRAPHY

1. Damm M, Temmel A, Welge-Lüssen A, et al. Olfactory dysfunctions. Epidemiology and therapy in Germany, Austria and Switzerland [in German]. *HNO* 2004;52:112–120.
2. Wilson DA, Best AR, Sullivan RM. Plasticity in the olfactory system: lessons for the neurobiology of memory. *Neuroscientist* 2004;10:513–524.
3. Damm M, Pikart LK, Reimann H, et al. Olfactory training is helpful in postinfectious olfactory loss: a randomized, controlled, multicenter study. *Laryngoscope* 2014;124:826–831.
4. Hummel T, Rissom K, Reden J, Hähner A, Weidenbecher M, Hüttenbrink K-B. Effects of olfactory training in patients with olfactory loss. *Laryngoscope* 2009;119:496–499.
5. Buschhüter D, Smitka M, Puschmann S, et al. Correlation between olfactory bulb volume and olfactory function. *Neuroimage* 2008;42:498–502.
6. Rombaux P, Mouraux A, Bertrand B, Nicolas G, Duprez T, Hummel T. Olfactory function and olfactory bulb volume in patients with postinfectious olfactory loss. *Laryngoscope* 2006;116:436–439.
7. Ashburner J, Friston KJ. Voxel-based morphometry—the methods. *Neuroimage* 2000;11:805–821.
8. Bitter T, Gudziol H, Burmeister HP, Mentzel H-J, Guntinas-Lichius O, Gaser C. Anosmia leads to a loss of gray matter in cortical brain areas. *Chem Senses* 2010;35:407–415.
9. Bitter T, Brüderle J, Gudziol H, Burmeister HP, Gaser C, Guntinas-Lichius O. Gray and white matter reduction in hyposmic subjects—a voxel-based morphometry study. *Brain Res* 2010;1347:42–47.
10. Yao L, Pinto JM, Yi X, Li L, Peng P, Wei Y. Gray matter volume reduction of olfactory cortices in patients with idiopathic olfactory loss. *Chem Senses* 2014;39:755–760.
11. Peng P, Gu H, Xiao W, et al. A voxel-based morphometry study of anosmic patients. *Br J Radiol* 2013;86:20130207.
12. Hummel T, Hummel C, Welge-Lüssen A. Assessment of olfaction and gustation. In: Management of Smell and Taste Disorders: A Practical Guide for Clinicians. Stuttgart, Germany: Thieme; 2013:58–75.
13. Hummel T, Kobal G, Gudziol H, Mackay-Sim A. Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. *Eur Arch Otorhinolaryngol* 2007;264:237–243.
14. Hummel T, Sekinger B, Wolf SR, Pauli E, Kobal G. "Sniffin" Sticks': Olfactory performance assessed by the combined testing of odour identification, odor discrimination and olfactory threshold. *Chem Senses* 1997;22: 39–52.
15. Schriever VA, Mori E, Petters W, Boerner C, Smitka M, Hummel T. The "Sniffin' Kids" test—a 14-item odor identification test for children. *PLoS One* 2014;9:e101086.
16. Sorokowska A, Albrecht E, Hummel T. Reading first or smelling first. Effects of presentation order on odor identification. *Atten Percept Psychophys* 2015;77:731–736.
17. Haehner A, Mayer A-M, Landis BN, et al. High test–retest reliability of the extended version of the "Sniffin' Sticks" test. *Chem Senses* 2009;34: 705–711.
18. Ashburner J. A fast diffeomorphic image registration algorithm. *Neuroimage* 2007;38:95–113.
19. Yousem DM, Geckle RJ, Bilker WB, Doty RL. Olfactory bulb and tract and temporal lobe volumes. Normative data across decades. *Ann N Y Acad Sci* 1998;855:546–555.
20. Rombaux P, Grandin C, Duprez T. How to measure olfactory bulb volume and olfactory sulcus depth? *B-ENT* 2009;5(suppl 13):53–60.
21. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 2002;15: 273–289.
22. Altundag A, Cayonu M, Kayabasoglu G, et al. Modified olfactory training in patients with postinfectious olfactory loss. *Laryngoscope* 2015;125: 1763–1766.
23. Rombaux PH, Duprez T, Hummel T. Olfactory bulb volume in the clinical assessment of olfactory dysfunction. *Rhinology* 2009;47:3–9.
24. Curtis MA, Kam M, Nannmark U, et al. Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *Science* 2007;315: 1243–1249.
25. Yousem DM, Geckle RJ, Doty RL, Bilker WB. Reproducibility and reliability of volumetric measurements of olfactory eloquent structures. *Acad Radiol* 1997;4:264–269.
26. Hummel T, Urbig A, Huart C, Duprez T, Rombaux P. Volume of olfactory bulb and depth of olfactory sulcus in 378 consecutive patients with olfactory loss. *J Neurol* 2015;262:1046–1051.
27. Bitter T, Gudziol H, Burmeister HP, Mentzel H-J, Gaser C, Guntinas-Lichius O. Volume alterations in the gray matter of anosmic subjects. Lessons we can learn from voxel-based morphometry [in German]. *HNO* 2011;59:248–254.
28. Yao L, Yi X, Wei Y. Gray matter alteration in isolated congenital anosmia patient: a voxel-based morphometry study. *Eur Arch Otorhinolaryngol* 2013;270:2569–2573.
29. Smitka M, Puschmann S, Buschhueter D, et al. Is there a correlation between hippocampus and amygdala volume and olfactory function in healthy subjects? *Neuroimage* 2012;59:1052–1057.
30. Erickson KI, Voss MW, Prakash RS, et al. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci* 2011;108: 3017–3022.
31. Valenzuela MJ, Jones M, Caroline Rae WW, Graham S, Shnier R, Sachdev P. Memory training alters hippocampal neurochemistry in healthy elderly. *Neuroreport* 2003;14:1333–1337.
32. Pellegrino R, Hähner A, Bojanowski V, Hummel C, Gerber J, Hummel T. Olfactory function in patients with hyposmia compared to healthy subjects—an fMRI study. *Rhinology* 2016;54:374–381.
33. Poellinger A, Thomas R, Lio P, et al. Activation and habituation in olfaction—an fMRI Study. *Neuroimage* 2001;13:547–560.
34. Plailly J, Howard JD, Gitelman DR, Gottfried JA. Attention to odor modulates thalamocortical connectivity in the human brain. *J Neurosci* 2008; 28:5257–5267.
35. Van Der Werf YD, Tisserand DJ, Visser PJ, et al. Thalamic volume predicts performance on tests of cognitive speed and decreases in healthy aging: a magnetic resonance imaging-based volumetric analysis. *Cogn Brain Res* 2001;11:377–385.
36. LaBerge D, Buchsbaum MS. Positron emission tomographic measurements of pulvinar activity during an attention task. *J Neurosci* 1990;10:613–619.
37. Sobel N, Prabhakaran V, Hartley CA, et al. Odorant-induced and sniff-induced activation in the cerebellum of the human. *J Neurosci* 1998;18: 8990–9001.
38. Cerf-Ducastel B, Murphy C. fMRI activation in response to odorants orally delivered in aqueous solutions. *Chem Senses* 2001;26:625–637.
39. Savic I, Gulyas B, Larsson M, Roland P. Olfactory functions are mediated by parallel and hierarchical processing. *Neuron* 2000;26:735–745.
40. Gottfried JA. Smell: central nervous processing. In: Hummel T, Welge-Lüssen A, eds. Advances in Oto-Rhino-Laryngology. Basel, Switzerland: Karger; 2006:44–69.
41. Rolls ET. The functions of the orbitofrontal cortex. *Brain Cogn* 2004;55: 11–29.
42. Kollndorfer K, Fischmeister FPS, Kowalczyk K, et al. Olfactory training induces changes in regional functional connectivity in patients with long-term smell loss. *Neuroimage Clin* 2015;9:401–410.
43. Reden J, Mueller A, Mueller C, et al. Recovery of olfactory function following closed head injury or infections of the upper respiratory tract. *Arch Otolaryngol Head Neck Surg* 2006;132:265–269.
44. Welge-Lüssen A, Wolfensberger M. Olfactory disorders following upper respiratory tract infections. In: Hummel T, Welge-Lüssen A. eds. Taste and Smell: An Update. Basel, Switzerland: Karger Publishers; 2006: 125–132.
45. Kalpouzos G, Chételat G, Baron J-C, et al. Voxel-based mapping of brain gray matter volume and glucose metabolism profiles in normal aging. *Neurobiol Aging* 2009;30:112–124.
46. Kollndorfer K, Kowalczyk K, Hoche E, et al. Recovery of olfactory function induces neuroplasticity effects in patients with smell loss. *Neural Plast* 2014;2014:e140419.