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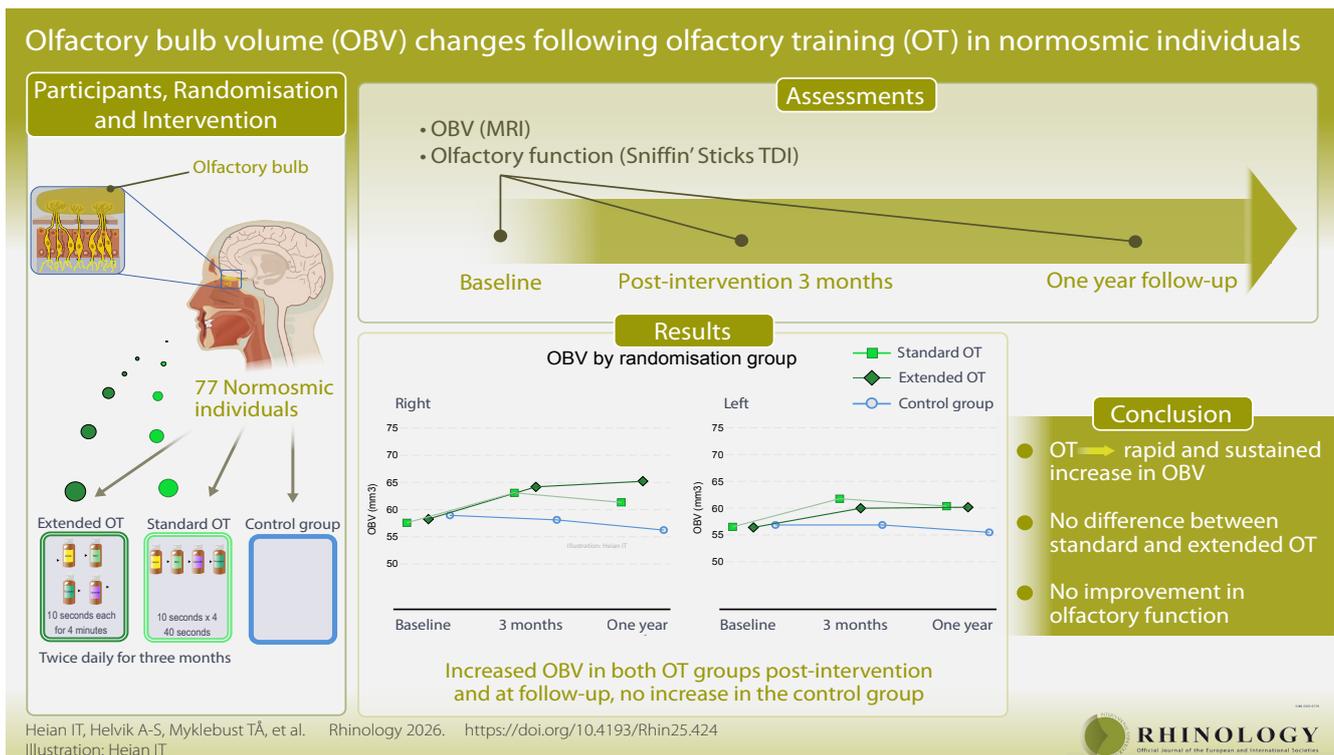
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Olfactory bulb volume (OBV) changes following olfactory training (OT) in normosmic individuals

Abstract

Background: Olfactory training (OT) has been linked to changes in olfactory function and structural modifications in the olfactory bulb (OB); however, the neuroplastic potential in the OB remains unclear. In this pilot study, we investigate how OT with different exposure lengths influences olfactory bulb volume (OBV) and olfactory function in individuals with normosmia. **Methodology**: Seventy-seven normosmic individuals were assigned to either standard OT, extended OT, or a control group. The intervention groups performed OT for three months, sniffing four odours - eucalyptus, lavender, mint and lemon – for 10 seconds per bottle, twice daily, totalling either 40 seconds (standard OT) or 4 minutes (extended OT), while the control group did not perform any OT. OBV (manual segmentation of 3-Tesla magnetic resonance images) and olfactory function (Sniffin' Sticks test) were assessed at baseline, post-intervention and at one-year follow-up. **Results**: OBV increased significantly in both the standard and extended OT groups after the intervention and at follow-up, compared to controls. There were no differences between the training methods and no significant changes in olfactory function. **Conclusions**: In normosmic individuals, OBV increased after both standard and extended OT, with no differences between training methods. The volume increase was evident at three-month assessment and persisted at one-year follow-up, indicating that neuroplastic changes induced by OT occur rapidly and may extend beyond the duration of the training itself, an effect not previously reported. However, the OBV changes were not accompanied by improvements in olfactory function.

Key words: olfactory training, olfactory bulb, neuronal plasticity, magnetic resonance imaging, olfaction disorders

Introduction

The olfactory system has a unique neuroplastic capacity [1,2]. This is fundamental for preserving olfactory function throughout life, and mitigating the adverse effects that age, sinonasal disease, infections, trauma, neurodegenerative disorders, and toxins can have on olfaction [3].

Neuroplasticity is evident throughout the entire olfactory pathway, from peripheral to central regions, influenced by both bottom-up and top-down modulation [1,2]. Unique among sensory systems, this plasticity is enhanced by continuous integration of newborn neurons in the olfactory epithelium [4] and possibly in the olfactory bulb [5] throughout life. The neuroplasticity can be triggered as a response to damage or disease, or by sensory stimulation, such as olfactory training (OT) [1,2,6,7]. OT is a well-documented treatment for olfactory dysfunction (OD) and has proven to be effective, largely regardless of aetiology [3,6,8]. OT has the potential to improve olfactory function, and this improvement often correlates with structural and functional changes in olfactory processing areas of the brain [6].

The olfactory bulb (OB) serves as the initial relay station in the olfactory pathway, playing a central role in transmitting and processing olfactory information, and is found to have remarkable plasticity [2]. The olfactory bulb volume (OBV) is correlated to olfactory function both in patients [9,10] and in healthy subjects [11-13], with larger volumes being associated with better olfactory function [12]. Small OBV has been associated with unfavourable prognostic outcomes in some conditions [3,14,15].

The neuroplasticity of the OB is explored in a few magnetic resonance imaging (MRI) studies. In patients with chronic rhinosinusitis, surgical treatment resulted in improved olfactory function and increased OBV [7]. The same was found after OT in patients with idiopathic OD and OT after laryngectomy [16,17]. In patients with posttraumatic and postinfectious OD, OT resulted in improved olfactory function, but unchanged OBV [18-20]. In healthy individuals, lateralised OT led to an increase in OBV on both sides, indicating the top-down influence on OBV [21]. Similarly, sommelier training in students caused increased OBV [22]. However, olfactory function did not improve in these two studies.

Despite the observed correlation between OT, olfactory function and structural changes in the OB, the underlying mechanisms of this neuroplastic phenomenon, as well as the long-term effects of OT on OBV, remain unclear. Understanding olfactory plasticity may improve OT protocols for individuals with OD. Furthermore, studying normosmic individuals can uncover this plasticity potential without the influence of disease-related factors.

In this study, we aim to explore how OT with different exposure lengths influences OBV and olfactory function in normosmic individuals, both in the short and long term. Additionally, we will investigate factors influencing OBV, including age, sex, allergy, endoscopic findings and olfactory function scores.

Materials and methods

In this pilot study, the participants were assigned to either perform OT with two different exposure lengths or to a control group. The Clinical Research Unit at the Norwegian University of Science and Technology provided a web-based program for randomisation. The participants were evaluated with Sniffin' Sticks test, nasal endoscopy and MRI at baseline, after three months and after one year. The clinical trial number was NCT02980718.

Participants

This predefined secondary analysis included 67 participants recruited from our main randomised controlled trial (n=200) [23], between January 2018 and December 2019, to participate in the MRI sub-study. Due to a shortage of control participants, an additional 10 controls were non-randomly recruited between May to October 2018, resulting in a total of 77 participants: 28 assigned to standard OT [24], 29 to extended OT [23] and 20 to a control group with no OT. This total exceeds the 45 participants originally planned in the study protocol (NCT02980718), as the substudy was expanded to increase the statistical power. Fifteen of the participants dropped out for different reasons (Figure 1). One participant was missing post-intervention OBV due to poor MRI quality (motion artefacts). The inclusion criteria were adults from 18 to 65 years old with normosmia, as indicated by a TDI score ≥30.75 [25]. Exclusion criteria were having diseases affecting olfaction (sinonasal diseases, recent sinonasal surgery, neurodegenerative diseases, multiple sclerosis or chronic obstructive pulmonary disease, as described in detail in [23]), subjects not being able to participate due to language limitations, practical implementation issues or mental conditions, and finally subjects who were unable to undergo MRI due to pregnancy, magnetic implants, or claustrophobia. The participants signed informed consent, wherein they indicated whether they wanted feedback on any incidental findings on MRI that should be investigated or treated. They did not receive any financial compensation for participation. The study was approved by the Regional Committee for Medical Research Ethics in Mid-Norway (reference number 2016/837), and investigations followed the principles of the Declaration of Helsinki/Hong Kong.

Olfactory training

In the two intervention groups, participants were instructed to perform OT for three months, with sessions twice daily of four bottles containing oils from eucalyptus, lavender, mint and lemon. Those in the standard OT group were instructed to sniff each bottle for 10 seconds, totalling 40 seconds, while those in
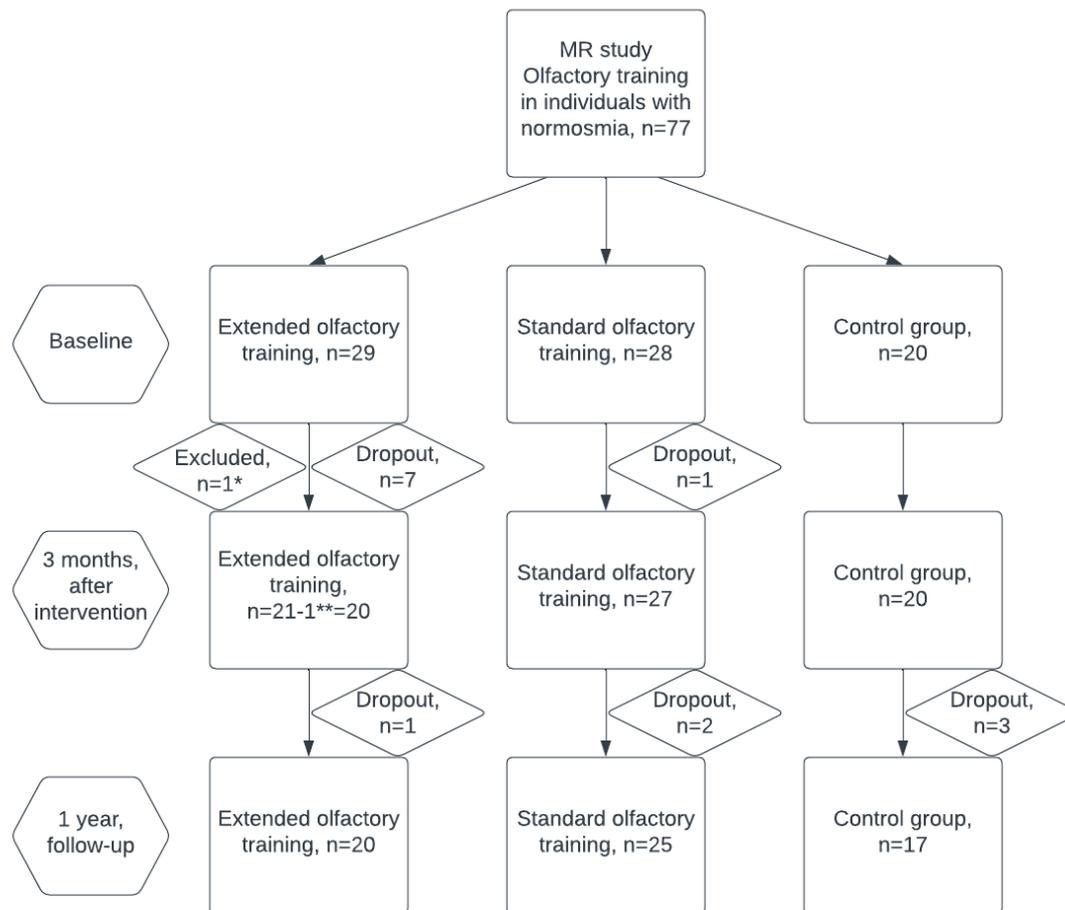
Figure 1. Inclusion and exclusion flowchart of the participants. * Excluded due to braces. ** One participant was not included in analyses due to poor MRI quality.

the extended OT group were instructed to sniff each bottle for 10 seconds and then rotate them without delay, for a total of 4 minutes. To ensure focused attention on the OT, participants in the intervention groups were asked to record their training sessions in a diary.

Olfactory assessment
Olfactory performance was assessed using the Sniffin' Sticks test (Burghart Messtechnik, Holm, Germany), to determine the odour threshold (T), discrimination (D) and identification (I) score [26]. The TDI score, summing scores from the T, D and I subtests, with a maximum of 48 points (each subtest with 16 points), was used to categorise participants as normosmic (score ≥30.75) [25]. The test is described in detail in a previous paper [23].

Olfactory bulb volume
MRI scanning was performed on a 3.0 Tesla scanner (MAGNE-TOM Skyra, Siemens) at St Olav University Hospital in Trondheim, Norway. OBV was assessed using coronal T2-weighted images with a slice thickness of 1.5 mm and an interslice gap of 5%, resulting in a slice distance of 1.575 mm. The left and right OBV

were manually measured by contouring the OB surface of each slice from anterior to posterior (Figure 2) on coronal slices using the radiological PACS software Sectra IDS7, where the anterior slice is where OB become visible, and the posterior slice is where there is a marked decrease in OB diameter [27]. Finally, all segmented surfaces were added and multiplied by the slice distance to calculate the OBV in mm^3, accounting for the interslice gap. OBV segmentations for the right and left sides were done twice by ITH, after discussion of the segmentation procedure [27] with a neuroradiologist (EMB). The mean OBV intra-rater difference was 1.5%. 10% of the segmentations were verified by EMB, of which none needed correction. Furthermore, in cases with an intra-rater variation of more than 10% (one case), EMB performed new segmentations. ITH was blinded to the study group, but not to the time of measurement or olfactory function scores. EMB was blinded to the study group and olfactory function scores, but not to the time of measurement. The final OBV recorded in the database was the mean of the measurements. Normal OBV was defined to be ≥ 58 mm^3 for those < 45 years and ≥ 46 mm^3 for those > 45 years [13].
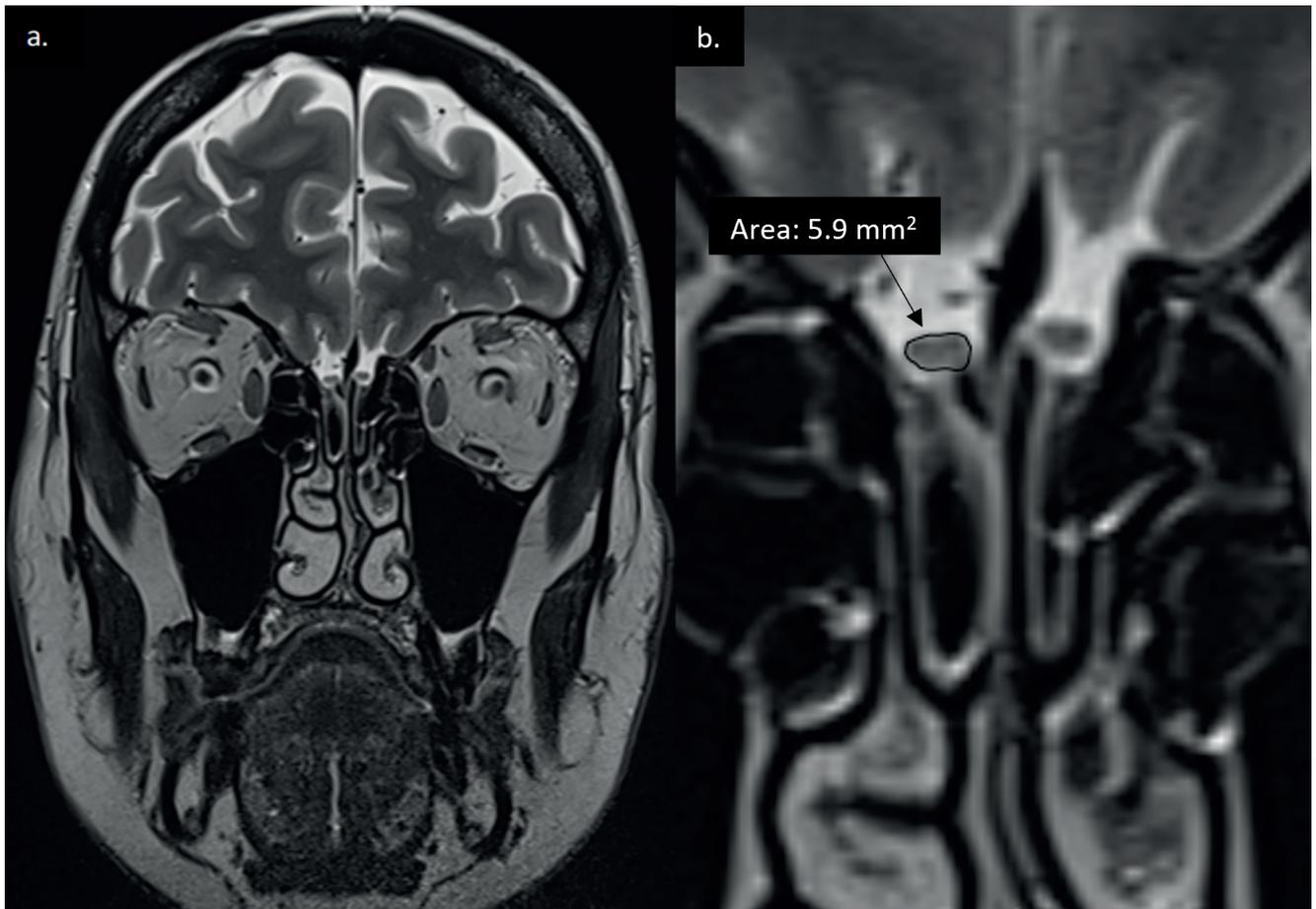
Figure 2. Coronal view of the brain MRI (3 Tesla) as seen on the PACS software: A) Zoomed out. B) Zoomed in on the olfactory bulbs, with the circumference of the right olfactory bulb delineated and the area calculated.

Variables

Background variables (age, sex, allergy symptoms, smoking, education) were assessed via a questionnaire, and self-reported olfactory function was evaluated on a 100 mm VAS scale. Allergy status was determined by skin prick test, where participants with a positive test and typical symptoms of hypersensitivity were classified as having allergic rhinitis. Nasal endoscopy was performed by an otolaryngologist, with findings scored using the modified Lund-Kennedy system, described in detail in a previous paper [23].

Statistical analysis

The analyses were conducted according to a predetermined analysis plan [28]. Stata version 18.0 was used for statistical analysis. Baseline characteristics were compared across the three groups using Chi-square tests and ANOVA. Normality assumptions were assessed for all continuous variables using test of normality (Shapiro-Wilk), histograms and Q-Q plots, confirming normality. To explore whether the data were Missing at Random, logistic regression analysis was performed, indicating some differences between dropouts and completers. The extended

OT group had a somewhat higher dropout rate compared to the other groups, suggesting that the data might be considered Missing Not at Random (MNAR). Mixed model analyses using multiple imputation (chained equations) were subsequently compared to analyses based on available data (Table S1 and Table 2). As the results revealed no substantial changes in estimates, the analyses based on available data without multiple imputation are presented here. Changes in OBV and olfactory function after intervention and at follow-up were estimated using linear mixed models, comparing the two intervention groups and the control group. Models included the following variables: study arm, follow-up time, OBV, olfactory function (T, D, I), age, sex, allergic rhinitis, smoking, education and endonasal endoscopic findings of mucus or oedema. Interaction effects between measurement time (baseline, after intervention, after one year) and OT regimen (extended OT, standard OT, control group) were assessed. To examine the effect of the intervention in subgroups, three-way interaction effects between study arm, follow-up time, and covariates of interest (age, sex, allergic rhinitis, endoscopic findings, and olfactory function scores) were estimated. Due to the low number of smokers and participants

Table 1. Demographics, descriptive statistics of the three study groups at baseline.

	Standard OT	Extended OT	Controls	P-value
N, number (%)	28 (36.4)	29 (37.7)	20 (26.0)	
Age, mean (SD)	38.7 (11.0)	37.6 (11.3)	36.1 (10.6)	0.72
Women [a], number (%)	18 (64.3)	20 (69.0)	17 (85.0)	0.27
Smoker [b], number (%)	1 (3.6)	3 (10.3)	0 (0.0)	0.25
Allergic rhinitis [c], number (%)	9 (32.1)	13 (44.8)	4 (22.2)	0.27
Education				0.66
High school, number (%)	4 (14.3)	2 (6.9)	2 (10.0)	
College/University, number (%)	24 (85.7)	27 (93.1)	18 (90.0)	
MLK, mean (SD)	0.3 (0.7)	0.7 (1.3)	0.3 (0.6)	0.12
Oedema/mucus [d], number (%)	4 (14.3)	8 (27.6)	3 (15.0)	0.38
T, mean (SD)	7.3 (1.8)	7.3 (1.5)	7.6 (2.2)	0.85
D, mean (SD)	13.6 (1.4)	13.6 (1.4)	13.4 (1.5)	0.85
I, mean (SD)	13.2 (0.9)	13.6 (1.5)	13.6 (1.1)	0.47
TDI, mean (SD)	34.0 (2.3)	34.4 (2.6)	34.5 (2.7)	0.79
VAS olfactory function, mean (SD)	62.4 (14.1)	69.4 (13.4)	70.8 (15.7)	0.08
OBV in mm^3, right, mean (SD)	57.5 (12.7)	58.3 (18.8)	59.0 (10.9)	0.94
OBV in mm^3, left, mean (SD)	56.5 (18.2)	56.4 (18.5)	56.9 (15.4)	1.00
OT diary sessions, mean (SD)	160.0 (22.2)	153.1 (19.0)	NA	0.27

P-values compare baseline characteristics across the three groups. VAS: visual analogue scale; MLK: modified Lund Kennedy endoscopy score; T: threshold; D: discrimination; I: identification; TDI: sum of the T, D, and I scores; OBV: olfactory bulb volume; OT: olfactory training; NA: Not Applicable. Note. [a] vs men, [b] vs non-smoker, [c] vs no allergic rhinitis, [d] vs no oedema/mucus.

Table 2. Estimated mean changes in olfactory bulb volume and olfactory function following olfactory training, relative to the control group, and differences in olfactory bulb volume between the two intervention groups.

	Standard OT		Extended OT		Differences Extended OT – Standard OT	
After intervention	Mean Δ (95% CI)	p-value	Mean Δ (95% CI)	p-value	Mean (95% CI)	p-value
Left OBV	5.5 (1.5, 9.5)	0.007*	3.8 (-0.5, 8.0)	0.08	-1.7 (-5.5, 2.2)	0.40
Right OBV	6.7 (3.2, 10.1)	<0.001*	7.5 (3.8, 11.1)	<0.001*	0.8 (-2.5, 4.1)	0.64
T	-0.01 (-1.0, 1.0)	0.99	-0.2 (-1.2, 0.8)	0.69		
D	-0.1 (-1.2, 1.1)	0.89	-1.1 (-2.3, 0.1)	0.07		
I	0.7 (-0.3, 1.6)	0.17	0.7 (-0.3, 1.6)	0.17		
TDI	0.5 (-1.2, 2.3)	0.56	-0.7 (-2.5, 1.1)	0.46		
Follow-up						
Left OBV	4.4 (0.3, 8.5)	0.03*	5.3 (1.0, 9.6)	0.02*	0.8 (-3.1, 4.7)	0.67
Right OBV	5.2 (1.7, 8.7)	0.004*	9.0 (5.4, 12.7)	<0.001*	3.8 (0.5, 7.2)	0.02*
T	-0.3 (-1.2, 0.7)	0.57	0.4 (-1.4, 0.6)	0.42		
D	-0.5 (-1.7, 0.7)	0.43	-0.2 (-1.4, 1.0)	0.79		
I	0.3 (-0.6, 1.2)	0.53	-0.2 (-1.2, 0.7)	0.65		
TDI	-0.3 (-2.1, 1.4)	0.72	-0.6 (-2.4, 1.2)	0.53		

Estimated mean changes in OBV (mm^3) and olfactory function after intervention (3 months) and at follow-up (one year), relative to controls, and differences in OBV between the two intervention groups, derived from linear mixed model on available data. OBV: olfactory bulb volume; T: Threshold; D: Discrimination; I: Identification; TDI: Sum of the T, D and I scores; CI: Confidence interval. * =significant.
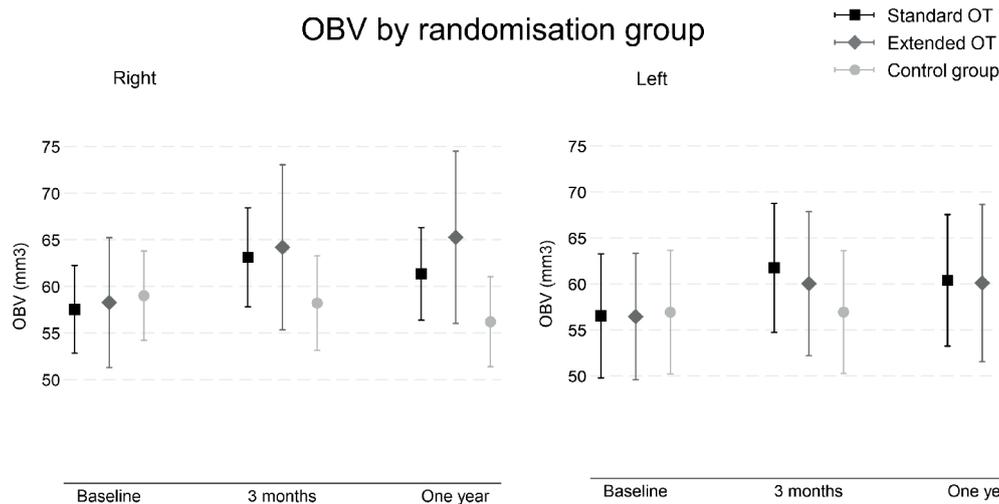
Figure 3. Right and left olfactory bulb volume by randomisation group and time points. The dots represent the mean, and the error bars indicate 95% confidence intervals. OBV: olfactory bulb volume; OT: olfactory training.

with lower education than college/university, these variables were excluded from further analysis. The significance level was set at 0.05.

Results

At baseline, no significant differences were observed in characteristics, olfactory function or OBV between the three randomisation groups (Table 1). All participants submitted the OT diary following the intervention.

Effects of OT on OBV and olfactory function

A linear mixed model, based on available data (n=77), was used to analyse changes in left and right OBV after intervention and at follow-up. Both OT groups had significantly larger OBV on both sides at both time points, compared to the control group, except for the left OBV after extended OT at three months (Figure 3 and Table 2). When comparing the two OT groups, right OBV was significantly larger after extended OT at one-year follow-up compared to standard OT, but no other significant differences were observed (Table 2). There were no significant changes in T, D, I, or TDI in either OT group compared to controls at any time point (Table 2).

Association of sex and age with OBV and changes in OBV

At baseline, OBV was significantly smaller in women compared to men (right; 54.2 vs. 67.8 mm³, p<0.001, left; 54.2 vs. 62.6 mm³, p=0.05), but there was no significant association between OBV and age (p>0.4). Three-way interaction analyses were performed to explore whether the effect of OT on OBV was influenced by baseline measures. No statistically significant effects were found for age or sex (Table 3), nor for other baseline measures (allergic rhinitis, endoscopic findings of mucus or oedema, or olfactory function scores at baseline (Table S2)) on OBV in the standard

or extended OT group compared to controls at three months or one year follow-up.

Discussion

In this study, we aimed to explore the impact of OT with different exposure lengths on OBV in normosmic individuals. Our results show that, compared to controls, OBV increased in both the standard and extended OT groups after the intervention and at follow-up, except for the left OBV at three months following extended OT. Extended OT resulted in a significantly larger increase in right OBV compared to standard OT at follow-up, but there were no other significant differences between the training methods. No changes in olfactory function were observed in the OT groups either after the intervention or at follow-up, compared to controls. Men had larger OBV than women, while age showed no effect on OBV. Neither sex, age, nor other cofounders influenced the changes in OBV in the different study groups.

Our findings of increased OBV following OT provide further evidence of the plasticity potential in the OB, as previously demonstrated in healthy individuals [21,22], in patients with idiopathic OD and after laryngectomy [16,17], as well as following sinus surgery in patients with chronic rhinosinusitis [7,29]. There is still no evidence of OBV changes following OT in patients with post-traumatic OD and postinfectious OD [18,19,30] which may indicate a reduced plasticity potential in the olfactory pathway after trauma- or infection-induced damage. However, OT has been shown to have a convincing effect on olfactory function in these patients [6]. Furthermore, our results indicate that OBV changes occur rapidly following OT, as the increase in OBV was already present at post-intervention assessment at three months.

The observed increase in OBV persisted at one-year follow-up in

Table 3. Three-way interaction analyses showing the effect of age and sex on OBV changes.

After intervention		Standard OT		Extended OT	
		Mean (95% CI)	p-value	Mean (95% CI)	p-value
Age	Left OBV	0.2 (-0.2, 0.5)	0.31	-0.3 (-0.6, 0.1)	0.15
	Right OBV	0.004 (-0.3, 0.3)	0.98	-0.2 (-0.5, 0.1)	0.27
Women [a]	Left OBV	3.5 (-6.2, 13.2)	0.48	0.2 (-10.0, 10.5)	0.96
	Right OBV	-0.1 (-8.4, 8.2)	0.98	-4.5 (-13.3, 4.3)	0.31
Follow-up					
Age	Left OBV	0.02 (-0.4, 0.4)	0.93	-0.3 (-0.7, 0.1)	0.11
	Right OBV	-0.02 (-0.3, 0.3)	0.93	-0.16 (-0.5, 0.2)	0.32
Women [a]	Left OBV	5.5 (-5.3, 16.3)	0.32	3.4 (-7.8, 14.5)	0.55
	Right OBV	4.1 (-5.1, 13.2)	0.38	-0.5 (-10.0, 9.1)	0.93

Estimated differences in OBV (mm³) based on three-way interaction effects, considering age and sex in the standard or extended OT group compared to controls after three months or one year follow-up. OBV: olfactory bulb volume. Note. [a] vs men.

both OT groups, suggesting that the structural changes in the OB are sustained over time. To our knowledge, this evidence of lasting neuroplastic changes of the OB after OT has not been demonstrated in prior studies. However, two studies exploring the long-term effects of OT in post-infectious and post-traumatic OD found that short-term OT resulted in a lasting improvement in olfactory function [31,32]. This suggests that the neuroplastic changes induced by OT may endure beyond the duration of the training itself, indicating potential for lasting improvements in olfactory processing, possibly through both peripheral and central neuronal modifications following OT [1,2,33,34].

We found no evidence to indicate that extended OT was superior to standard OT, as the only observed difference was a greater increase in right OBV in the extended OT group at follow-up, with no other significant differences between the training methods. This is supported by other studies, which demonstrate that intensified OT regimes do not necessarily enhance training effects [20,23,35-37]. Furthermore, extended OT is more time-consuming and may result in lower adherence to training [23].

Even if OBV increased after OT, there was no corresponding improvement in olfactory function, suggesting that structural growth does not always correlate with functional improvement, at least not in healthy subjects. This is supported by a study demonstrating that lateralised OT increased OBV bilaterally, without improving olfactory function [21], as well as a study on sommelier training in students, where OBV increased without a corresponding improvement in olfactory test scores [22]. However, OBV is considered a reliable indicator of olfactory function [9-13,38] and a key mediator in the relationship between the volume of central olfactory structures and olfactory function [10], and our results on healthy individuals may be due to a ceiling effect

[23]. The Sniffin' Sticks test was designed to distinguish OD from normosmia but may not be well suited for detecting changes in olfactory function within normosmic individuals. Notably, beyond its role on olfactory function, and likely due to the close relationship between olfactory-related and higher-order cognitive processing areas of the brain, OT is found to influence various cognitive processes [6,39], and the observed increase in OBV after OT may reflect these broader effects.

Although OBV was significantly larger in men than in women at baseline, as previously reported [10,40-42], sex did not influence the effect of OT on OBV. Despite fertile women having superior olfactory function compared to men [43,44], the OB in women is found to have a higher number of neurons and glial cells, suggesting that the number of neurons may serve as a more precise indicator of neural function than volume [45]. Regardless, the plasticity potential appears to be the same in men and women.

In our study in healthy participants, OBV did not change significantly with age, nor did age appear to influence the effect of OT on OBV. While many studies have shown a reduction in OBV with increasing age, this appears to be linked to the simultaneous decline in olfactory function [10,13]. The absence of age-related differences in OBV in our study is likely due to the inclusion of only individuals with normosmia. The unique neuroplasticity of the OB [2] may explain why it does not decrease with age, unlike whole-brain volume in cognitively normal individuals [46]. Notably, with increasing age, OBV plays an increasingly important role in linking central olfactory structures and olfactory function, making it particularly relevant in age-related olfactory changes and a potential marker for identifying individuals at risk of neurodegenerative disease [10].

This study has some strengths and limitations. One strength is the inclusion of a homogenous group of normosmic individuals with no influence of olfactory-related diseases, along with follow-up data to better explore the plasticity potential of the OB. Second, the study is a predefined secondary analysis based on data from a randomised controlled trial, which included two OT groups and a control group with no OT, where all participants underwent the same measurements at all three time points, allowing for consistent comparisons across groups. Third, the images were acquired using a 3-Tesla MRI scanner with an OB protocol, a reliable tool for assessing OBV and OB plasticity, with a good intra- and interobserver reliability [2]. However, measurement errors may have occurred, but a neuroradiologist verified a significant portion of the measurements. Furthermore, we did not evaluate OB shape, which can be relevant for olfactory outcomes [20], nor head size to standardise OBV [10]. Other limitations include the absence of a power calculation because no preliminary data were available. The sample size was determined by feasibility, and the results should therefore be interpreted with caution. The missing data were most likely MNAR, as those randomised to the more time-consuming extended OT had a higher dropout rate, which may have influenced the statistical analyses. However, analyses with and without imputation were conducted (Table 2 and Table S1), and no substantial differences in estimates were observed. Although participants received precise instructions on the training protocol and were encouraged to log each training session in a provided diary, adherence cannot be guaranteed and therefore remain a source of uncertainty.

This study is the first to explore the long-term effect of OT on OBV, as well as the impact of extended OT on OBV in healthy individuals, thus making an important contribution to the understanding of the plasticity potential of the OB. It may also have clinical implications for recommendations for olfactory recovery and imaging diagnostics in the assessment of olfactory disorders. Future studies should focus on long-term follow-up of OBV changes in individuals undergoing OT, as this may enhance our understanding of OBV's role in predicting olfactory recovery and guiding personalised treatment approaches.

Conclusion

In normosmic individuals, OBV increased following both standard and extended OT, with no compelling differences between the two training methods. The volume increase emerged early and was sustained at one-year follow-up, suggesting that the neuroplastic changes induced by OT develop rapidly and may persist beyond the duration of the training period. However, no corresponding improvement in olfactory function was observed. Men had larger OBV than women, while OBV remained unchanged with increasing age. Sex, age, and other covariates were not associated with changes in OBV across the different study groups.

Acknowledgements

Author contributions

ITH: Study design, data collection, MRI-based volumetric analysis, statistical analysis, paper drafting; ASH: Study design, statistical analysis, paper drafting; EMB: Study design, MRI-based volumetric analysis, paper drafting; TAM: Statistical analysis, paper drafting,TH, SN: Study design, paper drafting; MB: Study design, data collection; WMT: Study design, data collection, MRI-based volumetric analysis, statistical analysis, paper drafting.

Conflict of interest

None declared.

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Corrected Proof

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This manuscript contains online supplementary material

SUPPLEMENTARY MATERIAL

Table S1. Estimated changes and differences in Olfactory Bulb Volume following olfactory training, relative to control group, based on imputed dataset.

After intervention	Standard OT		Extended OT		Differences Extended OT – Standard OT	
	Mean Δ (95% CI)	p-value	Mean Δ (95% CI)	p-value	Mean (95% CI)	p-value
Left OBV	5.0 (-2.1, 12.0)	0.17	3.8 (-4.5, 12.1)	0.36	-0.8 (-8.5, 6.9)	0.76
Right OBV	5.9 (0.01, 11.8)	0.05*	7.0 (-0.2, 14.3)	0.06	0.2 (-7.1, 7.5)	0.74
Follow-up						
Left OBV	5.3 (-3.5, 14.0)	0.23	5.1 (-3.3, 13.5)	0.23	1.4 (-7.4, 10.2)	0.96
Right OBV	5.7 (-0.9, 12.2)	0.09	8.6 (2.4, 14.9)	0.007*	3.1 (-4.7, 11.0)	0.27

Estimated changes and differences in OBV (mm³) after intervention (3 months) and at follow-up (one year), relative to controls, derived from linear mixed model of imputed dataset. OBV: Olfactory Bulb Volume; CI: confidence interval. * =significant.

Table S2. Three-way interaction analyses showing the effect of allergy, endoscopic findings, and olfactory function scores at baseline, on OBV changes.

After intervention		Standard OT		Extended OT	
		Mean (95% CI)	p-value	Mean (95% CI)	p-value
Allergic rhinitis [a]	Left OBV	-2.8 (-11.7, 6.1)	0.54	-9.2 (-18.7, 0.4)	0.06
	Right OBV	4.3 (-3.3, 11.9)	0.26	-3.9 (-11.8, 3.9)	0.32
Oedema/mucus [b]	Left OBV	1.8 (-8.8, 12.5)	0.74	-5.4 (-15.4, 4.7)	0.29
	Right OBV	-1.5 (-10,6, 7.7)	0.76	-2.8 (-11.5, 5.9)	0.53
T1	Left OBV	-0.2 (-2.4, 1.9)	0.84	0.0 (-2.3, 2.3)	0.99
	Right OBV	-0.5 (-2.4, 1.3)	0.58	-0.3 (-2.3, 1.7)	0.79
D1	Left OBV	-0.6 (-3.3, 2.2)	0.68	2.3 (-0.9, 5.4)	0.15
	Right OBV	-0.7 (-3.1, 1.7)	0.56	-0.66 (-3.4, 2.1)	0.64
I1	Left OBV	-0.8 (-4.6, 2.9)	0.66	-2.5 (-5.8, 0.8)	0.14
	Right OBV	0.1 (-3.1, 3.3)	0.95	-2.4 (-5.2, 0.4)	0.09
TDI1	Left OBV	-0.5 (-2.2, 1.1)	0.53	-0.2 (-1.8, 1.3)	0.77
	Right OBV	-0.6 (-2.0, 0.8)	0.41	-0.9 (-2.2, 0.4)	0.18
Follow-up					
Allergic rhinitis [a]	Left OBV	1.5 (-8.1, 11.1)	0.76	-7.7 (-17.4, 1.9)	0.12
	Right OBV	-2.1 (-10.3, 6.1)	0.62	-6.5 (-14.7, 1.8)	0.13
Oedema/mucus [b]	Left OBV	10.0 (-1.6, 21.5)	0.09	0.6 (-10.5, 11.7)	0.91
	Right OBV	7.3 (-2.6, 17.3)	0.15	6.6 (-2.9, 16.2)	0.18
T1	Left OBV	0.6 (-1.6, 2.9)	0.57	0.5 (-1.9, 2.8)	0.69
	Right OBV	-0.17 (-2.1, 1.8)	0.86	0.19 (-1.8, 2.2)	0.86
D1	Left OBV	2.4 (-0.3, 5.2)	0.09	3.3 (-1.3, 7.9)	0.07
	Right OBV	0.9 (-1.5, 3.4)	0.46	1.3 (-1.6, 4.2)	0.38
I1	Left OBV	1.4 (-2.3, 5.3)	0.44	1.8 (-1.4, 5.1)	0.26
	Right OBV	0.6 (-2.6, 3.9)	0.71	-1.6 (-4.4, 1.1)	0.24
TDI1	Left OBV	1.5 (-0.1, 3.2)	0.08	1.4 (-0.1, 3.0)	0.06
	Right OBV	0.2 (-1.2, 1.6)	0.78	-0.1 (-1.4, 1.3)	0.91

Estimated differences in OBV (mm³) based on three-way interaction effects, considering allergy, endoscopic findings, and olfactory function scores at baseline in the standard or extended OT group compared to controls after three months or one year follow-up. OBV: olfactory bulb volume; T1: threshold at baseline; D1: discrimination at baseline; I1: identification at baseline; TDI1: sum of the T, D, and I score at baseline. Note. [a] vs no allergic rhinitis, [b] vs no oedema/mucus.