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ORIGINAL RESEARCH

Changes in olfactory bulb volume following lateralized olfactory training

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Abstract Repeated exposure to odors modifies olfactory function. Consequently, "olfactory training" plays a significant role in hyposmia treatment. In addition, numerous studies show that the olfactory bulb (OB) volume changes in disorders associated with olfactory dysfunction. Aim of this study was to investigate whether and how olfactory bulb volume changes in relation to lateralized olfactory training in healthy people. Over a period of 4 months, 97 healthy participants (63 females and 34 males, mean age: 23.74 ± 4.16 years, age range: 19–43 years) performed olfactory training by exposing the same nostril twice a day to 4 odors (lemon, rose, eucalyptus and cloves) while closing the other nostril. Before and after olfactory training, magnetic resonance imaging (MRI) scans were performed to measure OB volume. Furthermore, participants underwent lateralized odor threshold and odor identification testing using the "Sniffin' Sticks" test battery.

OB volume increased significantly after olfactory training (11.3 % and 13.1 % respectively) for both trained and untrained nostril. No significant effects of sex, duration and frequency of training or age of the subjects were seen. Interestingly, PEA odor thresholds worsened after training, while olfactory identification remained unchanged.These data show for the first time in humans that olfactory training may involve top-down process, which ultimately lead to a bilateral increase in olfactory bulb volume.

Keywords Olfaction · Olfactory bulb · Plasticity · Regeneration · Training

Introduction

The olfactory bulb (OB) is not only an olfactory relay station towards central processing structures, but it has a functionality that might resemble primary sensory cortices (Cleland and Linster 2005). In parallel with improving magnetic resonance imaging (MRI) capabilities, an increasing number of studies described variations of the human OB volume. In healthy subjects, OB volume was found to correlate to the measured olfactory function and to vary as a function of age (Yousem et al. 1998; Buschhuter et al. 2008; Mazal et al. 2014; Hummel et al. 2013a). In fact, lateralized differences in OB volume in healthy subjects correlate to lateralized olfactory function (Hummel et al. 2013a). Further reflecting olfactory function fluctuations, OB volume variations were shown in different types of olfactory pathologies, as posttraumatic, postviral or sinonasal olfactory loss (Hummel et al. 2015; Askar et al. 2015; Altundag et al. 2014; Patterson et al. 2015; Rombaux et al. 2006; Rombaux et al. 2008; Rombaux et al. 2010b; Haehner et al. 2008; Gudziol et al. 2009; Mueller et al. 2005; Yousem et al. 1996; 1999). They mainly involve changes below the level of the OB, suggesting that peripheral olfactory input might modulate OB volume. However, central pathologies as depression (Negoias et al. 2010; Croy et al. 2013; Negoias et al. 2015), schizophrenia (Turetsky et al. 2000; Nguyen et al. 2011; Rupp 2010), temporal lobe epilepsy (Hummel et al. 2013b), multiple sclerosis (Goektas et al. 2011), Alzheimer's disease (Thomann et al. 2009) or

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idiopathic normal pressure hydrocephalus (Podlesek et al. 2012) also seem to influence OB volume, either by directly affecting the OB or by top down modulation mechanisms.

Although well characterized for non human mammals, especially rodents, the presumed neurogenesis mechanisms to explain human OB volume variation are still under debate (for review see (Huart et al. 2013)). Strong evidence for OB plasticity comes from longitudinal data on olfactory deprivation or exposure. In rodents, closing one nostril leads to decrease in the ipsilateral OB (von Gudden 1870; Benson et al. 1984; Cummings et al. 1997; Coppola 2012). Similarly in humans, olfactory deprivation after laryngectomy (Veyseller et al. 2012) or after nasal obstruction of different causes (Askar et al. 2015; Altundag et al. 2014) leads to a reduced OB volume.

On the other hand, it is well known that exposure to odors induces improvement of olfactory function. Repeated exposure to odors in healthy subjects has been shown to significantly increase olfactory sensitivity (Engen and Bosack 1969; Rabin and Cain 1986; Dalton et al. 2002), or to improve the recovery of patients with postviral olfactory loss (Damm et al. 2014; Hummel et al. 2009) and prevent olfactory deterioration in older people (Schriever et al. 2014). Further on, patients with Parkinson's disease performing "olfactory training" (OT) were shown to improve significantly their olfactory ability (Haehner et al. 2013). Additional evidence comes from data on subjects performing lateralization training that significantly improved their ability to lateralize olfactory stimuli compared to subjects performing Sudoku problems (Negoias et al. 2013). Interestingly, Mainland et al. showed that lateralized exposure to androstenone lead to an increase in sensitivity of both trained and untrained side (Mainland et al. 2002). Few morphological correlates for olfactory training effects have been described to date: patients with chronic rhinosinusitis (CRS) with polyps showed an OB volume increase after nasal polyps surgery (Gudziol et al. 2009) while perfumers show gray matter changes in olfactory brain areas suggesting that repeated olfactory exposure counteracts the effects of aging (Delon-Martin et al. 2013). In the same line, OB volume seems to be significantly bigger in early-blinded subjects as compared to healthy subjects, probably as a compensatory mechanism by positive reinforcement of olfactory cues in every day life (Rombaux et al. 2010a). Thus, assuming that olfactory input manipulation induces morphological changes at the OB level, we set out to explore the influence of repeated exposure to odors on OB volume in healthy subjects. Further on, trying to shed light onto possible mechanism responsible for changes in OB volume, we opted for preforming lateralized olfactory training. If the OB were affected exclusively by bottom up mechanisms, lateralized changes in OB volume would be expected. If the changes would occur on both trained and untrained nostril, according to the observation of Mainland et al. (Mainland et al. 2002), one should consider more complex bottom-up and top-down mechanisms.

Material and methods

The study followed the Declaration of Helsinki on Biomedical Research Involving Human Subjects and was approved by the Ethics Committee from the Technical University of the Dresden Medical School (EK85032011). All participants provided written informed consent and received symbolic financial compensation for their participation.

Subjects

A total of 97 participants (63 females and 34males, mean age: 23.74 ± 4.16 years, age range: 19–43 years) were included in the study after being recruited via posters placed in the vicinities of the university clinic / by word of mouth and screened for exclusion criteria (age < 18 years; olfactory impairments; smoking; history of any medical condition known to interfere with olfactory function: neurological or medical comorbidity, severe head trauma, chronic drug abuse, acute or severe chronic rhinitis or sinusitis; inability to comply with the procedure, claustrophobia, pregnancy, or need for ferromagnetic devices / presence of traces of metal that could interfere with the MRI scan). A detailed medical history review and nasal endoscopy were performed to exclude sources of olfactory disorders. A mini mental state examination (MMSE, (Folstein et al. 1975)) was employed to screen for major cognitive impairment. Further on, subjects evaluated their olfactory and taste functions as well as the nasal patency on an 8-point scale ranging from "excellent" to "complete loss". Subjects were required to perform olfactory training over a period of 4 months, as first described by Hummel and coworkers (Hummel et al. 2009), where olfactory training was shown to produce an improvement in overall olfactory function in patients with olfactory loss. Before and after training, measurements of olfactory function and OB volume were performed. Data about individual importance of olfaction in daily life was also collected before and after training by means of a standardized questionnaire (Croy et al. 2010), consisting of 18 items. The questionnaire assesses 3 subscales: the emotions, memories and evaluations that are triggered by the sense of smell ("association-scale"), the level a person uses his or her sense of smell in daily life ("application-scale") and the consequences persons draw from their daily olfactory impressions ("consequence-scale").

Assessment of olfactory function

Olfactory threshold and identification were tested separately for the left and right nostril using the "Sniffin' Sticks" battery

(Hummel et al. 1997) based on pen-like odor dispensers. These two tests were selected in order to investigate olfactory function at both threshold and suprathreshold levels. Other suprathreshold olfactory tasks like odor discrimination were not included because the procedure would have become very time-consuming and only measurements of threshold and identification were found to correlate with the OB volume in previous studies (Buschhuter et al. 2008). To present an odor, the pen's cap was removed by the experimenter for approximately 3 s and the tip of the pen is placed approximately 2 cm in front of the nostril. Odor thresholds were determined for phenyl ethyl alcohol (PEA, a rose-like odor) diluted in propylene glycol, with altogether 16 numbered dilutions, number 1 representing the strongest, and number 16 the weakest odor. The dilution series started from a stock solution of 4 % PEA in propylene glycol; this was diluted in a ratio of 1 volume PEA to 2 volumes of propylene glycol. Odors were presented in triplets of pens (3-alternative forced choice paradigm; 3-AFC), with one pen among each triplet containing diluted PEA and two containing only propylene glycol, serving as blanks. The interval between presentations of individual pens of a triplet was approximately 3 s; the entire procedure for any triplet required roughly 20 s. Subjects were blindfolded with a sleeping mask to prevent visual identification of the odor-containing pens. Thresholds were determined using a single staircase technique: two successive correct identifications of the pen containing the odor or one incorrect response triggered a reversal of the staircase to the next higher or the next lower dilution step, respectively. Seven reversals had to be obtained (Hummel et al. 1997; Ehrenstein and Ehrenstein 1999). Odor thresholds were determined as the average dilution of the last four staircase reversals. Odor identification (Hummel et al. 1997) was determined by presenting the subjects 16 pens containing different odorants. The subjects' task was to identify the odorant out of a list with 4 items in a forced choice procedure. The identification scores were the counts of correctly identified pens. Testing was performed according to one of the 2 following sequences and randomized across subjects (Thresholds right > Identification right > Thresholds left > Identification left or Thresholds left > Identification left > Thresholds right > Identification right). The untested nostril was sealed using soft, odorless tape (microfoam; 3 M, Saint Paul, MN, USA). Subjects were instructed to breathe in through the free nostril and out through the mouth.

Olfactory training procedure

Participants were instructed to perform one-nostril olfactory training following a standardized procedure for 4 months (Hummel et al. 2009). They were asked to sniff 4 odorants for approximately 10 s each, twice a day, in the morning and in the evening: phenyl ethyl alcohol (PEA, rose-like), eucalyptol (eucalyptus), citronellal (lemon) and eugenol (cloves). These odorants were chosen to be representative of the 4 odor categories claimed by Henning (Henning 1916) in his work on the "odor prism" („Geruchsprisma"), where he tried to identify primary odors (compare (Amoore 1977)). These categories are flowery: „blumig" (e.g., rose), foul: „faulig", fruity: „fruchtig" (e.g., lemon), aromatic: „wuerzig" (e.g., cloves), burnt: „brenzlich", and resinous: „harzig" (e.g., eucalyptus). Training subjects received four brown glass jars (total volume 50 mL) with one of the four odors in each (1 mL each, soaked in cotton pads to prevent spilling). All jars were labeled with the odor name. Each training session included exposing always the same nostril to each of the 4 odorants for 10 seconds. The assigned training nostril was randomized across subjects. The untrained nostril was gently closed with the index finger, without applying much pressure. Subjects were provided with written instructions including visual depiction of the training procedure. Additionally, a "training diary" was included, where subjects were asked to evaluate their overall olfactory ability, number of training sessions performed and the intensity of each odorant once a week. Further on, they could provide observations about nose symptoms or eventual changes in the quality or intensity of the odorants. The intention here was to reinforce the continuous interest in the training procedure.

Assessment of OB volume

MRI measurements were performed with a 1.5-Tesla scanner (Sonata Vision; Siemens, Erlangen, Germany) using an 8 channel-head coil. The protocol included a whole brain anatomical sequence without interslice gap (5-mm-thick standard T1-weighted 3D sequence) for every participant to rule out any organic brain disorders. The OB sequence included acquisition of 2-mm-thick T2-weighted fast spin-echo images, with 2 by 2 mm voxel dimension, without interslice gap in the coronal plane covering the anterior and middle segments of the base of the skull. Images were offline processed and left and right OBs limits were drawn manually on each coronal slice using the AMIRA 3D visualization and modeling system (Visage Imaging, Carlsbad, USA). OB volumes were calculated by planimetric manual contouring (surface in mm^2) and all surfaces were added and multiplied by 2 (2-mm slice thickness) to obtain a volume in cubic millimeters. The field of view was 256x256mm^2. The sudden change of diameter at the beginning of the olfactory tract was used as the distal demarcation of the OB, as suggested by Yousem and colleagues (Yousem et al. 1998; Yousem et al. 1997). The described procedure was used in multiple studies focusing on OB volumetrics, with consistent results, e.g. (Buschhuter et al. 2008; Croy et al. 2013; Hummel et al. 2013a; Hummel et al. 2013b; Negoias et al. 2010). OB measurements were performed at least two times by the same experimenter (KP)

who was blinded to the time of measurement, trained nostril or subjects' olfactory test results. In case the two measurements diverged by more than 10 % the measurement of the OB was performed a third time. This was the case in 51 from 388 measurements (97 subjects* 2 MRI scans*2 measurements each). The two of the three volumes that were least different were then used for further analysis.

Statistical analysis

Data were analyzed using SPSS 22 (SPSS Inc., Chicago, Ill, USA). Data was verified for normal distribution. The normally distributed olfactory threshold and OB volume data for the trained and untrained nostril, before and after training were compared using paired samples t-tests. An ANOVA with "training" (before and after) and "nostril" (trained and untrained) as within-subjects factors and "sex" as between-subjects factor was used to test sex differences for OB volume and olfactory thresholds. Non-parametric tests (the Sign test) were used for the non-normally distributed identification scores and "importance of olfaction" data. The correlation between OB volume and olfactory function was calculated employing Pearson's correlation coefficient. Alpha level was set at 0.05, testing was performed two-tailed.

Results

Regarding documented *compliance to training*, the mean number of performed olfactory trainings sessions was 204 ± 53 (ranging from 25 to 291 sessions) corresponding to a mean frequency of 11 ± 2 sessions per week (ranging from 2 to 14 sessions) over a mean period of 18.4 ± 2.7 weeks (ranging from 2 to 23 weeks).

The olfactory training elicited a statistically significant median increase in perceived *importance of olfaction* score ($Z = 2.4$ points, $p = .01$). This reflected differences in the „application-scale", representing the level a person uses his or her sense of smell in daily life ($Z = 2.1$, $p = .04$). No correlation between duration, frequency and overall number of training sessions and improvement in the importance of olfaction score was found. No sex differences were observed.

Olfactory thresholds (see Table) were significantly higher after training, equivalent to a decrease in sensitivity for both trained ($t(96) = 3.84$, $p < .001$) and untrained nostril ($t(96) = 2.90$, $p = .005$). No effect of training for *olfactory identification* scores was seen (see Table).

OB volume after training was significantly bigger for both trained ($t(96) = 7.53$, $p < 0.001$) and untrained ($t(96) = 8.9$, $p < 0.001$) nostril. No difference in effect size between the trained and the non- trained nostril was found ($t(1,96) = -1.23$, $p > 0.05$). An increase in OB volume after training was observed in 79/97 subjects on both trained and nontrained side, while in 18 cases OB got smaller. All in all, OB volume increased by 11.3 % in the trained nostril and 13.1 % in the untrained nostril (see Fig. 1 and Table 1).

No correlation between OB volume and olfactory function measurements was seen. No significant effect was found comparing the influence of gender, duration and frequency of training or age of the subjects.

Discussion

The main finding of the present study was the significant increase in the OB volume after lateralized OT for both trained and untrained nostril. This is to our knowledge the first longitudinal study to show OB volume modulation after systematic exposure to odors in normosmic subjects.

Generally, proof of volumetric changes in olfactory regions emerged to date mainly from data on olfactory deprivation or deficits (Bitter et al. 2010; Huart et al. 2013), while few focused on olfactory exposure (Royet et al. 2013). The plasticity of the OB has been demonstrated in a wide variety of studies in relation to various types of diseases (for review (Huart et al. 2013)). Nevertheless only a handful of these studies offered proof of OB morphological changes based on longitudinal data. One example is Gudziol et al. showing an increase in OB volume in patients with nasal polyps 3 months after sinus surgery (Gudziol et al. 2009). Further on, in a series of 13 hyposmic patients, the change in olfactory function measured between 3 months and 6 years after initial diagnosis correlated with OB volume, indicating that patients who improve their olfactory function also show an increase in OB volume (Haehner et al. 2008). Finally, a recent study showed a decrease in OB volume in patients after laryngectomy, reflecting the olfactory deprivation induced in these patients (Veyseller et al. 2012). These studies, together with cross-sectional data on peripheral olfactory dysfunctions and animal data, emphasize the importance of olfactory input in modulating OB volume and stand for the bottom-up theory of OB volume change. Our data of increased OB volume after repeated olfactory exposure in normosmic subjects seems to further support this idea. However, the change in OB volume did not occur only for the trained nostril, but for both trained and untrained nostrils. Consequently, central mechanisms, i.e. by top down influences, must also be involved in OB volume modulation. Previous work on olfactory loss in patients with temporal lobe epilepsy (Hummel et al. 2013b) or various others central diseases (i.e. psychiatric conditions as depression or schizophrenia, neurodegenerative diseases as Parkinson or Alzheimer, multiple sclerosis, etc. see Introduction) support this idea. Such top-down processes are likely when considering the strong top-down projections from the olfactory cortex to the OB (Neville and Haberly 2004). To this respect, an interesting question is whether the

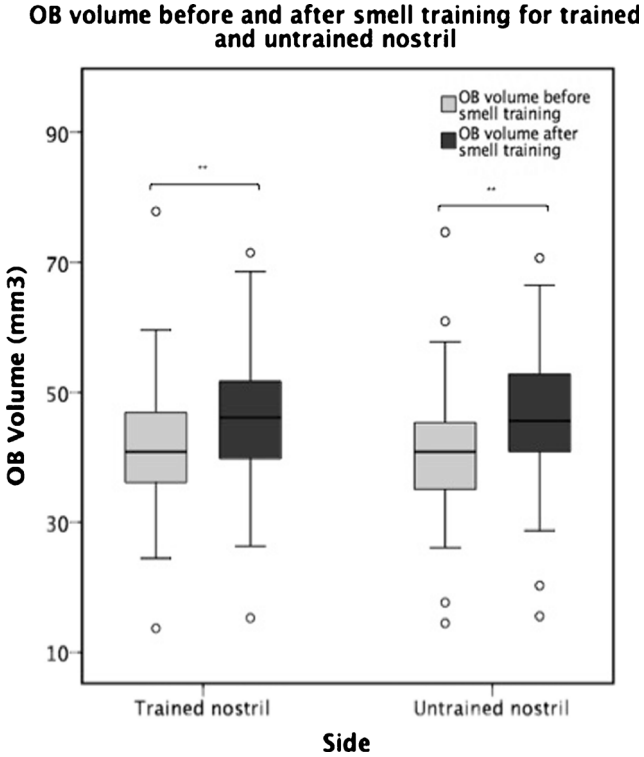

Fig. 1 Depiction of olfactory bulb volume for trained and untrained nostril, before and after training ("**" represent a level of significance <0.001, circles represent outliers)

mechanisms affecting the volume of the OB and leading to olfactory loss could be influenced, so that olfactory function could be strengthened again (for example in the specific case of temporal lobe epilepsy, whether OB volume would increase once the disease is successfully treated, as after surgery). Though reported in animals, connections between both OB via the stria olfactoria medialis have been shown to have little or no function in humans (Cleland and Linster 2003). Therefore it is less likely, that the observed effects are due to this connection.

Contrary to expected, general olfactory function not only did not improve after training in this group of young, healthy subjects, but PEA odor thresholds actually worsened. In patients with olfactory loss, OT typically induces an increase in olfactory function (Hummel et al. 2009; Damm et al. 2014; Altundag et al. 2015; Konstantinidis et al. 2013; Fleiner et al. 2012; Kollndorfer et al. 2014; Haehner et al. 2013; Geissler et al. 2014; Mori et al. 2015), although one study in older people failed to demonstrate such an effect (Schriever et al. 2014). In subjects with specific anosmias, repeated exposure to the anosmic items led to an increase in their sensitivity (Wang et al. 2004; Cain et al. 1995; Engen and Bosack 1969; Livermore and Laing 1996; Croy et al. 2015). Interestingly, failures to increase general olfactory sensitivity in healthy subjects have been reported repeatedly (Livermore and Hummel 2004; Frasnelli and Mercier 2015). This is however the first study to report a decrease in sensitivity after OT. While the mechanisms behind this phenomenon are unclear, possible explanations relate to olfactory overexposure that might lead to a loss of interest in the repeatedly performed olfactory tasks, although the analysis of the self-reported OT diary shows a good compliance to training. It may also be that we are dealing with a ceiling effect in that it becomes more difficult to demonstrate an olfactory function improvement in healthy participants, as they already score very high in the test even before manipulation of olfactory sensitivity. Further studies are necessary to investigate this contrasting finding.

Table 1 Means, standard deviations (SD) and mean difference between variables, as well as significance level (p-value) after paired t-tests for olfactory bulb volume (OB, mm3) and threshold scores as well as Sign test for identification score, for trained and untrained nostril, before and after olfactory training (OT)

		Trained nostril		Untrained nostril	
		Before OT	After OT	Before OT	After OT
OB volume (mm^3)	mean	41.5	46.2	40.7	46.1
	SD	8.9	10.1	8.7	9.6
	mean diff. (±SD)	4.7 ± 6.1		5.3 ± 5.9	
	t-test	0.000		0.000	
Thresholds	mean	9.3	7.9	8.7	7.8
(points, maximum = 16)	SD	1.9	3.4	2.4	3.1
	mean diff. (±SD)	−1.4 ± 3,6		−0.9 ± 2,9	
	t-test	0.000		0.005	
Identification	mean	14.2	14.4	14.3	14.5
(points, maximum = 16)	SD	1.3	1.4	1.4	1.4
	mean diff. (±SD)	0.2 ± 1.3		0.2 ± 1.6	
	Sign test	0.104		0.222	

Means, standard deviations (SD) and mean difference between variables, as well as significance level (*p*-value) after paired T-tests for olfactory bulb volume (OB, mm^3) and threshold scores as well as Sign test for identification score, for trained and untrained nostril, before and after olfactory training (OT)

Among the limitations of the study we mention the lack of a control group. Specifically, in the present study the contralateral bulb was thought to serve as a control. However, as indicated by the results of this study, the contralateral OB is also subject to changes induced by the experimental manipulation. Thus, future studies should carry an independent control group. A further limitation is related to compliance to OT. The administered training diary was meant to maintain and stimulate the compliance to training but it is based on self-reports. The importance of compliance and of an accurate reporting of performed number of training sessions was strongly emphasized, nevertheless the actual compliance cannot be verified.

Conclusion

In conclusion, lateralized OT leads to a bilateral increase in the OB volume indicating the possible presence of top-down influences. Largely in contrast to work in patients with olfactory loss, OT actually led to a decrease in olfactory sensitivity in healthy subjects, while no effect was shown on olfactory identification ability. This discrepancy requires further investigations.

Compliance with ethical standards

Funding The study did not receive external funding.

Conflict of interest None of the authors declares any conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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