NeuroImage 189 (2019) 45–54

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/neuroimage

Smell training improves olfactory function and alters brain structure

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ARTICLE INFO

Keywords:
odor training
Olfactory perception
Olfactory system
Neuroplasticity
MR imaging

ABSTRACT

Training and repeated exposure to odorants leads to enhanced olfactory sensitivity. So far, the efficacy of intensive olfactory training on olfactory function in a healthy population and its underlying neurobiological basis remain poorly known. This study investigated the effects of a 6-week intensive and well-controlled olfactory training on olfactory function and brain structure/neuroplasticity. Thirty-six healthy young individuals were recruited and randomly distributed in three groups: (1) 12 participants underwent daily intensive olfactory training of at least 20 min that included an (a) odor intensity classification task, an (b) odor quality classification task and an (c) target odor detection task, (2) 12 participants underwent an equivalent visual control training, and (3) 12 control individuals did not participate in any training. Before and after the training period, all participants performed a series of olfactory tests and those from groups 1 and 2 underwent structural magnetic resonance (MR) imaging, from which we obtained measures such as cortical thickness and tissue density.

Participants improved in the respectively trained tasks throughout the 6-weeks training period. Those who underwent olfactory training improved general olfactory function compared to control participants, especially in odor identification, thus showing intramodal transfer. Further, MR imaging analysis revealed that olfactory training led to increased cortical thickness in the right inferior frontal gyrus, the bilateral fusiform gyrus and the right entorhinal cortex.

This research shows that intensive olfactory training can generally improve olfactory function and that this improvement is associated with changes in the structure of olfactory processing areas of the brain.

1. Introduction

Contrary to what was thought for a long time, the adult brain exhibits an impressive degree of neural plasticity. Sensory loss (Merabet and Pascual-Leone, 2010; Reichert and Schöpf, 2018), stroke (Borstad et al., 2013; Jones, 2017; Wilkins et al., 2017), brain tumors/irradiation (Brockmann et al., 2011; Duffau, 2008; Fisicaro et al., 2016; Merabet and Pascual-Leone, 2010), but also learning and training (Chang, 2014) lead to neural reorganization. Throughout the lifespan, experience-driven neural plasticity is necessary to cope with and adapt to ongoing environmental modifications. Over the last two decades, numerous studies have highlighted that acquiring and mastering skills, via an intensive training over the life-span (i.e., becoming an expert in a certain domain) or a specific short-term training paradigm (i.e., in the frame of a scientific study) are associated with functional and anatomical modifications in corresponding brain areas. The effects of experience and training have been investigated in various domains, such as music, sport, and games. For example, musicians exhibit changes in brain structure, such as an increased cortical density in auditory and motor cortex (Bermudez et al., 2009; Bermudez and Zatorre, 2005; Draganski et al., 2004; Gaser and Schaulg, 2003a,b; Kleber et al., 2007; Kleber et al., 2010; Maguire et al., 2000; Schlaug, 2015; Sluming et al., 2002; Zarate and Zatorre, 2008). Longitudinal studies juggling training in young (Draganski et al., 2004; Driemeyer et al. 2008) and in older participants (Boyke et al. 2008) suggest similar effects of motor training on brain structure. Moreover, intensive learning of medical students (Draganski et al., 2006), being a taxi driver in London (Maguire et al., 2000s) and an 8-week intensive memory training in elder participants (Engvig et al., 2010), are all associated with structural alterations of memory areas. Taken together, specific long and short-term training induces structural and functional

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https://doi.org/10.1016/j.neuroimage.2019.01.008
Received 4 October 2018; Received in revised form 10 December 2018; Accepted 4 January 2019
Available online 7 January 2019

changes in corresponding brain areas, and this neuroplasticity can occur throughout lifespan.

Contrary to other domains, we know much less about the effects of odor learning and odor expertise on the adult human brain. The relatively scarce literature that exists suggests that similar mechanisms apply to the sense of smell: olfactory specialists, e.g., perfumers (Delon-Martin et al., 2013) and sommeliers (Banks et al., 2016), have denser olfactory processing areas compared to untrained individuals. Specifically, professional perfumers exhibited increased gray matter volume in the orbitofrontal cortex which was positively correlated with years of experience (Banks et al., 2016; Delon-Martin et al., 2013). Compared to controls, perfumers who imagine smells (Plailly et al., 2012) and sommeliers who taste wine (Castriota-Scanderbeg et al., 2005; Pazart et al., 2014) exhibit distinct activation patterns in olfactory processing regions (piriform cortex, orbitofrontal cortex) and hippocampus. Such functional adaptations may lead to the observed structural changes. These studies therefore suggest that sustained olfactory training and experience lead to functional and then structural reorganization of olfactory brain areas of odor experts. In non-expert individuals with a normal sense of smell, a short-term olfactory training improves olfactory performance (Dalton et al., 2002), and repeated exposure to an odorant enhances odor detection (Dalton et al., 2002; Doty et al., 1981; Engen, 1960; Rabin and Cain, 1986). Along the same lines, olfactory training of a few seconds daily is considered as a behavioral therapy in patients with olfactory dysfunction (Abolmaali et al., 2002; Damm et al., 2014; Fleiner et al., 2012; Haehner et al., 2013; Hummel et al., 2009; Konstantinidis et al., 2013; Mueller et al., 2005; Rombaux et al., 2006; Schriever et al., 2014b).

On a microscopic level, sensory experience leads to synapse formation and spine sprouting, and increases cell genesis of glial or neuronal cells (Trachtenberg et al., 2002), which macroscopically may result in an increase of gray matter density or thickness. Modern neuroimaging tools enable us to measure structural characteristics of the brain such as cortical thickness (Ad-Dab'bagh et al., 2006) or gray matter density (Ashburner and Friston, 2000) on the whole brain. These methods showed that cortical thickness in olfactory processing areas is associated with performance in olfactory tasks (Frasnelli et al., 2010) and that individuals suffering from congenital anosmia or hyposmia due to different etiologies exhibit an altered architecture of these same structures (Abolmaali et al., 2002; Bitter et al., 2010a,b; Collet et al., 2009; Frasnelli et al., 2013; Haehner et al., 2008; Ibarretxe-Bilbao et al., 2010; Mueller et al., 2005; Rombaux et al., 2009a; Rombaux et al., 2009b; Rombaux et al., 2006; Rupp et al., 2005; Wattendorf et al., 2009). Specifically, the volume of core olfactory areas such as the piriform cortex, the orbitofrontal cortex (OFC), and the insular cortex were correlated with olfactory performance (Frasnelli et al., 2010; Seubert et al., 2013). Additionally, healthy individuals possess a bigger olfactory bulb than individuals with a reduced/absent sense of smell (Rombaux et al., 2006). In individuals with a normal sense of smell, olfactory bulb volume is correlated with olfactory performance (Buschhuter et al., 2008). However, m ost of the studies mentioned above suffer from one or two of the following problems: (1) the lack of precise control over the olfactory training; (2) the lack of a longitudinal component in the study.

The aim of this pilot study was therefore to examine the effects of a well-controlled 6-week olfactory training on olfactory function and on structural measures of the brain, with a particular focus on the olfactory processing areas and olfactory tasks. While participants performed specific tasks during training, we investigated a generalized improvement of olfactory function, extending to olfactory tasks which had not been exercised (intramodal transfer). In order to do so we measured olfactory function on six olfactory tasks in an olfactory training group and two control groups before and after a 6 weeks training period and carried out structural MRI. Since very little data is available on the effects of olfactory training on structural brain measures, we decided to test for cortical thickness and density to determine if one measure is more sensitive to detect training effects than the other.

We hypothesized that a short-term intensive odor training (1) leads to a better performance in the training tasks (training-specific effect), (2) leads to a better performance in non-exercised olfactory tasks (intramodal transfer), (3) alters cortical density/thickness in olfactory and other brain areas and (4) that the changes in performance are correlated with changes in brain anatomy.

2. Methods

2.1. Participants

Thirty-six healthy participants (21 women and 15 men; mean [range] age = 24 [18–35] years) with normal olfactory function were included into the study. Exclusion criteria were neurological or psychiatric diseases, pregnancy, claustrophobia or impaired color vision. Participants were asked to refrain from smoking, eating, or drinking (except water) during the hour prior to training and testing.

All participants gave written consent, as required by the local ethical review board which approved all behavioral procedures and the use of individual MRI scans (CMER RNQ 15-16-10).

3. Material

3.1. Training

Participants were randomly distributed across three groups: (1) Olfactory training group (OT): 12 participants (7 women, 5 men) followed a strict olfactory training paradigm consisting of daily visits to the lab for 6 weeks, (2) Visual training control group (VTC): 12 participants (6 women, 6 men) completed an equivalent visual training paradigm, and (3) Control group (C): 12 participants (8 women, 4 men) did not receive any training.

The training sessions took place in a well-ventilated experimental room in our laboratory at the University du Québec à Trois-Rivières (UQTR). Trained participants (OT and VTC groups) were invited to visit the lab for daily training sessions over 6 weeks. Training sessions lasted between 20 and 30 min and consisted in the following 3 tasks: (1) Intensity classification, (2) Quality classification, and (3) Target detection. These tasks were carried out daily (Monday through Friday). For the weekend, we instructed participants to perform a reduced training session with task 1 at home.

Olfactory Training. The goal of the olfactory training was to expose participants to (a) odorants in general and (b) to a specific target odor in order to achieve a controlled, steady and repeated odor exposure. The target odor was either phenyl ethanol (PEA; n = 6) or n-butanol (BUT; n = 6). All of the odorants were contained in opaque glass bottles. For each task, participants were instructed to respond as fast as possible while maintaining accuracy and were allowed to smell as much as they wanted. (1) Odor intensity classification task: We asked participants to order 16 odor samples of the target odor according to its concentration (from the lightest to the strongest concentration of PEA and BUT: 4%; 2%; 1%; 0,5%; 0,25%; 0,125%; 0,0625%; 0,03125%; 0,0156%; 0,0078%; 0,0039%; 0,00195%; 0,000977%; 0,000488%; 0,00024%; 0,00012%; propylene glycol was used as solvent), (2) Odor quality classification task: We asked participants to order 11 odor samples according to the concentration of the target odor (4%) mixed with citrus odor (ratio target odor: citrus, ranging from 0:100 to 100:0), (3) Target odor detection task: We asked participants to identify whether the target odor was present among a set of 14 samples. Seven bottles contained only one non-target odor (e.g., cola, peach, citrus, etc.) whereas seven other bottles contained the target odor mixed with each of these odors (50:50 mixtures of isointense components). If participants terminated the three tasks in less than 20 min, they completed task 1 again. If participants completed task 1 two times, only their performance during the first administration was analyzed.

Visual Training Control. Participants in this group carried out visual control tasks, based on colored paper. Similar to the olfactory training

group, stimuli (here, colored papers) were contained in opaque glass bottles and participants could only observe them one at the time but as often as they wanted. Tasks were designed to be equivalent to the olfactory training tasks and similar instructions were given. (1) Color intensity classification task: We asked participants to order 16 pieces of colored papers of a target color (gray hues) according to its colorimetric intensity (from the lightest to the darkest gray), (2) Color quality classification task: We asked participants to order 11 color samples according to the color gradient of a target color (pink) mixed with the green color (ratio pink: green, from 0:100 to 100:0), (3) Target color detection task: We asked participants whether a target color (purple color with a specific hue) was recognized among 14 purple hues (7/14 were the target purple hue). More specifically, participants had to observe the target color only once, as long as they wanted, and then they had to answer if yes or no it was the same target purple hue. If participants terminated the three tasks in less than 20 min, they completed task 1 once again. If participants completed task 1 two times, only their performance during the first administration was analyzed.

In order to assess a task specific training effect, we computed a performance score for each task:

$$performance\ score = \frac{correct\ responses}{time}$$

Where "correct responses" was the number of consecutive correct responses for tasks 1 and 2 and the total of correct responses for task 3, and "time" was time needed to complete for each task.

3.2. Behavioral tasks

In order to assess the generalized effect of training, we measured a total of six olfactory behavioral tasks (see Table 1 for an overview), based on the Sniffin'Sticks olfactory test kit (Hummel et al., 1997; Kobal et al., 2000) (Burghart, Wedel, Germany) and the UPSIT (Doty et al., 1984). We did this before and after the training in all three groups of participants.

(1) PEA detection threshold and (2) BUT detection threshold: We assessed separate odor detection thresholds for PEA and BUT by using the Sniffin'Sticks and following standardized procedures (Hummel et al., 1997). In short, we assessed detection thresholds for each target odor with a single-staircase, 3-alternative forced-choice procedure. The experimenter sequentially presented three odorized pens for 2s in a randomized order. We asked participants to identify the pen containing the target odor (two pens contained the solvent and the third pen contained the target odor at a specific concentration). The staircase began at the lowest concentration of the target odor (among 16 concentrations). Reversal of the staircase was triggered when the odor was properly detected in two successive trials whereas subsequent reversal of the staircase was performed when the target odor was not correctly perceived. Scores for the odor threshold refer to the mean of the last four of seven staircase reversal points and can range from 1 to 16.

(3) Odor discrimination: Odor discrimination were assessed by using an extended version of the Sniffin' Sticks discrimination task (Frasnelli et al., 2010a). Three pens were sequentially presented for 2s in a randomized order; two containing the same odor and the third containing the target odor. The target odors were identified in a row of 32 triplets of odors. Scores for odor discrimination task can range from 0 to 32.

We assessed participants' capacity to identify odors using two different tasks:

(4) Free odor identification. For this task we adapted the identification subtest of the Sniffin'Sticks battery. Unlike the standard procedure, where participants choose among four descriptors for each of 16 odors, we asked participants to identify odors without cues (free identification). To assess intramodal transfer, we removed 2 out of 16 odorants (i.e., coca cola and lemon) of the analysis, as olfactory trained subjects were exposed to them throughout the olfactory training (task 3). We counted the number of correct responses (1 point for the correct identification of a given odor, 0.5 points for the correct identification of the category of a given odor); scores for this free odor identification test can range from 0 to 14. We used two different sets of odors for session 1 and 2.

(5) Cued odor identification. For this 4-alternative forced-choice odor identification test we used the UPSIT, a scratch-and-sniff test based on microencapsulated odorants printed on paper sheets, which are released upon scratching. In order to avoid a learning effect between both sessions (before and after training), we selected 20 odors amongst the 40 of the UPSIT, in a pseudorandomized and counterbalanced fashion for each participant. For the session after the training we then tested the remaining 20 odors. Participants identified the odors with the help of four descriptors per odor (Doty et al., 1984). Scores for this adapted version of the UPSIT therefore can range from 0 to 20.

(6) Odor memory: We assessed the ability of the participants in recognizing odors after 24 h. A set of 8 odors from the Sniffin' Sticks test were selected (which participants had smelled in the free identification task) as well as 8 odors from an extra-set of sticks (new odors). The experimenter asked whether the odor has been presented during the odor identification task performed the previous day. We counted the number of hits (0–8) and correct rejections (0–8) and calculated d', in accordance to signal detection theory (Snodgrass and Corwin, 1988).

Table 1
Behavioral tasks assessing olfactory function.

#	Assessed task	Material *Procedure*
1	Odor threshold 1 for phenyl ethanol	Sniffin' Sticks *Standard*
2	Odor threshold 2 for n-butanol	Sniffin' Sticks *Standard*
3	Odor discrimination	Sniffin' Sticks *Adapted: 32 items instead of 16*
4	Free odor identification 1	Sniffin' Sticks *Adapted: free instead of cued identification*
5	Cued odor identification 2	UPSIT *Adapted: 20 items instead of 40*
6	Olfactory memory	Sniffin' Sticks *Adapted*

3.3. Brain imaging

We carried out the magnetic resonance imaging sessions at the 3.0 T S Trio scanner of the Unité de Neuroimagerie Fonctionnelle (UNF) at the Institut Universitaire de Gériatrie de Montréal (IUGM) of Université de Montréal. We acquired a T1-weighted structural volume (voxel size: $1.0 \times 1.0 \times 1.0$ mm), using an MPRAGE sequence (repetition time 2530 ms, echo time 1.64 ms, flip angle 7°, 176 contiguous sagittal slices, in-plane field of view 256 mm).

3.4. General procedure

Before and after the 6-week training period, all participants took part in two sessions in which we measured olfactory function behaviorally. Therefore, we assessed odor thresholds, odor discrimination, odor identification (on day 1) and odor memory (on day 2) for all 36 participants. In addition to this, both (1) the olfactory training group (n = 12) and (2) the visual control group (n = 12) underwent magnetic resonance

(MR) imaging in two separate sessions (before and after training; see Table 2 for an overview).

4. Data analysis and statistics

4.1. Behavioral data

We used SPSS 20.0 (IBM) to analyse behavioral data. Since participants of the olfactory training group had different target odors (either PEA, n = 6 or n-BUT, n = 6) we first compared these two subgroups to each other with separate two-sample t-tests for the six behavioral tasks (i.e, BUT threshold, PEA threshold, odor discrimination, free identification, cued identification, odor memory) at the end of training. We did not observe any difference between both subgroups and therefore merged them together.

Task specific training effect. In order to assess task specific training effects, we carried out the following transformations. First, we averaged performance scores per week. Second, we indexed these scores to the mean of week 1, where the value for week 1 was set a 100. If, for example, the score for week 2 was 50% above that of week 1, we assigned a value of 150, etc. We then computed a repeated measures ANOVA with week (6 levels: weeks 1–6) and training task (3 levels: (1) intensity classification, (2) quality classification, (3) target detection) as within-subject factor (wsf) and group (2 levels: olfactory training group, visual training control group) as between-subject factor (bsf).

General training effect (intramodal transfer). In order to evaluate the effect of training on olfactory function, we calculated the difference between scores before and after training (post-pre) for each of the six olfactory tasks. In order to increase statistical power, we compared the two control groups to each other with two sample t-tests (visual control group and no training control group forming visual + control group). After verifying that they did not exhibit any significant difference for any variable, we merged both control groups. Next, we z-transformed each of the 6 variables and verified normal distribution using Kolomogorov-Smirnoff test. We then computed a repeated measures ANOVA with task (6 levels: (1) PEA threshold; (2) BUT threshold; (3) odor discrimination; (4) free identification; (5) cued identification; (6) odor memory) as wsf and group (2 levels: olfactory training group; visual + control group) as bsf. We used t-tests for post hoc analyses, with Bonferroni correction for multiple comparison, unless stated otherwise. The alpha level was set at 0.05.

4.2. Imaging data

We analyzed images using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) in MATLAB unless stated otherwise.

4.3. Preprocessing

We used the CAT12 toolbox (C. Gaser, Structural Brain Mapping group, Jena University Hospital, Jena, Germany) implemented in SPM12. First, using the longitudinal segmentation tool, structural images from the 1st and 2nd visits were realigned, segmented and normalized to MNI space. Briefly, this tool first realigns the two structural images, calculates a mean structural image and corrects the realigned images for signal inhomogeneities (bias-correction) with regard to the reference image. The mean image is then segmented and spatial normalization parameters are estimated using this segmentation. Normalization parameters are applied using DARTEL spatial registration to the segmented bias-corrected images from the 1st and 2nd visit. Finally, the segmented normalized images from the 1st and 2nd visit are again realigned and underwent quality control (using CAT12 quality control tools).

Table 2
Overview over procedures.

Group	Behavioral testing 1	MRI 1	training	Behavioral testing 2	MRI 2
olfactory training	x	x	x (olfactory)	x	x
visual training control	x	x	x (visual)	x	x
no training control	x			x	

4.4. Cortical thickness

CAT12 uses a projection-based thickness (PBT) approach that uses tissue segmentation to estimated the white matter distance and projects the local maxima (equal to the cortical thickness) to other gray matter voxels by using a neighbour relationship described by the white matter distance (Dahnke et al., 2012). This results in separate cortical thickness data for the left and right hemispheres. This cortical thickness data was finally resampled and smoothed using a 15 mm FWHM kernel. Each participant's cortical thickness data was entered in a second-level analysis using SPM flexible factorial design with the wsf visit (2 levels: pre-training, post-training) and the bsf group (2 levels: olfactory training group; visual training control group). We also evaluated cortical thickness changes between pre- and post-training for the olfactory training group by conducting a second level comparison on the wsf visit (post--training – pre-training). Results were thresholded at $p < 0.05$ using a FWE correction. We also provide results with a lowered criterion ($p < 0.001$) for predicted regions.

4.5. Voxel based morphometry

Cortical density was defined as the relative concentration of gray matter within a voxel. Voxel based morphometry data was resampled and smoothed using a 8 mm FWHM kernel. Each participant's data was entered in a second-level analysis using SPM flexible factorial design with the wsf visit (2 levels: pre-training, post-training) and the bsf group (2 levels: olfactory training group; visual training group). We also evaluated cortical density changes between pre- and post-training for the olfactory training group by conducting a second level comparison on the wsf visit (post-training – pre-training). Results were thresholded at $p < 0.05$ using a FWE correction. We also provide results with a lowered criterion ($p < 0.001$) for predicted regions.

4.6. Relation between behavioral and imaging data

In order to assess possible associations between changes in performance and changes in brain morphometry related to the training, we tested the correlation between imaging and behavioral measures within the olfactory training group. First, whole brain second level analyses were first conducted by testing the correlation between changes in morphometry metrics (post-training – pre-training) and changes in performance (post-training – pre-training). Results were thresholded at $p < 0.05$ using a FWE correction. Second, ROI based analyses were conducted. Specifically, ROIs were defined as anatomic regions (from the aparc_2009s atlas available in CAT12, see Destrieux et al., 2010), where the peak of significant clusters from the Visit × Group interaction analysis were located. For each participant, morphometry measures were extracted for all voxels within these ROIs and averaged for the post-training and pre-training visits. Then, the differences between post-training and pre-training for morphometry and behavioral measures were entered into a correlation analysis where we corrected for multiple tests (within ROI) by setting a Bonferroni threshold of $.05/6 = 0.008$.

5. Results

5.1. Task specific training effect

The repeated measure ANOVA revealed a significant effect of week ($F(5,105) = 6.663$; $p = 0.004$; Greenhouse-Geisser corrected). Post hoc t-tests showed a significant difference between (1) week 1 and all other weeks (all $p < 0.022$; uncorrected); (2) week 2 on the one hand and weeks 4–6 on the other hands (all $p < 0.031$; uncorrected; Fig. 1) indicating a significant improvement of scores over time. We did not observe any effects of group or training task, nor any interaction between any of the variables.

5.2. General effect of training

The repeated measure ANOVA revealed a significant effect of group ($F(1,34) = 6,56$; $p = 0.014$); with the olfactory training group having significantly better overall results on the six non-trained tasks than the visual + control group. We next evaluated both groups on all six individual tasks; While being nominally superior on five of the six tasks, the olfactory training group obtained a significantly better score for the free identification task ($p = 0.006$; Fig. 2). We did not observe any effect of task or interactions.

Half of the participants in the olfactory training group had PEA as target odor, whereas the other half had BUT as target odor. We therefore investigated whether detection thresholds for the target odor were more affected than for the non-target odor, by using a paired *t*-test. We did not observe any significant difference.

5.3. Brain imaging

Data from one participant had to be excluded from further analysis due to movement artifacts.

Cortical thickness: when applying FWE correction, the ANOVA revealed a significant interaction between group and visit at two clusters, one located in the right inferior frontal gyrus and one in the left occipital cortex (see Table 3 for detailed information). When we lowered the threshold to $p < 0.001$ uncorrected, we observed significant differences in two additional clusters namely in the right frontal operculum and in the right superior temporal gyrus stretching into the superior temporal sulcus.

When only comparing between visits for the olfactory training group, we observed again a significant effect in the right inferior frontal gyrus

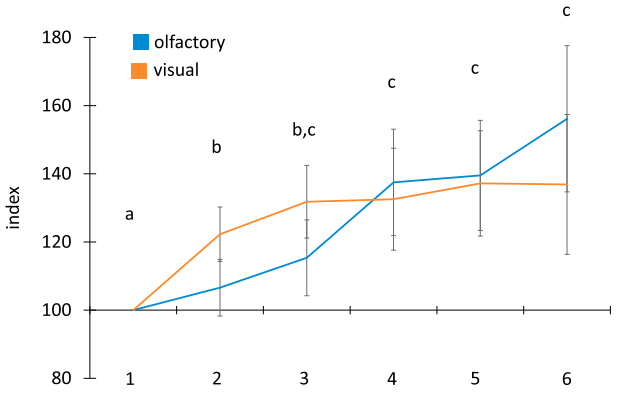

Fig. 1. Task specific training effect. Scores were averaged for per week (week 1–6) and task and indexed to week 1 (week 1 = 100) for the olfactory training group (blue) and the visual training group (orange). Error bars indicate standard error of the mean. Letters indicate significant differences between weeks (bars with different letters were significantly different from each other; for example, bars with "a" are significantly different from bars with "b", but both are not significantly different from bars with "a, b").

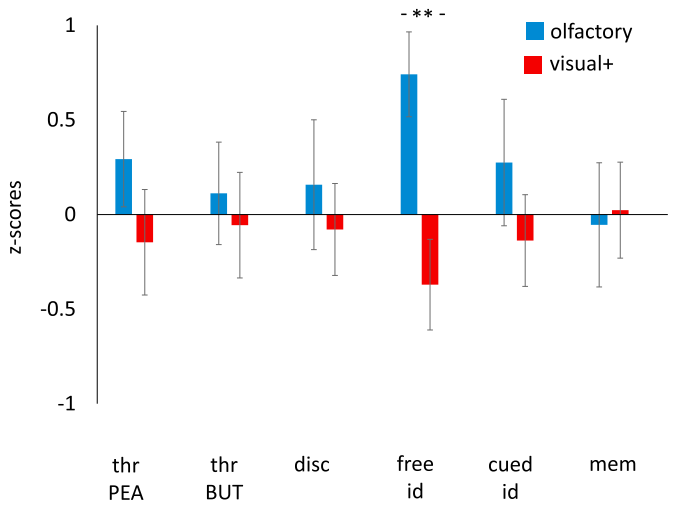

Fig. 2. General effect of training. Z-scores for 6 untrained olfactory tasks (thr PEA: detection threshold for phenyl ethanol detection; thr BUT: detection threshold for n-butanol; disc: odor discrimination; free id: uncued odor identification; cued id: cued odor identification; mem: olfactory memory) for the olfactory training group (blue) and the combined control group (visual training control group and untrained control group). Error bars indicate standard error of the mean. Asterisks indicate significant group difference.

Table 3
Significant effects of olfactory training on cortical thickness. Structures in bold were significant at a $p < 0.05$, FWE level, the remainder at a $p < 0.001$ uncorrected level. Coordinates (x, y, z) are in MNI space. BA: Brodmann area.

Contrast	Region	T	Coordinates		
			x	y	z
Interaction: group x time ($Olf_{post\text{-}training}$ - $Olf_{pre\text{-}training}$) - ($Vis_{post\text{-}training}$ - $Vis_{pre\text{-}training}$)	**R inferior frontal gyrus** (triangular portion) BA 45	5.94	54	29	1
	L occipital cortex BA 18	6.53	−8	−102	9
	R inferior frontal gyrus (opercular portion) BA 44	4.20	49	14	4
	R superior temporal gyrus BA 22	3.69	59	−12	−6
Olfactory group: effect of time ($Olf_{post\text{-}training}$ - $Olf_{pre\text{-}training}$)	**R inferior frontal gyrus** (triangular portion) BA 45	6.94	54	30	2
	L inferior temporal gyrus BA 21	4.12	−55	−19	−32
	R inferior frontal gyrus (opercular portion) BA 44	4.09	51	15	6
	R anterior fusiform gyrus BA 20	3.77	33	−12	−35
	L anterior fusiform gyrus BA 20	3.72	−34	−12	−34
	R entorhinal cortex	3.70	22	−6	−31

with the conservative criterion. At the more liberal threshold, we additionally observed effects in the left inferior temporal gyrus, the right inferior frontal gyrus, bilateral fusiform gyrus and the right entorhinal cortex (Fig. 3).

Voxel based morphometry: We did not observe any cluster with a significant interaction between group and visit when applying FWE correction ($p < 0.05$). A lower threshold ($p < 0.001$, uncorrected) yielded five small clusters, two of which were located in the occipital cortex bilaterally; three were located in the precentral gyrus bilaterally. Focusing on differences between visits for the olfactory training group, we only observed significant effects using the liberal threshold including in the bilateral inferior frontal gyrus (see Table 4 for detailed

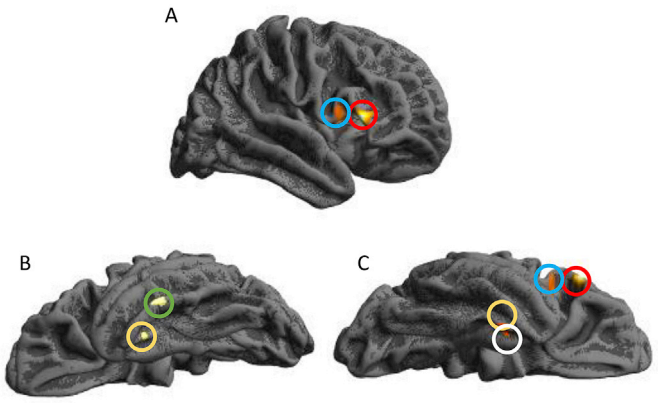

Fig. 3. Effect of olfactory training on cortical thickness: Comparison between before and after training in the olfactory training group with increased cortical thickness highlighted. A. frontolateral view of the right hemisphere. B. basal view of the left hemisphere. C. basal view of the right hemisphere. FWE $p < 0.05$: red circle: right inferior frontal gyrus (triangular portion); uncorrected $p < 0.001$: blue circle: right inferior frontal gyrus (opercular portion); green circle: left inferior temporal gyrus; yellow circle: bilateral fusiform gyrus; white circle: right entorhinal cortex.

Table 4
Significant effects of olfactory training on voxel based morphometry. All structures were significant at a $p < 0.001$ uncorrected level. Coordinates (x, y, z) are in MNI space. BA: Brodmann area.

Contrast	Region	T	Coordinates		
Interaction: group x time ($Olf_{post\text{-}training}$ - $Olf_{pre\text{-}training}$) - ($Vis_{post\text{-}training}$ - $Vis_{pre\text{-}training}$)	L striate area BA 17	5.46	−9	−99	−2
	L precentral gyrus BA 6	4.74	−39	−2	38
	L precentral gyrus BA 4	4.38	−14	−18	66
	R precentral gyrus BA 4	4.24	39	−16	40
	R occipital area BA 18	3.91	2	−78	6
Olfactory group: effect of time ($Olf_{post\text{-}training}$ - $Olf_{pre\text{-}training}$)	L superior frontal gyrus BA 10	7.35	−21	69	10
	L middle frontal gyrus BA 10	5.04	−36	57	20
	L superior frontal gyrus BA 10	4.85	−28	64	−9
	R middle temporal pole BA 38	4.80	38	22	−39
	R cerebellum	4.65	27	−87	−45
	L inferior orbital frontal BA 47	4.58	−28	32	−3
	R middle frontal gyrus BA 6	4.41	27	0	46
	L middle frontal gyrus BA 6	4.30	−24	3	48
	R caudate BA 48	4.28	10	24	−4
	R occipital pole BA 18	4.18	15	−104	10
	L inferior frontal gyrus BA 46	4.18	−51	45	3
	R inferior frontal gyrus BA 47	4.17	30	30	−4
	R supplemental motor area BA 6	4.10	15	−6	60
	L inferior temporal gyrus BA 20	4.07	−45	−21	−21
	R supplemental motor area BA 6	4.05	16	9	62

information).

5.4. Correlation between behavioral and brain measures

Regarding the olfactory group, none of the correlations at the whole brain level reached the statistically significant threshold. ROI correlation analysis between behavioral changes and brain measures yielded no significant correlation that passed Bonferroni correction for either ROIs (see Table 5 where we also provide the correlation results for the visual training group and the whole sample). However, we noted a tendency for a positive correlation between the increase in performance in the olfactory memory task and increases in CT in the left occipital cortex ROI ($r = 0.81$, $p = 0.009$).

6. Discussion

In this paper, we describe three major results. First, intensive and well-controlled 6-week olfactory and visual trainings both led to improvement in performance specific to the trained tasks. Second, olfactory training, but not visual training led to a generalized improvement of olfactory function, i.e., showed a transfer to olfactory tasks which had not been trained. We observed the largest effect on free odor identification. Third, this olfactory training was associated with a significant increase in cortical thickness and, to a lesser extent, cortical density of several brain regions, including the right inferior frontal gyrus and areas in the temporal lobe.

6.1. Behavioral measures

We observed a specific and generalized effect of olfactory training on olfactory function, including a transfer to non-trained tasks.

As expected we observed a specific training effect (i.e., an improvement of performances over weeks in the tasks performed during the training) as olfactory training has proved to have such an effect in multiple studies. For example, participants with specific anosmia (e.g., androstenone, isovaleric acid, lyral) have seen sensitivity to odors they could barely perceive increase after a repeated exposure to them (Croy et al., 2015; Möller et al., 1999; Wang et al., 2004). Similar effects have been shown in patients with generalized olfactory dysfunction (Haehner et al., 2013; Hummel et al., 2009), and in non-expert individuals with a normal sense of smell (Dalton et al., 2002; Rabin and Cain, 1986), with repeated exposure to some odorants leading to an increase in the sensitivity to these specific odorants.

Further, such an effect of olfactory training enhancing olfactory function in general has repeatedly been shown for patients with olfactory alteration or loss (Hummel et al., 2009; Damm et al., 2014; Altundag et al., 2015; Konstantinidis et al., 2013; Fleiner et al., 2012; Kollndorfer et al., 2014, 2015; Haehner et al., 2013; Geissler et al., 2014). More precisely, a meta-analysis recently reported positive effect of olfactory training on general olfactory function, with large effects of training on a global olfactory score, odor discrimination and odor identification for patients with olfactory diseases of all types, and small-to-moderate effect on odor sensitivity (Sorokowska et al., 2017). However, its effectiveness on olfactory function in general remained understudied in individuals with a normal sense of smell where results are more heterogeneous. In fact, while one study did not show any generalized effect of olfactory function in individuals with a normal sense of smell (Livermore and Hummel, 2004), a second paper reported even a decrease in sensitivity after olfactory training (Negoias et al., 2017), and other studies reported enhanced odor sensitivity in young and healthy older individuals (Mori et al., 2015; Schriever et al., 2014a). Interestingly, neuroanatomical and electrophysiological studies have demonstrated that repeated odor exposure in humans can increase olfactory bulb volume (Negoias et al., 2017) and increase amplitudes of recordings from the olfactory epithelium (Livermore and Hummel, 2004; Wang et al., 2004).

Our results are therefore contrasting some of these earlier reports, as we observed a significant improvement of olfactory function. The effect in our study was mostly driven by an improvement in the free

Table 5
Correlation analysis between brain changes in cortical thickness and behavioral changes in performance.

Group	Region of interest	PEA threshold	n-BUT threshold	Discrimination	Free identification	Olfactory Memory	Cued identification
		r	*r*	*r*	*r*	*r*	*r*
Olfactory training group	R inferior frontal gyrus	−0.30	−0.19	−0.33	0.14	0.44	0.14
	L occipital cortex	−0.16	−0.18	−0.37	−0.52	0.81	−0.16
Visual training group	R inferior frontal gyrus	0.17	0.18	−0.11	−0.15	0.02	−0.06
	L occipital cortex	0.08	0.66	0.30	0.36	0.13	−0.10
Whole group	R inferior frontal gyrus	0.11	0.08	−0.12	0.11	0.17	0.05
	L occipital cortex	0.05	0.35	0.01	0.12	0.45	−0.16

Regions of interest (ROIs) were defined as regions where significant interaction (Group X Visit) were found in our whole brain analyses.
No correlation passed Bonferroni correction.

identification task. This difference may, at least partly, explained by the fact that our daily 20 min training procedure, including three complex tasks, mobilized higher cognitive abilities than the procedure usually used, consisting in passively smelling 4 odorants, twice a day. Further, we did not observe a target odor specific improvement of the detection threshold, which is congruent with the literature on patients with olfactory dysfunction: training mainly improved their performance in higher order tasks such as odor identification and discrimination rather than odor detection thresholds (Fleiner et al., 2012; Haehner et al., 2013; Sorokowska et al., 2017). Odor detection threshold having less cognitive demand than tasks like identification (Hedner et al., 2010), a possible explanation would be that this short and intensive olfactory training does not impact olfactory sensitivity, but processing of olfactory stimuli at a higher cognitive level. In our study, the positive effect of olfactory training on olfactory function may not only be related to peripheral changes, but seems to also be linked to central changes; i. e., improved cognitive processing of odor stimulation and increased attention to odors. The underlying mechanisms have yet to be discovered. We can note, however, that our results showed an improvement in the free identification task, but not in the cued one; the absence of improvement in this task might be due to a ceiling effect as healthy and young individuals usually already achieve high scores in the UPSIT which is designed to distinguish patients with reduced olfactory function from individuals with normal olfactory function.

6.2. Brain imaging

We observed an enhancement of cortical thickness and, to a lesser degree, density due to olfactory training. The link between brain anatomy and olfactory function has been investigated in earlier studies; this literature can be subdivided into four categories of papers. The first set of articles investigated healthy individuals. They show a correlation between olfactory function and neuroanatomical measures such as volume of the olfactory bulb (Buschhuter et al., 2008; Seubert et al., 2013) and the density or thickness of cortical structures, including olfactory processing areas such as the orbitofrontal, piriform and insular cortex, but also regions which are not classically associated with olfactory processing such as precentral, postcentral and superior temporal gyri (Frasnelli et al., 2013; Segura et al., 2013; Seubert et al., 2013). A second set of papers compared olfactory specialists such as perfumers and sommeliers with healthy controls. Here, specialists, which can be seen as individuals with year-long training, exhibited denser cortex in the orbitofrontal (Delon-Martin et al., 2013), entorhinal and insular cortex (Banks et al., 2016). The third set of papers examined differences between patients with acquired loss of olfactory function with healthy controls. Here, next to reduced volumes of the olfactory bulb (Rombaux et al., 2006; Rombaux et al., 2008; Rombaux et al., 2010), patients showed thinning of olfactory processing areas such as piriform, insular, orbitofrontal, anterior cingulate cortex and parahippocampal gyrus (Bitter et al., 2010a,b; Gellrich et al., 2017; Peng et al., 2013; Yao et al., 2014). Furthermore, they exhibited thinning in additional brain regions such as subcallosal, superior and middle temporal, middle occipital, fusiform gyri as well as medial prefrontal and cingulate cortex (Bitter et al., 2010a,b; Gellrich et al., 2017; Peng et al., 2013; Yao et al., 2014). Finally, one report investigated the effect of 12 weeks of olfactory training in patients with olfactory dysfunction and found density in hippocampus and thalamus to increase with olfactory function (Gellrich et al., 2017). In summary, these studies indicate a link between olfactory function and neuroanatomical measures, with better olfactory function being related to thicker and denser cortex in olfactory processing areas and other brain regions. We add to this by showing that olfactory training affects brain structures also in healthy participants. We will discuss the most important findings in the following.

We observed the most significant effect of training on cortical thickness in the triangular portion of the right inferior frontal gyrus (IFG). In addition, when focusing exclusively on the olfactory training group, we also found increases in cortical density of the bilateral IFG between session 1 and 2, but only at a liberal threshold. While the IFG is commonly reported to be activated after olfactory stimulation, its triangular portion is not typically associated with olfactory processing. Nevertheless, several studies reported involvement of this structure at nearly identical coordinates in olfactory tasks. For example, when judging familiarity of odors, participants exhibited activations of the triangular portion of the IFG (Plailly et al., 2005). The authors interpreted this to reflect the involvement of the region in selection and integration of semantic memory. Three independent studies reported activation to olfactory stimulation to be larger in the triangular portion of the IFG in patients with Parkinson's Disease who exhibit untypically preserved olfactory function compared to controls (Hummel et al., 2010; Welge-Lussen et al., 2009; Westermann et al., 2008). Finally, one study found a positive correlation between density of this structure and the ability to identify odors in patients with corticobasal syndrome (Pardini et al., 2009). While it is difficult to generalize from studies on patients with neurodegeneration this seems to suggest that the triangular portion of the IFG is involved in higher order processing of olfactory function, in line with our finding. However, we did not observe an association between improvement of olfactory function and changes in cortical thickness in the IFG. This suggests that the effect of olfactory training on this particular structure is an all-or-nothing effect. The association between olfactory improvement on the memory test and thickness of the occipital cortex showed a strong correlation although not significant when using a stringent correction. This result is puzzling since this data was obtained in the group of olfactory training. Future studies should investigate these possible links.

Next, we observed an effect of olfactory training in the right superior temporal gyrus (STG). This is in line with earlier reports: in healthy individuals, odor identification and STG thickness are correlated (Frasnelli et al., 2010), whereas in patients with anosmia its density is reduced (Bitter et al., 2010b; Peng et al., 2013). ERP source localization and functional MRI show that STG is involved in early processing of olfactory stimuli (Lascano et al., 2010), especially more complex ones (Pellegrino et al., 2017). Our study suggests that olfactory training affects the STG, possibly caused by the repeated evaluation of complex stimuli.

Further, we observed an effect of training on the bilateral fusiform

gyrus. This structure has repeatedly been shown to have a reduced volume in patients with anosmia and hyposmia (Bitter et al., 2010a,b; Peng et al., 2013). In fact, fMRI shows that the fusiform gyrus is involved in odor recognition (Cerf-Ducastel and Murphy, 2006) and correct odor identification (Kjelvik et al., 2012). Again, our results suggest that repeated odor recognition and identification led to an increase of cortical thickness in the fusiform gyrus.

Finally, we also observed a significant increase in thickness of the right entorhinal cortex following olfactory training. The implication of the entorhinal cortex in olfactory processing is well known (Zald and Pardo, 2000), especially with regards to olfactory memory (Wilson et al., 2014). A recent fMRI study showed that odor category learning is associated with the appearance of specific activation patterns in the entorhinal and piriform cortex (Qu et al., 2016). Regarding anatomical measures, its gray matter volume is correlated with the ability to identify odors in patients with different degrees of olfactory dysfunction (Segura et al., 2013).

In summary our results are in line with a notion that olfactory training increases cortical thickness in brain regions involved in olfactory identification, learning, and memory. In addition, there appeared a link between olfactory training and thickness of the occipital cortex.

Voxel based morphometry revealed no effects of training with a conservative threshold. With a more liberal threshold, visual and precentral areas were found to be changed by olfactory training. Due to the small sample size this result in non-olfactory regions has to be taken with caution, but we have shown that olfactory ability is correlated with thickness of right pericentral areas (Frasnelli et al., 2010). While the exact implication remains unknown, it may have to do with motor control of sniffing. Future studies should show if some people are better sniffers due to a cerebral anatomical predisposition.

There are some limitations to this pilot study. First, the sample size was small, which reduced the power of our statistical analysis. This might have impacted our ability to find significant correlations between olfactory performance and cortical thickness. Next, the duration of the training of 6 weeks was short compared to most other studies, where olfactory training lasted commonly from 2 to 8 months (Fleiner et al., 2012; Hummel et al., 2009; Haehner et al., 2013; Kollndorfer et al., 2014). Moreover, the control group who did not receive training did not undergo MRIs session before and after 6 weeks, limiting any interpretation of their result. However, the biggest strength of our report is the control we had over different aspects of our study. First, we had two control groups. Because the visual training control paradigm was similar to the olfactory training one, we can affirm that any found effect was specifically due to the olfactory training, and not to any unspecific training. Second, since training was carried out in the lab and lasted at least 20 min, we were able to exactly control participants' exposure to odors. This is a great advantage of our study compared to most other ones, where training was typically carried out at home and lasted 20 s on each of 4 odors, twice a day (Fleiner et al., 2012; Hummel et al., 2009; Haehner et al., 2013; Kollndorfer et al., 2014).

In conclusion, our findings confirm that olfactory training can improve olfactory function (Sorokowska et al., 2017), and that changes can occur rather fast as a 6-week training was long enough to observe an improvement. These changes may be related to modifications occurring directly in the brain. Although a recent study investigated longitudinal effects of olfactory training in patients with post-infectious olfactory (Konstantinidis et al., 2016), the question of the effects of the olfactory training duration on behavioral and cerebral changes and the stability of theses changes is still open. Further studies with, ideally, a larger sample size, should in various populations investigate: (1) the effects of a longer olfactory training on olfactory function and on the brain, (2) the effects of olfactory training on olfactory bulb size, (3) the stability of olfactory improvement beyond the training period, and (4) brain structure connectivity by mapping white matter tractography in the brain using diffusion tensor imaging (DTI). Understanding the underlying mechanisms of neuroplasticity in the olfactory system could be useful to develop efficient ways to use olfactory training as a therapeutic approach for patients with olfactory dysfunction.

Declarations of interest

none

Acknowledgements

We would like to thank Sherezada Ochoa, Joelle Robert, Florence Gingras-Lessard for their help with testing. This research was supported by grants from Natural Sciences and Engineering Research Council of Canada (RGPIN-2015-04597), Bio-Imaging Network of Quebec (#/5886; Fonds de Recherche du Québec - Santé) and the Université du Québec à Trois-Rivières Research Chair in Chemosensory Neuroanatomy (JF).

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