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Olfactory training induces changes in regional functional connectivity in patients with long-term smell loss

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ABSTRACT

Recently, olfactory training has been introduced as a promising treatment for patients with olfactory dysfunction. However, less is known about the neuronal basis and the influence on functional networks of this training. Thus, we aimed to investigate the neuroplasticity of chemosensory perception through an olfactory training program in patients with smell loss.

The experimental setup included functional MRI (fMRI) experiments with three different types of chemosensory stimuli. Ten anosmic patients (7f, 3m) and 14 healthy controls (7f, 7m) underwent the same testing sessions. After a 12-week olfactory training period, seven patients (4f, 3m) were invited for follow-up testing using the same fMRI protocol. Functional networks were identified using independent component analysis and were further examined in detail using functional connectivity analysis.

We found that anosmic patients and healthy controls initially use the same three networks to process chemosensory input: the olfactory; the somatosensory; and the integrative network. Those networks did not differ between the two groups in their spatial extent, but in their functional connectivity. After the olfactory training, the sensitivity to detect odors significantly increased in the anosmic group, which was also manifested in modifications of functional connections in all three investigated networks.

The results of this study indicate that an olfactory training program can reorganize functional networks, although, initially, no differences in the spatial distribution of neural activation were observed.

1. Introduction

Anosmia, the complete loss of the ability to perceive odors, is a common disorder that has wide-ranging effects on everyday life (for review see Croy et al., 2014). However, treatment options for patients with olfactory dysfunction are still limited. Recently, a new and promising treatment option for patients who suffer from smell loss was developed — olfactory training (Hummel et al., 2009a). In a large multicenter study, olfactory training was proven successful, especially in patients with olfactory dysfunction following an upper respiratory tract infection (Damm et al., 2014). Previous studies revealed not only an increase in smell performance induced by an olfactory training program in patients with olfactory dysfunction, but also showed a neural plasticity effect (Kollndorfer et al., 2014). Before the training, the authors found a diverse network of functional connections for the piriform cortex that involved a broad range of non-olfactory brain areas. After training, these connections declined, suggesting the reorganization of functional connections of the piriform cortex induced by the olfactory training. However, the effect of olfactory training on processing pathways of chemosensory stimuli remains unclear.

The olfactory processing pathway is unique among our senses, as every form of chemosensory perceptional input is a result of an interaction between the olfactory and the trigeminal systems. This processing partnership has several reasons: the olfactory system is responsible for odor quality perception, whereas the trigeminal system conveys sensations in the nasal cavity, such as stinging, burning, temperature, or pain. In addition, the trigeminal system provides an important warning system to protect the airways from harm (Hummel et al., 2003, 2009; Kleemann et al., 2009). Results of functional imaging studies have shown the interaction between the olfactory and the trigeminal

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systems on a cerebral level, as trigeminal stimulation activates pain processing areas, such as the insula, the anterior cingulate cortex, or the primary somatosensory cortex (Bensafi et al., 2008; Iannilli et al., 2008, 2007), as well as olfactory-related areas, such as the orbitofrontal cortex (Albrecht et al., 2010). The close interaction of these two systems is of immense interest in patients with olfactory disorders. Patients who suffer from anosmia – the complete loss of the sense of smell – therefore lose the connection to one of the 'cerebral partners' that process chemosensory information, while the other partner continues to function. Frasnelli et al. (2010) have shown that trigeminal perception is reduced in patients with olfactory dysfunction.

The present study was designed to address the following aims. First, we aimed to investigate the specificity and sensitivity of the chemosensory system in patients with anosmia. The second aim was to investigate the effect of olfactory training on the chemosensory processing networks. These research aims were targeted on an fMRI experiment involving different chemosensory stimuli. To investigate the specific alterations of the chemosensory system (aim 1), three compounds were chosen to evoke various sensations in the nasal cavity: 1) carbon dioxide (CO_2), which is perceived as completely odorless and creates a burning and stinging sensation; 2) menthol, which is perceived as a fresh odor, and evokes a cooling sensation in the nose; and 3) cinnamaldehyde, which smells like cinnamon and causes a warm sensation. These three trigeminal compounds differ in three domains: First, all three compounds target different trigeminal receptor subfamilies. CO_2 activates the TRPV1 receptor (Julius et al., 1997), menthol stimulates the TRPM8 receptor (Peier et al., 2002), and cinnamaldehyde targets the TRPA1 receptor (Bandell et al., 2004). Second, they evoke distinct sensations in the nose. And third, CO_2 is perceived as odorless, whereas menthol and cinnamaldehyde have a clear olfactory quality. The diversity of functional connectivity in networks that process different chemosensory inputs was tested in healthy controls and in patients with anosmia, based on the three chemosensory compounds. For the second aim, neuronal as well as olfactory performance modifications, induced by olfactory training in the anosmic patient group, were investigated. To test this, patients with long-term smell loss due to infection underwent the fMRI experiment before and after a 12-week olfactory training period. We hypothesized that training induces alterations of functional connectivity in olfaction-related networks.

2. Materials and methods

All experiments in this study were approved by the Ethics Committee of the Medical University of Vienna. All subjects were informed about the aims of the study and gave their written, informed consent prior to inclusion.

In this study, we performed two experiments (see Fig. 1), which are described in detail in the following paragraphs.

2.1. Experiment 1

Experiment 1 included two cohorts of subjects, healthy control subjects and patients with anosmia. Nineteen healthy subjects (ten female, nine male) participated. All healthy subjects had normal olfactory function and had no history of neurological or psychiatric diseases. Five subjects had to be excluded from further processing due to incomplete fMRI measurements. Fourteen subjects (seven female, seven male; mean age, 30.1 years; SD, 6.7) completed all measurements and underwent further analysis. For the second cohort, 11 patients with smell loss after an upper respiratory tract infection were screened. One anosmic patient had to be excluded from the data set due to incomplete fMRI measurements, resulting in a total of 10 patients with smell loss (seven female, three male; mean age, 43.4 years; SD 14.1), with a mean disease duration of 4.1 years (SD 3.0), who were then included in the final analysis. All anosmic patients were examined by an ENT, which included an endoscopic examination of the nasal cavity, to determine the cause of olfactory dysfunction. To prevent any influence of trauma-induced alterations of functional brain networks, only patients diagnosed with anosmia after an infection of the upper respiratory tract, were included in this study. Further measurements of olfactory function, as described below, were performed to assess the severity of olfactory dysfunction (Kobal et al., 2000).

All participants completed three scanning sessions, one for every stimulus: 1) CO_2 (50% v/v); 2) cinnamaldehyde (75% v/v dissolved in 1,2-propanediol; Sigma Aldrich, Germany); and 3) menthol (2.5 g; Sigma Aldrich, Germany). Chemosensory stimuli were delivered in an event-related design, using a computer-controlled, air-dilution olfactometer compatible with magnetic resonance imaging (MRI), which was constructed at the Center for Medical Physics and Biomedical

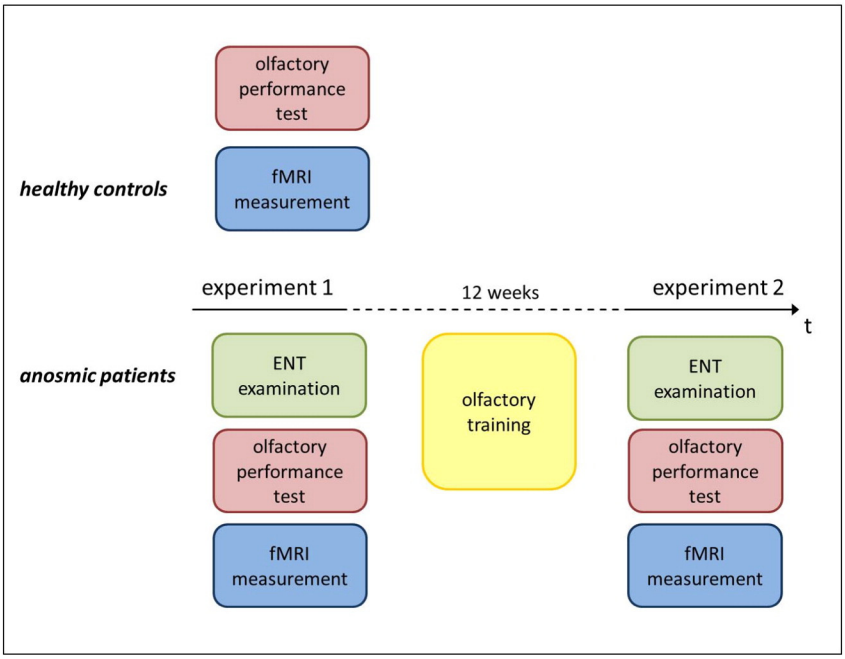

Fig. 1. Schematic description of experimental procedures.

Engineering (Medical University of Vienna). Outlets of all stimulation channels were combined in a single air-line, with a nose applicator at the end of the air-line. All stimuli were applied monorhinally to the left nostril. All stimuli were presented for 500 ms, with an interstimulus interval (ISI) of 30 s, resulting in 20 trigeminal pulses per scanning session, and a total scanning time of 30 min. During fMRI measurements, all participants were instructed to breathe through the mouth, and to avoid any respiratory airflow in the nose (velopharyngeal closure breathing technique; Kobal, 1981). Furthermore, all participants were asked to keep their eyes closed (Wiesmann et al., 2006) during all three scanning sessions. After completing the scanning sessions, patients were asked to evaluate the intensity of stimuli on a visual analog scale ranging from 0 to 100.

2.2. Experiment 2

After completing experiment 1, anosmic patients were instructed to perform the olfactory training over a period of 12 weeks at home. After 12 weeks, patients were invited for a follow-up visit, at which their olfactory performance was tested again. All anosmic patients of experiment 1 were invited to perform a follow-up fMRI experiment after completing the training. Three patients rejected participation in the follow-up scanning session due to personal reasons. Thus, seven anosmic patients participated in experiment 2 (four female, three male; mean age, 41.6 years; SD 12.9; mean disease duration 4.6 years; SD 3.2). All patients completed two testing sessions. After that, fMRI measurements were obtained, as well as a stimulus intensity rating identical to that in the scanning session of experiment 1.

2.3. Olfactory performance measurement

For olfactory performance assessment, the Sniffin' Sticks test battery (Burghart Instruments, Wedel, Germany), a clinically approved test battery, was used. This test battery comprises three subtests: odor detection threshold; odor discrimination; and odor identification. The Sniffin' Sticks battery uses pen-like devices for odor presentation (Hummel et al., 1997; Kobal et al., 1996, 2000). All tests were carried out using a standardized computerized test protocol (Hummel et al., 2012). The odor detection threshold of *n*-butanol is performed using a single-staircase, three-alternative, forced-choice procedure. In the second step, the odor discrimination task consists of 16 triplets of odorants (two pens contain the same odorant; the third pen contains an odd odorant). The participants' task is to detect the odd pen in a forced-choice procedure. The odor identification task is composed of 16 common odors presented in a multiple-choice answer format. Each odor contains a list of four descriptors. Scores for the odor detection threshold range from 1–16, and, for the other two subtests, a score between 0 and 16 may be achieved. Thus, the results of all three subtests are summed to obtain a TDI (Threshold Detection Identification) score. Anosmia was determined based upon clinical definitions (Kobal et al., 2000). Specifically, anosmia was defined by a TDI score of 17 or less. In addition, all subjects rated the intensity of all 16 odors of the identification task to gain a deeper insight into alterations of odor perception caused by the olfactory training.

2.4. Olfactory training

The olfactory training was performed at home over a period of 12 weeks (Hummel et al., 2009b). Patients could select four of six odors for the olfactory training: cinnamon (cinnamaldehyde; 30% v/v dissolved in 1,2-propanediol); vanilla (vanillin; 1 g dissolved in 1 ml 1,2-propanediol); orange (orange oil); rose (phenylethyl alcohol; PEA); menthol; and banana (isoamyl acetate; 1% v/v dissolved in 1,2-propanediol). Thus, all patients received four brown glass jars (50 ml total volume), filled with 1 ml of the respective odorant (soaked in cotton pads to prevent spilling), and labeled with the name of the odor. All six odors were selected almost equally frequently. Orange was selected most frequently (by eight patients), and rose, cinnamon, and menthol were selected least, by six patients. All patients were instructed to expose themselves to each of the four odors twice a day and take a deep sniff of every odor. Further, included patients were advised to keep a diary over the 12-week training period to monitor whether the training was performed regularly. Patients were asked to fill in a form for each training day, where they would indicate whether they performed the training twice a day, as well as the time of performance, and to record special occurrences during the training. In addition, all patients were contacted weekly by the experimenter to maintain compliance and motivation over the full training period.

2.5. Behavioral data

In addition to sociodemographic data, such as age, gender, educational background, and self-rating of olfactory performance, data regarding depressive symptoms and social phobia were collected using psychological questionnaires. Previous studies have already determined correlations between major depression as well as social phobia and decreased olfactory performance. Therefore, two additional questionnaires were administered to exclude patients with clinically relevant depressive and social phobia symptoms. Depressive symptoms were evaluated using the Beck Depression Inventory II (BDI-II; Beck et al., 1996), and the presence of social phobia symptoms was assessed using the Social Phobia Inventory (SPIN; Connor, 2000). Patients with a BDI-II raw score of 9 and above (mild depressive symptoms) or a SPIN score of 21 and above (mild social phobia symptoms) were excluded.

2.6. Statistics

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA), version 20.0. For all test scores, mean and standard deviation (SD) were calculated. To compare olfactory performance values between anosmic patients and healthy controls, both before and after the smell training, the nonparametric Wilcoxon test was performed due to the small sample size. The alpha level for all statistical tests was set at $\alpha = .05$.

2.7. Imaging methods

fMRI measurements were performed on a 3 Tesla Trio System (Siemens Medical Solution, Erlangen, Germany) using a 32-channel head coil. Functional images were acquired using an optimized 2D single-shot, gradient-recalled, echo-planar imaging (EPI) sequence, including online distortion correction with point-spread function-mapping (Zaitsev et al., 2004). Thirty-six slices (2.7 mm thickness, 0.5 mm gap) were acquired, aligned parallel to the AC–PC line, with an echo time (TE)/ repetition time (TR) of 32/2000 ms and a field of view (FOV) of 210×210 mm.

2.8. fMRI data analysis

fMRI data were preprocessed using SPM12b (http://www.fil.ion.ucl.ac.uk/spm/), implemented in MATLAB (Matlab 7.14.0, Release 2012a, Mathworks Inc., Sherborn, MA, USA), and included motion correction, spatial normalization to an MNI template, and spatial smoothing.

To address all the research aims, postprocessing of fMRI data included several steps.

We investigated the spatial extent of the processing networks for all three stimuli and compared patients to anosmics. We also investigated the functional connectivity of these networks and compared both groups. We then analyzed the networks for all three stimuli for the patient group (pre- vs. posttraining) to assess the effect of the olfactory training on the spatial extent of the networks. In addition, we

investigated the effect of the treatment on functional connectivity of these networks.

To study the processing networks for each stimulus compound for both subject groups (experiment 1), and for the patients after training (experiment 2), we performed independent component analysis (ICA). ICA is a statistical, data-driven method that allows the identification of activation networks without the predefinition of a hemodynamic response function or a stimulus time course (see, for example, Schöpf et al., 2010). It allows for separation of independent sources into their underlying components, assuming that the fMRI data set may be modeled as a constant number of spatially independent components that are linearly mixed and spatially fixed. ICA has already been applied in numerous paradigm-based fMRI studies, and, for chemosensory experiments, its superiority to pure hypothesis-driven tools has been shown (Frasnelli et al., 2012; Schöpf et al., 2011).

Group ICA was performed for all three scanning sessions (for all three stimuli) for all subjects conjointly using the Group ICA for fMRI Toolbox (GIFT; http://icatb.sourceforge.net; Calhoun et al., 2001). Separate ICAs were calculated for experiment 1 and experiment 2, as not all subjects in experiment 1 participated in experiment 2. The number of independent components (ICs) was estimated using the minimum description length (MDL) criterion (Li et al., 2007), as implemented in GIFT. After a reduction of dimension using principal component analysis (PCA) in two consecutive reduction steps, group ICA was performed using the Infomax algorithm (Bell and Sejnowski, 1997). The statistical reliability of estimated ICs was tested using the ICASSO toolbox (Himberg et al., 2004), implemented in GIFT. Using ICASSO, the IC estimation was calculated 20 times, varying the initial conditions of the algorithm as well as the bootstrapped data sets. The reliability of the identified ICs was assessed by clustering the results of each run. In a last step, differences between the three substances were calculated using one-way ANOVA (FWE-corrected, $p < 0.05$) with SPM12b. The comparison of signal intensity was performed using MarsBaR v0.44 (Brett et al., 2002).

In a second step, to compare the connectivity of the networks accordingly, seed regions for functional connectivity analysis were defined as the global maximum of the respective ICs for healthy controls with a 10 mm sphere (Maldjian et al., 2004, 2003), with the center of the seed region located at the following MNI coordinates (see Supplementary Fig. 1): 1) olfactory network: −14,14,2 (caudate nucleus); 2) integrative network: −34,22,10 (insular cortex) and 3) somatosensory network: −58,−42,36 (supramarginal gyrus). This was designed to compare the functional connectivity of preselected seed regions between healthy controls and anosmic patients. The same seed regions were used for the healthy controls and anosmic patients, in order to ensure the comparability of functional connections. ROI-to-ROI functional connectivity analysis was performed using the CONN toolbox, v14.d (http://www.nitrc.org/projects/conn) (Whitfield-Gabrieli and Nieto-Castañon, 2012) implemented in MATLAB. Within the CONN toolbox, additional preprocessing steps were performed. First, nuisance parameters that were extracted from the motion-correction process were regressed out. Further, the mean time course from the preselected seed region was correlated with the time course of all other ROIs in the brain (Brodmann areas). The results provided separate spatial maps of functional connectivity within the respective seed region for the two subject groups (experiment 1) and the two measurement time points (experiment 2).

3. Results

3.1. Olfactory performance measurement and behavioral data

Healthy controls achieved significantly higher TDI scores compared to anosmic patients at the first testing session ($p < .001$). Detailed results of olfactory performance measures and sociodemographic variables of anosmic patients are presented in Supplementary Table 1. All patients performed the smell training for 12 weeks. The mean time period between the two testing sessions was 13 weeks. According to their diaries, all patients performed the olfactory training regularly, twice per day. A comparison of olfactory performance measurements revealed a significant improvement in the odor detection threshold ($p = .028$, improvement of olfactory sensitivity). No significant difference between the two testing sessions was obtained for the odor discrimination task ($p = .916$) or the odor identification task ($p = .673$). Detailed results of the behavioral data are presented in Table 1. Subject groups did not differ significantly in the distribution of gender ($\chi^2 = .757$) or educational background ($\chi^2 = .172$). Furthermore, no differences were identified in depressive and social phobia symptoms between healthy controls and anosmic patients before or after the training.

3.2. fMRI experiments in healthy controls vs anosmics (experiment 1)

Functional data for all three stimuli, for both healthy controls and anosmic patients, were submitted to a combined group ICA estimation, which resulted in 50 independent components. For further analysis, we included within-brain activations of the reported components. For all the subsequent analyses, all three investigated substances were analyzed together as three sessions, as no significant differences were detected, either for anosmic patients or for healthy controls.

ICA revealed three major networks that overlapped significantly between the two subject groups. These networks were labeled the (1) olfactory network, (2) the somatosensory network, and (3) the integrative network, based on a trigeminal processing study in healthy controls (Kollndorfer et al., 2015). The olfactory network was centered in the putamen/caudate nucleus, bilaterally, which comprised characteristic olfactory processing areas, including the piriform cortex, the entorhinal cortex, the amygdala, as well as parts of the thalamus (see Supplementary Table 2). Importantly, this network was relatively symmetric in both hemispheres, even though the stimuli were applied only to the left nostril. Subject groups did not differ in the spatial distribution of this network (see Fig. 2A).

The somatosensory network comprised pain-processing areas, such as the primary and secondary somatosensory cortices and the insula (see Supplementary Table 2). Although neuronal activation was found bilaterally, clusters were significantly larger in the right hemisphere. Again, no significant differences between the subject groups were determined after performing a two-sample T-test (see Fig. 2B).

The integrative network included clusters in the orbitofrontal cortex, the insula, the inferior parietal lobule, and the middle and superior temporal gyrus (see Supplementary Table 2). This network comprised several areas known to be involved in sensory integration processes, and showed a clear predominance for the left hemisphere, ipsilateral to

Table 1
Behavioral data for healthy controls and anosmic patients before and after the smell training.

	Healthy controls	Anosmic patients	
	Mean (SD)	Before training mean (SD)	After training mean (SD)
TDI-score	36.00 (2.05)[a]	11.82 (1.66)[a]	13.79 (4.21)
Odor threshold	8.50 (1.60)[a]	1.39 (0.61)[a,b]	3.07 (1.98)[b]
Odor discrimination	13.36 (1.78)[a]	5.57 (1.27)[a]	5.71 (1.98)
Odor identification	14.14 (1.35)[a]	4.86 (2.04)[a]	5.00 (2.16)
Mean intensity rating (odors of the identification task)	7.20 (1.08)[a]	2.11 (1.47)[a]	2.67 (1.69)
Subjective olfactory performance	2.92 (1.69)[a]	7.86 (1.07)[a]	7.14 (1.35)
Stimulus intensity (fMRI)	61.79 (19.99)	41.71 (27.17)	55.86 (23.18)
BDI	3.64 (3.71)	6.00 (3.11)	6.29 (2.63)
SPIN	16.93 (12.07)	13.71 (7.16)	13.72 (7.34)

[a] Marks significant differences between healthy controls and anosmic patients at the first testing session.
[b] Marks significant differences before and after the training in anosmic patients.

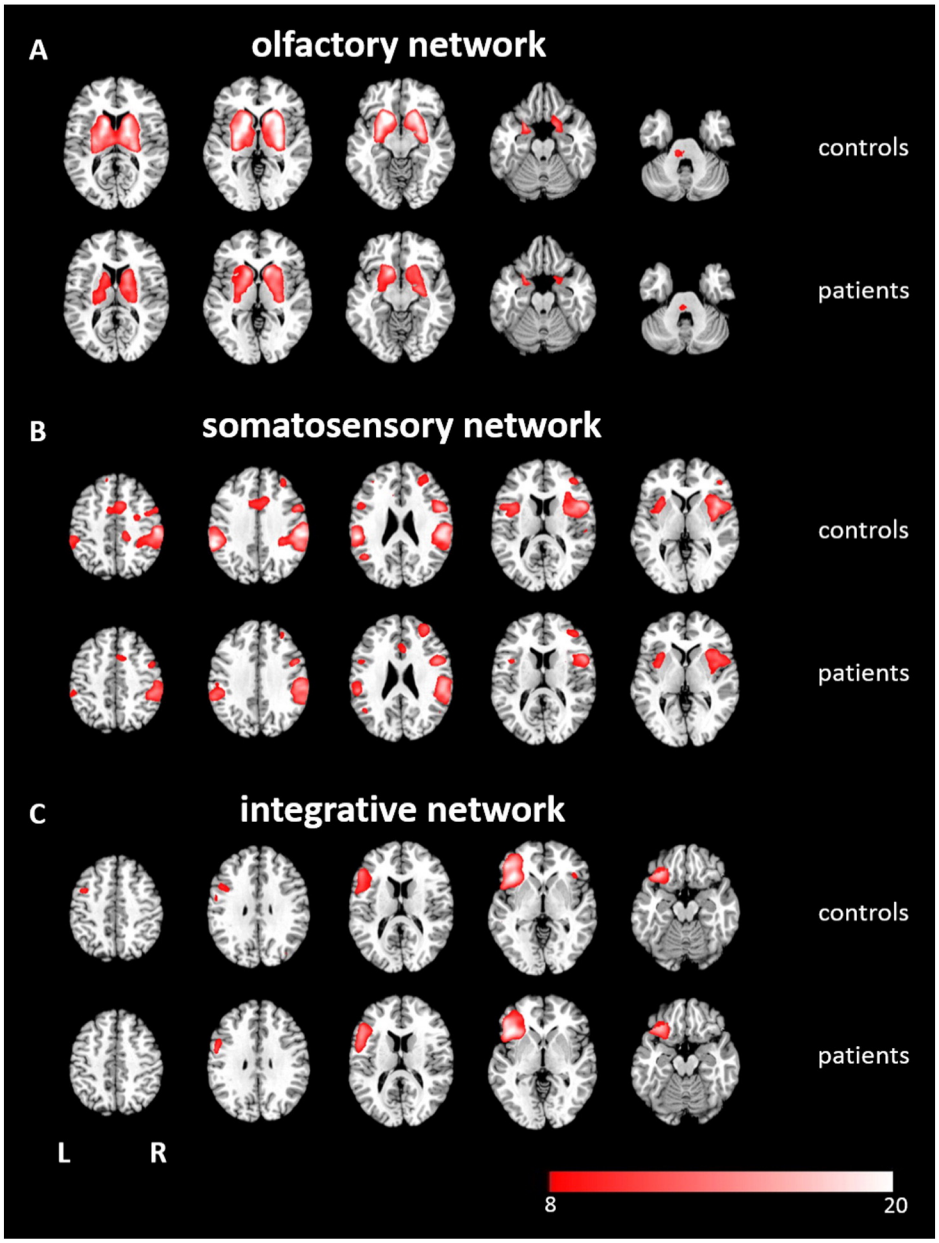

Fig. 2. Axial mean anatomical images overlaid with (A) the olfactory network, (B) the somatosensory network, and (C) the integrative network resulting from the combined group ICA ($p < 0.05$, FWE-corrected) for healthy controls and anosmic patients. As there were no significant differences observed between the three substances, the conjunction for all stimuli is presented.

the application of the stimuli. The comparison of stimulus-specific network patterns again showed no significant differences (see Fig. 2C).

Functional connectivity analysis was performed using the global maxima of the three identified functional networks as seed regions in healthy controls. Analysis revealed differences in the functional connectivity of the selected seed region between healthy controls and anosmic patients. In all three networks, a decreased number of connections to the seed region were observed, which was most prominent in the olfactory network (see Fig. 3 and Supplementary Table 3). In the olfactory network, eight connections were obtained for healthy controls, whereas none were determined for anosmic patients. For the somatosensory network, a reduction of 44.5% in connections was identified for anosmic patients (27 connections for healthy controls compared to 15 for anosmic patients). The highest decrease of functional connections was obtained for the somatosensory network. Here, 41 connections were determined for healthy controls and 13 for anosmic patients, which equaled a reduction of 68.3%.

3.3. fMRI experiments in anosmics before and after the smell training (experiment 2)

A comparison of the three networks resulting from ICA (olfactory network, integrative network, and somatosensory network) before and after the smell training revealed no significant differences induced by the training. As in experiment 1, no differences between the three substances were determined. We therefore analyzed all three stimuli together as three sessions at the group level. Although no differences were found regarding the spatial distribution of the identified networks, an increase in signal intensity was observed, especially in the olfactory network, but also in the somatosensory network (see Fig. 4 and Supplementary Table 4). Before the training, a mean signal change of −1.7% was calculated. In contrast, after completing the training program, a signal change of 3.1% was observed in the olfactory network. Differences for the integrative and the somatosensory networks were smaller, but, again, a higher percentage of signal change was found after the training

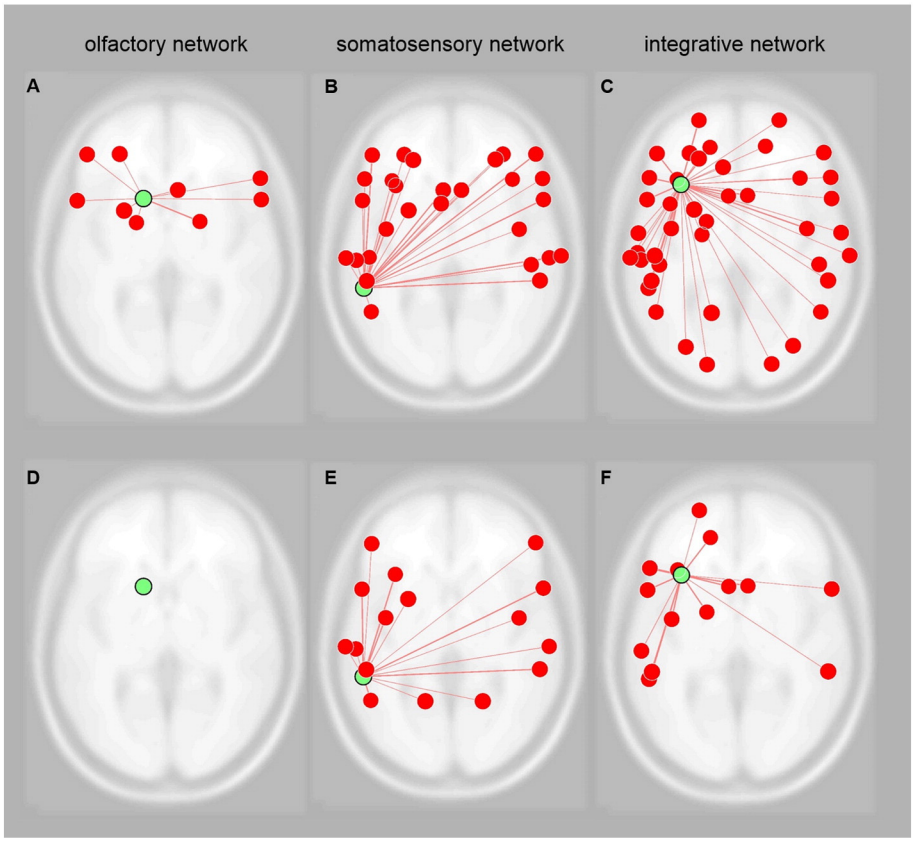

Fig. 3. Functional connectivity during chemosensory stimulation in healthy controls (A/B/C) and anosmic patients (D/E/F), overlaid on an axial template in MNI space ($p = 0.05$, FWE-corrected). The green dot represents the selected ROI (A/D (olfactory network): −14,14,2 (caudate nucleus); B/E (integrative network): −34,22,10 (insular cortex); C/F somatosensory network: −58,−42,36 (supramarginal gyrus)); the red dots capture the statistically significant functionally connected brain areas.

(before training: integrative network: 3.0%; somatosensory network: 1.0%; after training: integrative network: 4.2%, somatosensory network: 1.6%).

Functional connectivity analysis revealed differences in the functional connections of the selected seed regions for all three networks. For the olfactory network and the integrative network, in particular, there was an increase in the number of connections to the seed regions (see Fig. 5 and Supplementary Table 5). After completing the training, which induced a significant improvement of olfactory sensitivity, an increase in functional connectivity was observed for all networks. For the olfactory network, after the training, some connections emerged that were also observed for healthy controls, and are known to be part of the olfactory network, such as connections to the anterior entorhinal cortex, the inferior prefrontal cortex, and the primary somatosensory cortex. For the olfactory network, the number of functional connections increased from no connections to four. Before the training, eight connections for the center of the somatosensory network were determined, and, after the training, ten functional connections were identified (increase of 25%). In the integrative network, an increase of 69% in connections was observed (13 connections before and 22 connections after the training).

4. Discussion

The main aim of the study was to investigate the neural activation induced by chemosensory stimulation in patients with smell loss before and after smell training, compared with healthy controls. We were able to show that, compared to healthy controls, the networks processing chemosensory stimuli in anosmics are altered in their functional connectivity compared to healthy controls. After successful olfactory training for 12 weeks, which resulted in better sensitivity to detect odors, patients with no formal sense of smell showed extended functional connectivity in chemosensory processing networks.

For the networks that process chemosensory information for all three substances, we did not observe any differences in our study, which has already been shown for healthy controls (Kollndorfer et al., 2015). Although the three investigated substances cause different sensations in the nose (CO_2: burning, stinging, perceived as odorless; menthol: fresh, cooling sensation, with a minty odor; cinnamaldehyde: warm sensation, smells like cinnamon), the same functional network patterns for an olfactory, an integrative, and a somatosensory network were identified. Although anosmic patients were not able to perceive odors consciously, the same networks were activated during stimulation. As the spatial extent of the networks did not differ between the substances, we can assume that trigeminal stimuli follow a common processing pathway in the central nervous system. This finding indicates that the trigeminal system is still functional in patients with olfactory dysfunction. A close interrelation between the olfactory and the trigeminal processing pathways has been intensively investigated (Frasnelli and Hummel, 2007; Kleemann et al., 2009; Lundström et al., 2011). The results of our study support this assumption of a close interaction between the olfactory and the trigeminal systems, as all investigated substances, even the pure trigeminal stimulus, CO_2, activated the olfactory network. Based on the results of our study, we assume that the intact trigeminal pathway may trigger the recovery of olfactory function, which was observed after the completion of the olfactory training program. This hypothesis is supported by a finding discovered by Damm et al. (2014), who determined the superior effect of higher odor concentrations in the olfactory training program.

In the last several decades, a growing number of studies provide evidence of the brain's possibility to recover functions (Bende and Nordin, 1997). Recent findings in animal research have shown that the recovery

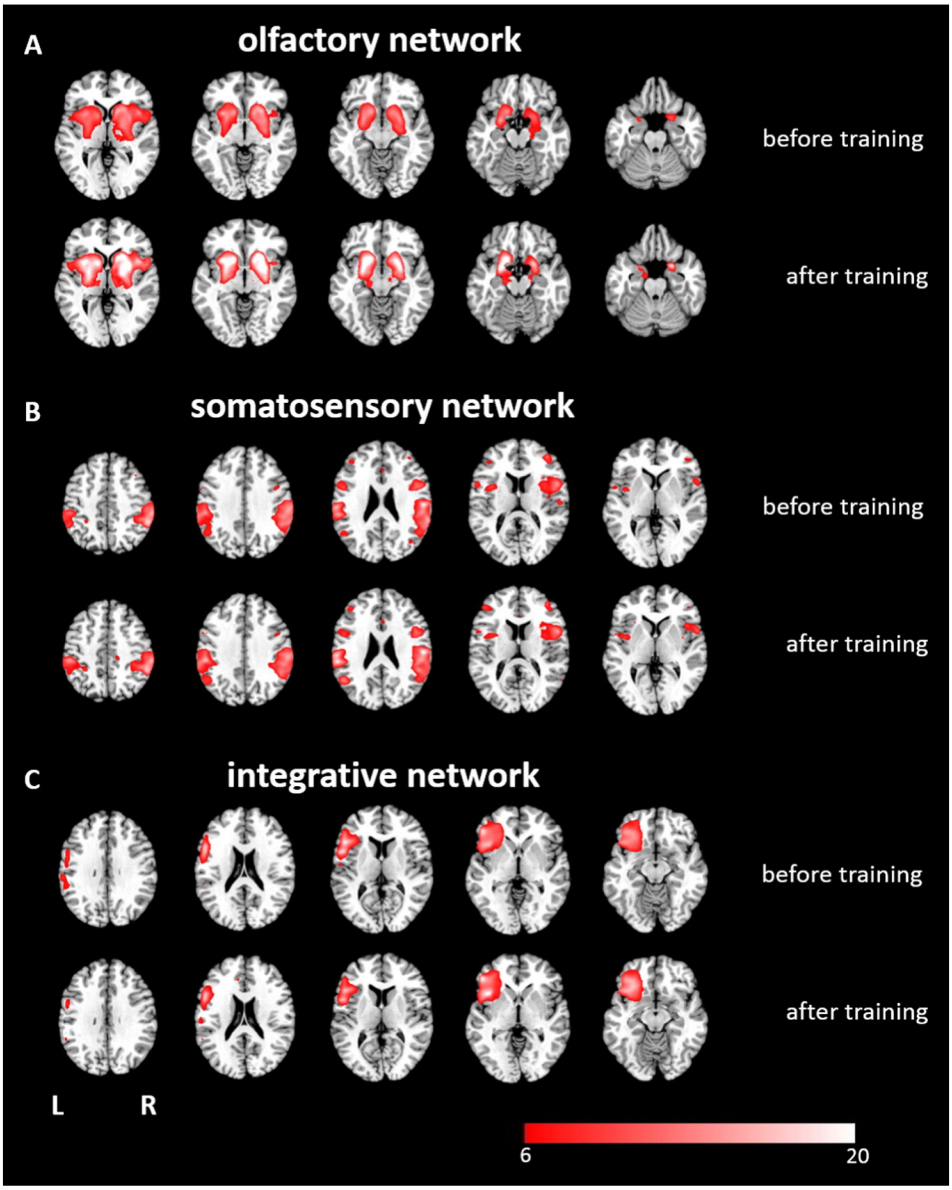

Fig. 4. Axial mean anatomical images overlaid with (A) the olfactory network, (B) the somatosensory network, and (C) the integrative network resulting from the combined group ICA ($p < 0.05$, FWE-corrected) for anosmic patients before and after the training. As there were no significant differences determined between the three substances, the combined results for all stimuli are presented.

is present not only at the behavioral level. Previous findings also suggest neurogenesis in the developing brain (Cadiou et al., 2014) and in the adult brain of mice (Cummings et al., 2014; Czarnecki et al., 2012). It has already been demonstrated that olfactory training may induce at least partial recovery of olfactory function in patients with olfactory dysfunction (Damm et al., 2014). These training effects were also present in a follow-up 3 months after completion of the study. Thus, it is assumed that the olfactory training induced long-term recovery of olfactory function. This finding is supported by the results of our study, suggesting the recovery of olfactory-specific functional connections after the olfactory training. Based on these findings, we assume that the high functional plasticity of the olfactory system might be the main reason for the success of olfactory training, as reflected by a significant increase in odor sensitivity. Based on our hypothesis, the regaining of olfactory function, especially the odor detection threshold, was represented by changes of functional connectivity in the olfactory and in the integrative network.

Previous research revealed structural changes in the brain in patients with smell loss. Bitter et al. (2010a, 2010b) found evidence of decreased gray and white matter in anosmic patients. Volume loss was detected not only in areas mainly responsible for odor processing, such as the PIR, but also in other brain areas, such as the anterior insula or the anterior cingulate cortex. The finding that a decrease in gray and white matter was also detected in patients with reduced olfactory function (Bitter et al., 2010a) indicates that gray and white matter volume reduction may be caused by a lack of sensory input. Although no significant differences in the spatial distribution of the three networks were determined, a reduced number of functional connections in anosmic patients in all investigated networks were observed. Thus, the key to gaining deeper insight into the functional networks of olfactory perception in this setting was the combination of different analysis modalities which covered classical functional connectivity analysis and ICA. The combination of data-driven and hypothesis-driven methods can have a huge impact on the significance of neuroimaging studies (Xu et al., 2015). Previously published data from the same patient group (Kollndorfer et al., 2014) has shown that olfactory training induces alterations in functional connectivity that emerge from primary olfactory areas, solely evoked by the act of sniffing, without any presentation of an olfactory stimulus. In contrast, this study investigated alterations in

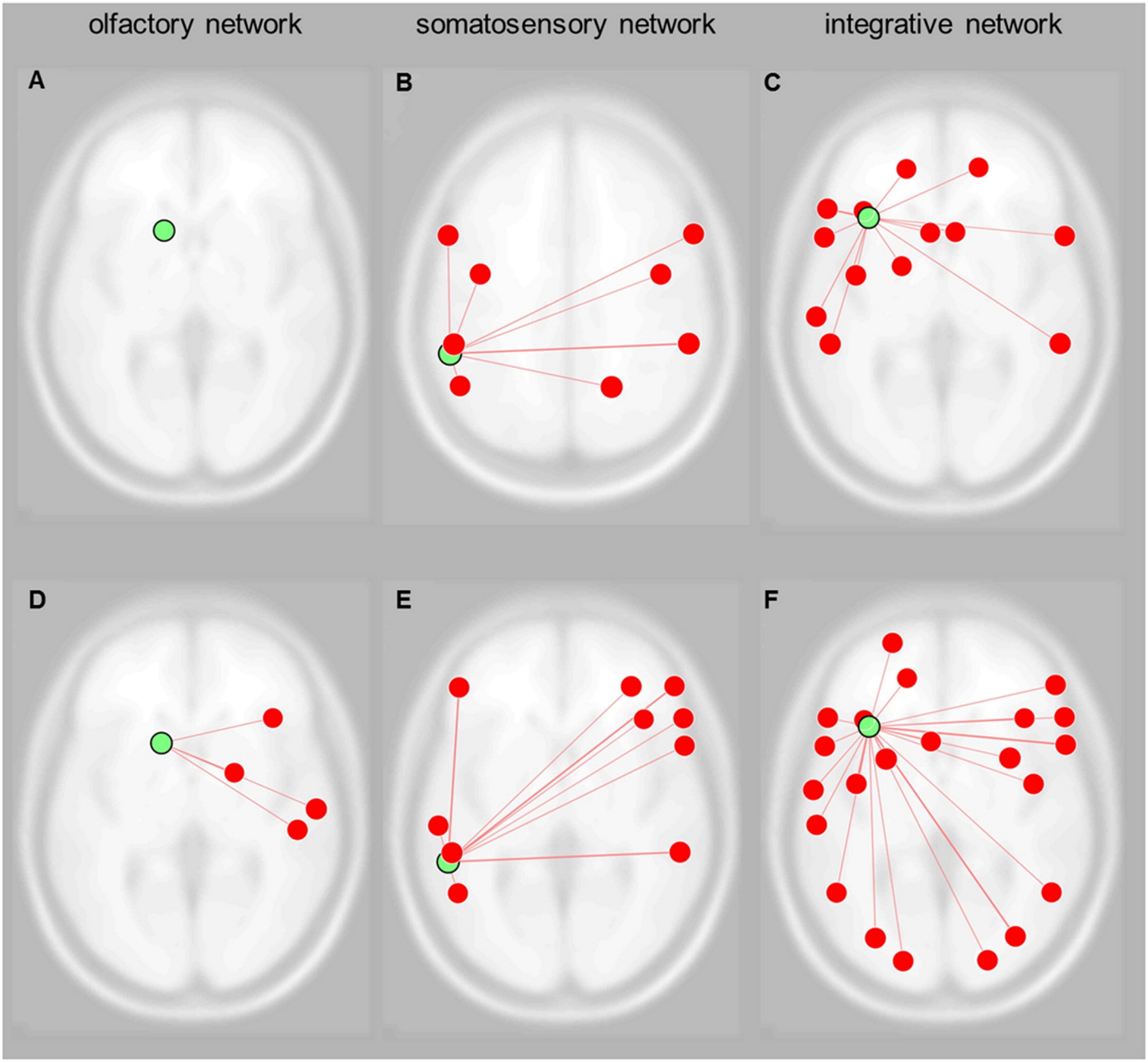

Fig. 5. Functional connectivity during chemosensory stimulation in anosmic patients before (A/B/C) and after the olfactory training (D/E/F), overlaid on an axial template in MNI space ($p = 0.005$, uncorrected). The green dot represents the selected ROI (A/D (olfactory network): −14,14,2 (caudate nucleus); B/E (integrative network): −34,22,10 (insular cortex); C/F somatosensory network: −58,−42,36 (supramarginal gyrus)); the red dots capture the statistically significant functionally connected brain areas.

functional connectivity, induced by olfactory training, in networks involved in olfactory and trigeminal stimulus processing. ICA has been proven to be a useful analysis tool for the investigation of functional activation patterns in chemosensory perception (Frasnelli et al., 2012; Schöpf et al., 2011). However, it is not appropriate for examining local functional connectivity. Thus, the multimodal analysis of the central processing pathways is of tremendous importance to gain deeper insight into sensory perception.

In this study, we were able to show that the functional connectivity of olfactory-related networks may be reorganized by training. After a 12-week training period, the number of connections to the seed region of the network increased, especially in the olfactory and the integrative networks. After the training, some functional connections in the olfactory network were re-established, among them functional connections to the anterior entorhinal cortex, the inferior prefrontal gyrus, and the primary somatosensory cortex. This finding is of particular interest, as it suggests that the olfactory training induces the reconstruction of the olfactory-specific processing network. However, during this time, no significant differences in the spatial distribution of the network patterns were detected. Training-induced behavioral improvement in olfactory performance has also been observed in patients with reduced olfactory function (hyposmia), and in healthy controls. Thus, future studies may shed light on the potential functional connectivity in patients with hyposmia.

A limitation of this study is the small sample size of the seven anosmic patients who completed all measurements. However, a very strict screening procedure was performed to include only patients with anosmia after an upper respiratory tract infection to prevent the influence of different causes of smell loss. We decided to investigate post-infectious anosmic patients, as previous studies have reported that olfactory

training was most successful in this patient group. When the study was designed, the recommended training period for smell training was 12 weeks (Hummel et al., 2009b). However, a multi-center follow-up study (Damm et al., 2014) revealed a greater improvement of olfactory performance after a training period of at least 18 weeks. Thus, studies investigating the effects of longer training periods on functional connectivity should follow. Furthermore, future studies may include a control group with placebo training, in order to investigate potential spontaneous reorganization effects. However, based on behavioral investigations of the olfactory training (Damm et al., 2014), and basic knowledge of functional reorganization in the brain, spontaneous reorganization of neural networks is not likely.

5. Conclusion

The results of our study support the assumption of an intact trigeminal pathway in patients with smell loss caused by an upper respiratory tract infection. Based on these findings, we assume that the intact trigeminal pathway plays an important role in the improvement of olfactory performance induced by olfactory training.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nicl.2015.09.004.

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