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Direktor: Herr Prof. Dr. Thomas Zahnert

Novel methods to assess olfactory processing

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List of Abbreviations

BOLD	Blood -oxygenated level dependent
EEG	Electroencephalogram
fMRI	Functional magnetic resonance imaging
CNV	Contingent negative variation
OFC	Orbitofrontal cortex
ROI	Region of interest
TBSS	Tract based spatial statistics
DWI	Diffusion weighted imaging
UPSIT	University of Pennsylvania Smell Identification
SPSS	Statistical Package of the Social Sciences
OB	Olfactory Bulb
ACC	Anterior cingulate cortex
PD	Parkinson’s disease
ICA/CA	Congenital anosmia
CT	Computed topography
FA	Fractional anisotropy
MD	Mean diffusivity
RD	Radial diffusivity
AD	Axial diffusivity

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Introduction of the topic

Olfaction

Olfactory System

As soon as odorants reach the olfactory mucosa, the signal transduction starts from the peripheral part (olfactory epithelium). Olfactory information then travels to the olfactory bulb (OB), which sends it to higher order brain processing regions, like piriform cortex or amygdala (Gottfried, 2010). Cortical regions receiving direct projections from OB are referred to as "primary olfactory areas" including the piriform cortex, and rostral entorhinal cortex, and periamygdaloid and anterior amygdaloid cortex. These areas are connected to other brain regions and are referred to as "secondary olfactory areas" which include hippocampus, parahippocampal gyrus, anterior cingulate cortex (ACC), insula, mediodorsal thalamus and orbitofrontal cortex (OFC) (Zhou et al., 2019), as shown in Figure 1.

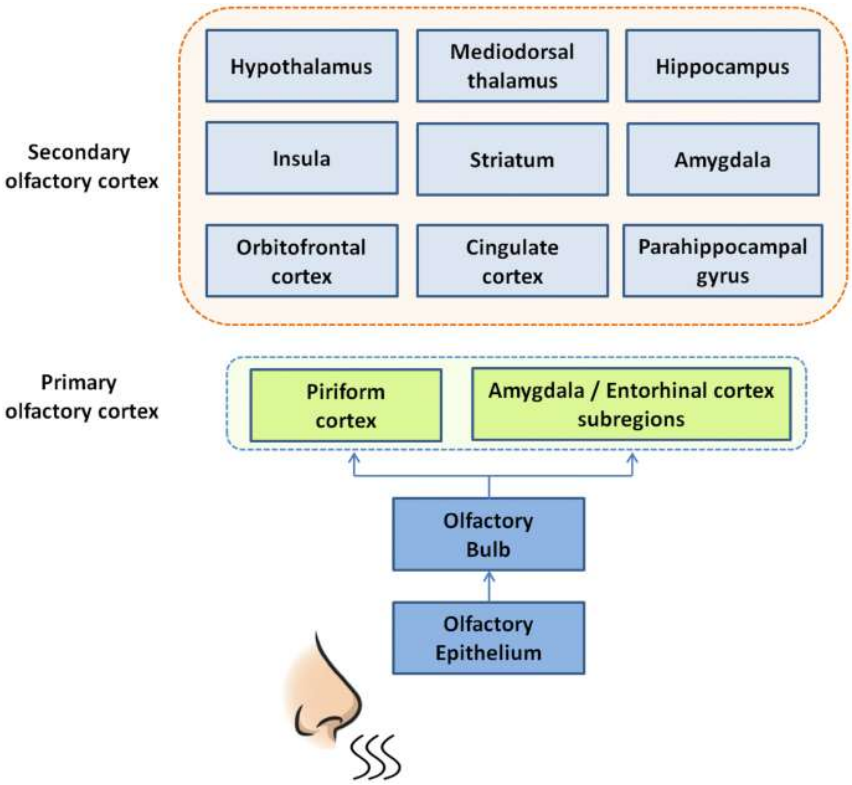

Figure 1 The human olfactory system

Various studies have focused not only on the anatomical aspects of the olfactory system but also on the functional aspects. It is very well known that piriform cortex receives projections directly from OB in rodents as well as in humans. However, in rodents, piriform cortex has a

clearly distinguishable functional and anatomical part (Grau-Perales et al., 2019; Yang et al., 2017). In contrast, it was only recently that Zhou and colleagues (2019) found that in humans information flows from the OB to the cortex, which includes the entorhinal cortex, and the temporal lobe pole, in a direct manner, parallel to the connection from the OB to the piriform cortex. Using parcellation techniques, they found that the piriform cortex has a frontal and a temporal part and is functionally connected to motor planning areas like caudate, putamen, and primary motor areas while the latter is more functionally connected to insula and supramarginal gyrus (Zhou et al., 2019). As can be seen in publication 3, we used diffusion tractography to visualise white matter streamlines between primary olfactory area, like piriform cortex and secondary olfactory areas, like OFC. We also found that these streamlines are linked to olfactory function in terms of odor threshold and odor identification score, which will be discussed further in this thesis under the heading “measuring olfactory function”.

Olfactory dysfunction

Olfaction is one of the key senses that warns us about hazards in our surroundings, which is among its primary functions. However, compared to other senses, like vision, it is still less clear how smell is interpreted by the brain. Although olfaction is considered very primitive in humans, this sense modulates eating behaviour (Boesveldt & de Graaf, 2017), kin recognition (Lundström et al., 2008) and partner selection (Sorokowska et al., 2018). Functionally, this sense is bimodal with trigeminal and olfactory systems working together (Cain, 1974; Hummel & Livermore, 2002).

Olfactory loss is characterised by a reduced ability to detect and smell odors in daily life. Olfactory dysfunction presents with various functionality ranging from hyposmia (reduced sense of smell) to anosmia (complete loss) (Hummel et al., 2016). Besides, approximately 1 in 10,000 people present with absence of olfactory bulb, which is known as congenital anosmia. However, a recent paper found 2 women without an OB performing at par in terms of olfactory abilities with healthy subjects (Weiss et al., 2020). Studies have shown a reduction in olfactory ability as a function of age (Dan et al., 2021). Major causes of olfactory loss include viral infections, traumatic brain injury, or chronic rhinosinusitis (Temmel et al., 2002). Early olfactory loss has been shown to be an important marker of Parkinson’s disease (PD) (Haehner et al., 2007). A recent imaging based study using diffusion weighted imaging found that advancement of PD is reflected by white matter changes in olfactory brain areas, like the piriform cortex and orbitofrontal cortex (Hummel et al., 2021). In Publication 2, we looked into ICA patients using water diffusion properties that can be traced using magnetic resonance imaging (MRI) and

found increased fractional anisotropy (FA) in OFC which indirectly points to the plastic nature of the brain.

Measuring olfactory function

Psychophysical

Smell loss is subjective unless tested objectively. Patient history plays an important role when it comes to the assessment of smell loss. However, it is often inaccurate because it is highly biased as patients might over or underrate their olfactory function. Hence, an objective measure is a must. We have come a long way since the Elsberg-Levy method of testing (Jones, 1953). Although the Elsberg-Levy test is quite inexpensive and easy to perform but users report bias as air is blasted in the nose affecting aerodynamics of the flowand hence results in un-reliable data. Since then, various tests have come up and are typically easy to use and effectively and objectively measure smell function. For example, the University of Pennsylvania Smell Identification Test (UPSIT) is a 40-item smell identification test with even shorter versions being available (Doty et al., 1984). The UPSIT is based on a forced choice paradigm where participants have to identify the odor from lists of four verbal descriptors. Sniffin' Sticks measure different dimensions of smell function, threshold (T), identification (I) and discrimination (D). This test also follows a forced choice paradigm and it is quick and reliable (Oleszkiewicz et al., 2019). Each sub-test has 16 items at different concentrations (T) and different smells (D and I) to obtain a more complete olfactory profile. The threshold test, using a staircase design, measures at what odor concentration a participant can distinguish between items with a certain odor concentration vs odourless items. Odor discrimination test measures the discrimination ability when presented with three items, one item containing a different odor. The identification test is a 16-item odor identification test with one correct answer that has to be identified from a choice of 4 options presented per odor. Together, the combined scores from individual tests form the TDI score, which allows to categorize patients as hyposmic, anosmic or normosmic (Oleszkiewicz et al., 2019). Sniffin' sticks were used for olfactory testing throughout the publications referenced here in the thesis.

Physiological

Electroencephalogram (EEG)

EEG-based olfactory event-related potentials were first recorded in humans in 1966 by Finkenzeller. Compared to other imaging modalities like CT and MRI, EEG has very high

temporal resolution with ms precision (Howseman et al., 1999). The basic principle of EEG relies on the fact that presentation of stimuli, olfactory related, initiates an electrical field that can be recorded by electrodes. EEG has been widely used for olfactory research and also for diagnostic purposes (Bonanni et al., 2006). Most recently the electrobulbogram, recorded from the OB, has been established. Since OB is the first structure where odor interpretation starts, it is interesting to see how this technology grows and allows us to use it clinically (Iravani et al., 2020). In publication 1, we employed a variant of studying EEG related responses called contingent negative variation (CNV) which is a negative variation produced in response to expected stimuli.

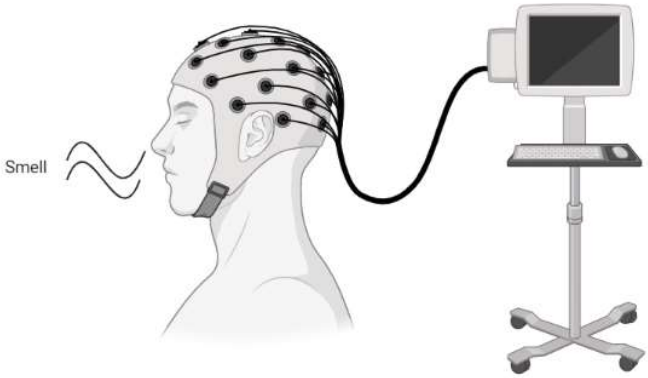

Figure 2 Typical olfaction-based electroencephalogram setup (created in Biorender.com).

Functional magnetic resonance imaging (fMRI)

fMRI is a non-invasive neuroimaging technique where functional aspects of brain processes can be understood. Compared to EEG, fMRI has a very high spatial resolution and we can focus on brain areas with a resolution as low a one millimetre (Katwal et al., 2013). Principle of fMRI acquisition depends on the intensity of the blood oxygenated level dependent (BOLD) signal, which is a measure of the ratio of deoxygenated blood to oxygenated blood. High spatial resolution makes it a better choice (compared to EEG) for looking into brain processes at the level of cortical and sometime at subcortical regions (with more enhanced protocols) as compared to EEG. One of the most important modulators in fMRI experiments is repetition time or TR. In the olfactory world, longer TR means higher chances of habituation which leads to reduction in signal intensity (Georgiopoulos et al., 2018). This is probably more important in the sense of smell than in other senses, like vision. Previous work in this area suggests using shorter TR of approximately 1s and the use of block design for stimulation. Shorter TR and shorter stimulation time enhance brain activations and shorten the latency to peaks

(Georgiopoulos et al., 2018). For stimulations, computer controlled olfactometers are used which provide for precise and timed odor delivery (Sommer et al., 2012).

Diffusion weighted imaging (DWI)

Communications between different brain regions are mediated by axons, which form the white matter of the brain. Such white matter tracts can be visualised using diffusion weighted imaging. DWI is based on random movements of water protons. DWI is routinely used as a diagnostic for more detailed investigations (Bammer, 2003). In particular in the field of olfaction, not much work has been done. Recently, COVID based studies showed altered diffusion metrices like MD, AD, RD and increased FA in white matter tracts near olfactory cortices (Lu et al., 2020). Fibre tractography, derived from DWI, has been extensively used in other senses for visualising white matter connections. However, it was not until 2011 that fibre tracking was used in olfaction. Skorpil et al. were the first to use fiber tractography to visualise white matter tracts generated from the olfactory tubercule (Skorpil et al., 2011). In this regard, publication 3, showcases our work on fiber tractography using probabilistic mapping in olfactory areas.

Novelty of methods in olfactory processing

Contingent negative variation (CNV)

It is well known that odors have modulating effects on individual's consciousness and, possibly more importantly, on emotions (Manley, 1993; Lorig et al., 1995). Although pleasantness is intimately related to the perception of odors, (Khan et al., 2007; Kadohisa, 2013) it appears problematic to objectively assess differences between odors in relation to differences in valence, especially when differences are subtle (Pichon et al., 2015). One possibility to assess odor pleasantness could be the use of ‘‘Contingent Negative Variation" (CNV). It was described in 1964 by Walter as the first ‘‘evoked response" obtained from the EEG by averaging stimulus-correlated EEG sections. It has been reported that arousal and sedation are associated with CNV. CNV is comprised of a slow cortical event-related potential which is recorded from the scalp following presentation of a stimulus (typically, a tone would be presented (S1 stimulus)) and subjects expecting a second event while preparing for a certain task to be performed in relation to the second event. Typically, the subjects' task would be to push a button following presentation of a second tone (S2 stimulus). Attention and expectation of the stimulus have been reported to be more associated with early CNV (S1 stimulus), and the late CNV (S2 stimulus) is supposed to be related to estimation, preparation and motor processing induced by stimulus expectancy (Yazawa et al., 1997). The developing negativity of the CNV is associated

with ‘‘increased preparedness for motor act or decision making” (Duschek et al., 2007). Torri and colleagues have suggested that CNV may help in gaining insights into the mood-elevating properties of odors (Torii et al., 1988). A study by (Hiruma et al., 2002) investigated the stimulating effect of Hiba oil (major constituents being terpinolene and 4-terpineol) on stress modulation which resulted in higher CNV amplitudes (Manley, 1993). However, its relation with pleasantness could not be described. Aim of the present study was to investigate the association between the valence of odors and CNV. We used odors that are typically rated as pleasant but still cover different areas of the olfactory space, namely peppermint, vanilla, musk, and orange. Because younger subjects generally have a better sense of smell compared to the older population, we hypothesized that this would be reflected in the modulation of CNV which should be more pronounced in younger compared to older subjects (Hummel et al., 1998).

Tract based spatial statistics (TBSS)

DTI is a robust tool to investigate structural integrity where one of the measures is fractional anisotropy (FA). Higher FA values indicate more axon myelination (Osuka et al. 2012). FA has been found to be a marker of improved function in various neurodegenerative diseases and recovery from traumatic brain injury (Alba-Ferrara and de Erausquin 2013; Wallace et al. 2018). Increased cerebral myelination has been associated with increased gray matter thickness and FA, both sharing a linear correlation (Kochunov et al. 2011). However, the effect has yet not been fully understood. The main purpose of the study was to investigate whether FA can explain the differences noted previously in OFC in CA (increased gray matter volume) and compare them with healthy controls. Voxel wise statistical analysis of the FA data was carried out using TBSS (Tract-Based Spatial
Statistics (Smith et al. 2006)), part of FSL. TBSS projects all subjects' FA data onto a mean FA tract skeleton, before applying voxel wise cross-subject statistics.

Probabilistic tractography

Fibre tracking within the olfactory system is complicated as the system is intimately connected with other brain regions, e.g., thalamus, cerebellum, and insula (Soudry et al., 2011). Another issue is that some regions that are directly involved in olfaction, such as piriform cortex or the entorhinal cortex, are structurally difficult to delineate. The piriform cortex, part of the olfactory cortex, has many projections to other brain regions such as the amygdala and hippocampus, playing a major role in converting sensory input into specific behavioural output (Chen et al., 2014; Diodato et al., 2016). On the other hand, the OFC, a secondary olfactory area, is not solely dedicated to olfactory processing but is, for example, strongly involved in sensory

integration (Wang et al., 2020). Whilst there is a consensus about olfactive information processing between the piriform cortex and OFC, there is less agreement about the role of the thalamus. Some authors (Shepherd, 2005) consider that the exact role of the thalamus in olfaction is not clear and that there seems to be no direct involvement of the thalamus in the connections between the piriform cortex and the OFC. In contrast, Courtiol and Wilson concluded that the thalamus serves as a crucial center for olfactory processing by forming a reciprocal connection with the OFC via the piriform cortex (Courtiol and Wilson, 2015). In order to assess the role of the connections between the piriform cortex, thalamus and OFC, we aimed to examine white matter stream-lines within the olfactory system using fibre tractography. Additionally, we explored how the processing of olfactory versus trigeminal odors (Joshi et al., 2021) impacted potential relations between these stream-lines.

Methods

Method 1: Publication 1 Olfactory Modulation of the Contingent Negative Variation to Auditory Stimuli

Subjects

Eighty participants were recruited for the study, which were classified into two groups. For final analysis only sixty-two participants were included. The results from the remaining eighteen subjects had to be removed from analysis due to a high number of artifacts, e.g., eye blinking (>50μV), motor artefacts, or large background noise or less trials (minimum 8). Groups included subjects under the age of 30 years (N – (no. of subjects) = 30, mean age = 23.1 ± 3.0 years, male (m) = 18, female (f) = 12; termed as younger population group) (YOUNG) and subjects over the age of 40 years (N= 32, Mean age = 52.3 ± 11.6 years, m = 17, f = 15; termed as older population) (OLD). All participants provided written informed consent and ethics approval was obtained prior to commencement of the study (EK340082017). All participants were over 18 years of age, had legal liability, were non-smokers and had a normal sense of smell as defined using the Sniffin' Sticks 16-item odor identification test (Oleszkiewicz et al., 2019). Participants also received nasal endoscopy to ascertain normal nasal anatomy and absence of major nasal pathologies. In addition, a standardized medical history was taken (Welge-Luessen et al., 2014). Female participants who were pregnant or breastfeeding were excluded. Participants with significant health impairments (e.g., Parkinson's disease, renal insufficiency) that can be associated with disorders in olfactory function or acute or pronounced chronic inflammation of the nose or paranasal sinuses were not included in the study.

Subjects were characterized for personality traits and for their interest in olfaction. To this end, they received the personality trait scale NEO Five-Factor Inventor (NEOFFI) (McCrae et al., 2005) that examines openness to experience, conscientiousness, extraversion, agreeableness, and neuroticism. Importance of Olfaction scale (IOQ) (Croy et al., 2010) was used to check how important smell was rated by the participants in day-to-day affairs.

Experimental design and recording of EEG/CNV

Figure 1 shows the experimental design for the study which took about 2 hours per subject. All subjects were asked not to eat and drink 1hr before commencement of the study. The first part of the experiment included testing with the Sniffin' Sticks test (a 16-item odor identification test) to ascertain normal sense of smell. Nasal endoscopy was performed to rule out any abnormal nasal anatomy. Then the NEOFFI and the IOQ was filled in.

The pre-session was followed by a main session where precise bilateral stimulation of the olfactory system was achieved using a computer-controlled olfactometer (total airflow 2l/min) (Sommer et al., 2012). We used odors that are typically rated as pleasant but cover different areas of the olfactory space, namely peppermint oil (20% dilution in propylene glycol: Sigma Aldrich, Deisenhofen, Germany: order number 398039), vanilla (10% dilution in propylene glycol), musk (undiluted), and orange (10% in propylene glycol) (all odors from Takasago, Tokyo, Japan; order numbers: peppermint ICC#016019; vanilla ICC#022007; musk ICC#013113; orange ICC#015026). During pilot tests, based on ratings from 10 experienced individual's odor intensities were adjusted to be approximately equal. Odor-less air was used for control (Air). After the session subjects were asked to rate the odor intensity, pleasantness and calmness associated with the odors on scales of 0 to 100, with 0 being the lowest intensity /very unpleasant/very calm and 100 being the highest intensity /very pleasant/highest degree of being excited.

EEG-segments of 5120ms length (including a pre-trigger period of 500 ms before the first acoustic stimulus) were recorded at a sampling frequency of 250Hz using a band-pass filter of 0.2-30Hz (16-channel amplifier; SIR: Röttenbach, Germany) from positions Fp2 (frontopolar, recording of vertical eye movements), and the midline positions Fz (frontal), Cz (central), and Pz (posterior), referenced against linked earlobes (A1+A2). Subjects sat on a comfortable chair with headphones (for auditory stimulation) and nasal cannula (for olfactory stimulation). Pairs of acoustic stimuli were presented via headphones. The first tone had a lower pitch (~ 500 Hz) (S1), the second was either the same deep or a higher pitch (~ 700 Hz) (S2). The interstimulus interval (ISI) between S1 and S2 was 2.4s. The interval between pairs of tones (S1S2) was 9.6s. The ISI of 2.4s was inspired by the previous study where a longer ISI was recommended to correctly detect the CNV (Lorig & Roberts, 1990).Subjects were instructed to click the mouse

button for the high tone. Their reaction time (RT) was recorded. This was achieved using E Prime 3.0 (Psychology Software Tools, Inc. [E-Prime 3.0] (2016) https://www.pstnet.com). The main experiment was comprised of 3 sessions with 50 pairs of auditory stimuli each (150 stimuli in total; 30 min in total). During each of the 3 sessions the 5 odors were presented in a randomized fashion continuously during the session, where each session lasted 10mins. The sequence of odors was kept the same for the 3 sessions. The order of odor presentation was randomized across subjects. Odor ratings are presented in table 1, which were rated by the subjects after the session. CNV and ERP amplitudes are presented in tables 2, 3, and 4, for background odors (intranasal odors given during the CNV recordings).

Signal Analysis and Data analysis

The data was heuristically evaluated with EP Evaluate (Kobal, Erlangen, Germany) based on LabVIEW 6.1 (National Instruments, Austin, TX, USA). For the CNV, data was additionally filtered offline with a low pass filter of 15Hz. The starting and ending points of the CNV were measured using markers placed on the curve, with the first marker placed at the EEG curve following the acoustic ERP (~200ms after onset of stimulus 1), and the second marker placed at the end of the negativity, at occurrence of stimulus two (~2400ms after onset of stimulus 1). In order to examine how focused the participants were during the task, we recorded the reaction times (RT). We also analyzed auditory event related potential (ERP) data. ERP consist largely, of negative N1 component, which is followed by positive component P2. We were interested in amplitudes between P2 and N1 (N1P2) (Figure 2).

Statistical analysis

The data were statistically evaluated with the program package SPSS (SPSS Statistics for Windows, vs. 27; IBM, Armonk, NY, USA). We found that the data were normally distributed by means of Shapiro-Wilk test, where we found $p = 0.12$. We also ran test for equal variance (Levene's test) and found no statistically significant difference in variance between our groups (YOUNG and OLD) for CNV measures at different recording site. We used mixed-model repeated measures ANOVA (full-factorial) for analyzing within subject variance using two factors, "odor" (peppermint, vanilla, musk, orange, and air) and "recording site" (Fz, Cz, and Pz). We also employed multivariate analysis of variance (MANOVA) to compare NEOFFI and IOQ scores among groups, YOUNG and OLD. Post-hoc analysis using Bonferroni corrections were used where necessary. Level of significance was ascertained at $p<0.05$.

Method 2: Publication 2 Subtle Differences in Brain Architecture in Patients with Congenital Anosmia

We present an investigation in 13 CA participants and 15 healthy controls using a ROI-based approach. Diffusion tensor imaging was performed using 3 T MRI scanner (Verio; Siemens Healthineers, Erlangen, Germany). An eight-channel receiver head coil was used for image data acquisition. DTI was acquired as 2D fast spin echo planar imaging with following specifications; TR = 71ms, TE = 6ms, Slice thickness = 2mm, FoV = 110 x 110, repetitions = 1, flip angle = 180°. Diffusion scans were acquired at b=0 and b=800 with number of diffusion directions = 20. Following written informed consent, participants underwent olfactory testing with the Sniffin' Sticks battery (odor threshold, discrimination, and identification: TDI score) (Oleszkiewicz et al., 2019). Masks for piriform cortex and orbitofrontal cortex (OFC) were adapted and thresholded from two published studies (Fjaeldstad et al., 2017; Seubert et al., 2013) using FSL edit mode (FMRIB software library v6.0.2) (Jenkinson et al., 2012) to include white matter areas and manual removal of any underline gray matter, if required. These ROIs were visually inspected by expert neuroradiologists, who also helped in normalisation and outline of the ROIs. We also analysed the FA values in piriform cortex (PFC) using the same approach. Voxel wise statistical analysis of the FA data was carried out using TBSS (Tract-Based Spatial Statistics (Smith et al., 2006)) , part of FSL. TBSS projects all subjects' FA data onto a mean FA tract skeleton, before applying voxel wise cross-subject statistics. Statistical analysis was carried out using SPSSv27 (Armonk, NY, USA: IBM Corp). We used Mann-Whitney U test, a non-parametric test given the sample of the study, where $r < 0.3$ represents a small effect, r between 0.3 – 0.5 medium effect and $r > 0.5$ a large effect ($r = z/\sqrt{n}$; z: standardised test statistic, n: number of samples).

Method 3: Publication 3 Tractography indicates lateralized differences between trigeminal and olfactory pathways

Participants

Thirty-eight healthy subjects (20 males, 18 females) were recruited to take part in the study after having provided written informed consent. The experiments were conducted according to the Helsinki declaration. The ethics committee at the medical faculty of the Technical University of Dresden approved the study design (approval number EK558122019). A detailed, structured medical history was conducted including questions regarding drinking habits, smoking, medications, or current disorders (Welge-Luessen et al., 2014). The sample size is always debatable in functional or structural studies. Still, considering previous work a sample above 30 is thought to provide reliable results (Joshi et al., 2021).

Olfactory testing

A normal sense of smell was ascertained using the “Sniffin’ Sticks” odor identification test with maximum score of 16. This test is based on a forced choice paradigm where subjects have to identify 16 odors at suprathreshold concentrations using flash cards with four verbal descriptors each (Oleszkiewicz et al., 2019). For olfactory activation in the MR scanner, we used four odors (provided by Takasago, Paris, France), two more trigeminally active stimuli and two more olfactory active stimuli. The trigeminal odors were peppermint (order number ABX321352) and spearmint (ABX321351A), the olfactory stimuli were cherry (ABX321603) and strawberry (ABX321354A). Prior to the administration in the MR environment each of the pure, undiluted odors was rated by the participants for their intensity and pleasantness using visual analogue scales (0 to 10, with 0 meaning no intensity perceived / extremely unpleasant and 10 meaning extremely intense / extremely pleasant). Threshold scores (1 to 8, with 1 meaning high threshold [relatively insensitive] and 8 meaning low threshold [very sensitive]) for each odor were also obtained from each participant using a 3-alternative forced choice task with odors presented in glass bottles using a staircase design (4 ml odor in 50 ml volume bottles with an opening of 4 cm diameter) (Hummel et al., 1997). Participants had to discriminate the odor-containing bottle from two others containing the solvent propylene glycol (Sigma-Aldrich, Deisenhofen, Germany, order number 398039). All tests were performed within a forced choice design.

The two trigeminal odors (peppermint and spearmint) did not differ statistically (t-test) in terms of threshold ($p=0.83$), or intensity ($p=0.45$) but differed in terms of pleasantness ($p=0.02$). Still, peppermint and spearmint were categorized and combined together as “trigeminal odors” activating both the olfactory and trigeminal systems (Han et al., 2020; Joshi et al., 2021; Krone et al., 2001). Their trigeminal nature was characterized by lateralization tests where 20 randomized odor pairs (in two squeeze bottles) are presented to each nostrils where one bottle contained no odor whereas the other did contain an odor (Frasnelli et al., 2011; Pellegrino et al., 2017). The two olfactory odors did not differ in intensity ($p=0.09$), pleasantness ($p=0.93$) and threshold ($p=0.53$) and were categorized and combined together as “olfactory odors” with little or no activation of the trigeminal system (Pellegrino et al., 2017).

Image acquisition

All image data was acquired on a 3T Prisma (Siemens Healthcare, Erlangen, Germany) MRI scanner, using a 32-channel head coil. A 3D magnetization prepared gradient echo T1 image was acquired (TR=2000ms, TE=1.95s, FoV=256mm x 256mm, slice thickness = 1.7mm and voxel size of 1x1x1mm). A diffusion weighted, generalized auto-calibrating partial parallel acquisition (GRAPPA) sequence image (TR=6070ms, TE= 100.8ms, multiband acceleration factor=2, 67 diffusion directions, b-value=1000s/mm^2, slice thickness=1.7mm, voxel

size=1.7x1.7x1.7 mm) was acquired. b0 images, no diffusion images, were also acquired in 6 directions which resulted in longer TR=12110ms and did not affect tensor fitting.

Image analysis

All image analysis was carried out in FSL, a FMRIB software from the Oxford center for functional magnetic resonance imaging of the brain version 6.0.2 more specifically, FDT (FSL diffusion toolbox) was used (Jenkinson et al., 2012). A standard pipeline was used for pre-processing of the diffusion data which included head motion, eddy current correction using eddy from FDT, and followed by removal of non-brain tissue using bet. Using Quality assessment of diffusion (QUAD) we assessed quality at subject level and Study-wise quality assessment of diffusion (SQUAD) for group level, where a cut-off value for mean displacement was set at >2mm in terms of absolute motion and >0.3mm in terms of relative motion for the whole study population (Bastiani et al., 2019). No dataset had to be discarded. BedpostX was used to map out diffusion parameters at each voxel (Behrens et al., 2007).

Fiber tracking

Tractography was performed using PROBTRACKX probabilistic tracking in FDT diffusion toolbox (Behrens et al., 2007). For seed space, a single mask option was chosen with piriform cortex as the mask. Further, since the mask was not defined in diffusion space, appropriate transforms were selected to further streamline the tracking. In optional targets, waypoint mask, exclusion mask and termination mask were chosen, where the midline mask was selected as exclusion mask (Lancaster et al., 2000). Waypoint and termination mask were always the same so that only those streamlines that passed the waypoint mask were accepted. Termination mask and waypoint mask were the same to stop tracking as soon as the waypoint condition was met which means that only those tracks should be accepted which start from the piriform cortex and pass through the orbitofrontal cortex or thalamus and terminate at the mask.

ROIs were used from already published data (Fjaeldstad et al., 2017; Seubert et al., 2013). They were mapped to each subject in two steps; since the ROIs were defined in structural space or rather MNI space, firstly FLIRT (Jenkinson & Smith, 2001) was used to obtain a linear transformation matrix between diffusion and structural space and then FNIRT (Simpson et al., 2015) was used to obtain non-linear transformation to store results in standard space. ROIs from Seubert et al. were derived using ALE from 40 different functional olfactory studies. These authors also mentioned that they initially had insular cortex included in the mask; it was removed in order to increase functional homogeneity of the OFC and piriform cortex mask. However, to make sure that excessive white matter areas were not included, ROIs from Fjaeldstad et al. which are derived from both functional and structural space were used and superimposed. Superimposing here means to overlay masks from Seubert et al. over the mask

from Fjaeldstad et al., considering the latter as standard and then manually removing excessive white matter areas. Each mask was then reviewed by an expert neuroradiologist (CG). The mask for the OFC included only the central part. Thalamus, as a mask, was chosen from Harvard-Oxford subcortical structural atlas (Frazier et al., 2005). This atlas does not divide the thalamus based on its nuclei because this atlas is largely taken from functional studies. We also thresholded the mask. All ROIs were thresholded using fslmaths (fslmaths mask.nii.sgz -thr 95) (Lancaster et al., 2000). For the purpose of calculating the streamlines between ROIs, the waytotal number was used. The waytotal number was derived as one of the metrics while running probtrackX, and it infers the total number of streamlines for all seed voxels (piriform cortex) which have not been rejected. The volume of the tract was computed. Simple tracking was performed from piriform cortex to orbitofrontal cortex or Thalamus and the waytotal number was used to determine how many streamlines made it to the termination mask.

Statistics

We used non-parametric Kruskal-Wallis ANOVA independent samples test with Mann-Whitney U test for pairwise comparisons using Bonferroni corrections. We ran the test for side (left and right) and track pathway. We also ran partial correlations between track pathways and identification scores, threshold scores, intensity, and pleasantness ratings. A p-value <0.05 was considered to be significant. All statistical analysis was performed using SPSS v27.0 (IBM Corporation, Armonk, NY, USA).

Contributions in the Publications

Publication 1: Conceptualization, Methodology, Conduct or Experiments, Formal analysis, Writing of the manuscript

Publication 2: Conceptualization, Methodology, Conduct or Experiments, Formal analysis, Writing of the manuscript

Publication 3: Conceptualization, Methodology, Conduct or Experiments, Formal analysis, Writing of the manuscript

List of Published Papers

Thaploo D, Zelder S, Hummel T. Olfactory Modulation of the Contingent Negative Variation to Auditory Stimuli. *Neuroscience*. 2021; 470:16-22. doi: 10.1016/j.neuroscience.2021.07.005. Impact Factor: 3.708

Thaploo D, Georgiopoulos C, Haehner A, Hummel T. Subtle Differences in Brain Architecture in Patients with Congenital Anosmia. *Brain Topogr*. 2022;35(3):337-340. doi:10.1007/s10548-022-00895-z. Impact Factor: 4.275

Thaploo D, Joshi A, Georgiopoulos C, Warr J, Hummel T. Tractography indicates lateralized differences between trigeminal and olfactory pathways. *Neuroimage*. 2022; 261:119518. doi: 10.1016/j.neuroimage.2022.119518. Impact Factor: 7.4

Publication 1 (First study) Olfactory Modulation of the Contingent Negative Variation to Auditory Stimuli

Abstract of Publication 1

Background: Although pleasantness is intrinsically related to the perception of odors it is difficult to objectively assess odor-induced pleasantness. To evaluate the effects of odors of different valences on the contingent negative variation (CNV) in a younger and an older population.

Methods: Data from 62 participants (27 men, 35 women) were included. Two age groups with Age group 1 (YOUNG) had 30 subjects within age 18–30 years and age group 2 (OLD) had 32 subjects with age >40 years. Pre-testing was performed to acquaint subjects with the experimental tasks and their environment. Subjects received “Sniffin’ Sticks” odor identification tests and a standardized medical history to ascertain normosmia. In addition, they also took questionnaires relating to importance of the sense of smell and personality traits. CNV was elicited with two auditory stimuli, S1 followed by S2 at an interval of 2.4 s. Subjects were asked to push a button as fast as possible after they perceived S2. EEG was recorded from 4 positions of the 10–20 system (Fp2, Fz, Cz, and Pz). Four odors plus odourless air was presented in randomized order.

Results: The following main results emerged: (1) Consistent with the literature CNV showed a typical topographical distribution with the largest amplitude over the front-central recording sites. (2) 69% of subjects had consistent CNV for all conditions. (3) for some odor conditions CNV amplitudes exhibit a weak relation to odor pleasantness and associations with calmness. (4) CNV amplitude correlated negatively with reaction times.

Conclusions: Overall, the results suggest the usefulness of CNV as an electrophysiological measure of cognition. However, in the present context, concomitantly applied odors of different hedonic tones exerted only minor effects on CNV. Thus, we conclude that odors have little or no effect on CNV.

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Olfactory Modulation of the Contingent Negative Variation to Auditory Stimuli

Divesh Thaploo,* Stefanie Zelder and Thomas Hummel

Smell & Taste Clinic, Department of Otorhinolaryngology, TU Dresden, Fetscherstrasse 74, 01307 Dresden, Germany

Abstract—Although pleasantness is intrinsically related to the perception of odors it is difficult to objectively assess odor-induced pleasantness. To evaluate the effects of odors of different valences on the contingent negative variation (CNV) in a younger and an older population. Data from 62 participants (27 men, 35 women) were included. Two age groups with Age group 1 (YOUNG) had 30 subjects within age 18–30 years and age group 2 (OLD) had 32 subjects with age >40 years. Pre-testing was performed to acquaint subjects with the experimental tasks and their environment. Subjects received ''Sniffin' Sticks" odor identification tests and a standardized medical history to ascertain normosmia. In addition, they also took questionnaires relating to importance of the sense of smell and personality traits. CNV was elicited with two auditory stimuli, S1 followed by S2 at an interval of 2.4 s. Subjects were asked to push a button as fast as possible after they perceived S2. EEG was recorded from 4 positions of the 10–20 system (Fp2, Fz, Cz, and Pz). Four odors plus odorless air was presented in randomized order. The following main results emerged: (1) Consistent with the literature CNV showed a typical topographical distribution with the largest amplitude over the front-central recording sites. (2) 69% of subjects had consistent CNV for all conditions. (3) for some odor conditions CNV amplitudes exhibit a weak relation to odor pleasantness and associations with calmness. (4) CNV amplitude correlated negatively with reaction times. Overall, the results suggest the usefulness of CNV as an electrophysiological measure of cognition. However, in the present context, concomitantly applied odors of different hedonic tones exerted only minor effects on CNV. Thus, we conclude that odors have little or no effect on CNV.

Key words: EEG, expectation, pleasantness, valence, smell, nose.

INTRODUCTION

It is well known that odors have modulating effects on individual's consciousness and, possibly more importantly, on emotions (Manley, 1993; Lorig et al., 1995). Although pleasantness is intimately related to the perception of odors, (Khan et al., 2007; Kadohisa, 2013) it appears problematic to objectively assess differences between odors in relation to differences in valence, especially when differences are subtle (Pichon et al., 2015).

One possibility to assess odor pleasantness would be the use of ''Contingent Negative Variation" (CNV). It was described in 1964 by Walter as the first ''evoked response" obtained from the EEG by averaging stimulus-correlated EEG sections. It has been reported that arousal and sedation are associated with CNV. CNV is comprised of a slow cortical event-related potential which is recorded from the scalp following presentation of a stimulus (typically, a tone would be presented (S1 stimulus)) and subjects expecting a second event while preparing for a certain task to be performed in relation to the second event. Typically, the subjects' task would be to push a button following presentation of a second tone (S2 stimulus). Attention and expectation of the stimulus have been reported to be more associated with early CNV (S1 stimulus), and the late CNV (S2 stimulus) is supposed to be related to estimation, preparation and motor processing induced by stimulus expectancy (Yazawa et al., 1997). The developing negativity of the CNV is associated with ''increased preparedness for motor act or decision making" (Duschek et al., 2007).

Torri and colleagues have suggested that CNV may help in gaining insight into the mood-elevating properties of odors (Torii et al., 1988). A study by (Hiruma et al., 2002) showed the stimulating effect of Hiba oil (major constituents being terpinolene and 4-terpineol) on stress modulation which resulted in higher CNV amplitudes (Manley, 1993). However, its relation with pleasantness could not been described.

Aim of the present study was to investigate the association between the valence of odors and CNV. We

*Corresponding author.
E-mail address: taploodivesh4@gmail.com (D. Thaploo).
Abbreviations: CNV, contingent negative variation; IOQ, Importance of Olfaction scale; NEOFFI, NEO Five-Factor Inventor.

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0306-4522/

used odors that are typically rated as pleasant but still cover different areas of the olfactory space, namely peppermint, vanilla, musk, and orange. Because younger subjects generally have a better sense of smell compared to the older population, we hypothesized that this would be reflected in the modulation of CNV which should be more pronounced in younger compared to older subjects (Hummel et al., 1998).

EXPERIMENTAL PROCEDURES

Subjects

Eighty participants were recruited for the study, which were classified into two groups. For final analysis only sixty-two participants were included. The results from the remaining eighteen subjects had to be removed from analysis due to a high number of artifacts, e.g., eye blinking (>50 μV), motor artefacts, or large background noise or less trials (minimum 8). Groups included subjects under the age of 30 years (*N* – (no. of subjects) = 30, mean age = 23.1 ± 3.0 years, male (*m*) = 18, female (*f*) = 12; termed as younger population group) (YOUNG) and subjects over the age of 40 years (*N* = 32, Mean age = 52.3 ± 11.6 years, *m* = 17, *f* = 15; termed as older population) (OLD). All participants provided written informed consent and ethics approval was obtained prior to commencement of the study (EK340082017). All participants were over 18 years of age, had legal liability, were non-smokers and had a normal sense of smell as defined using the Sniffin' Sticks 16-item odor identification test (Sorokowska et al., 2015). Participants also received nasal endoscopy to ascertain normal nasal anatomy and absence of major nasal pathologies. In addition, a standardized medical history was taken (Hummel et al., 2013). Female participants who were pregnant or breast-feeding were excluded. Participants with significant health impairments (e.g., Parkinson's disease, renal insufficiency) that can be associated with disorders in olfactory function or acute or pronounced chronic inflammation of the nose or paranasal sinuses were not included in the study.

Subjects were characterized for personality traits and for their interest in olfaction. To this end, they received the personality trait scale NEO Five-Factor Inventor (NEOFFI) (McCrae et al., 2005) that examines openness to experience, conscientiousness, extraversion, agreeableness, and neuroticism. Importance of Olfaction scale (IOQ) (Croy et al., 2010) was used to check how important smell was rated by the participants in day-to-day affairs.

Experimental design and recording of EEG/CNV

Fig. 1 shows the experimental design for the study which took about 2 h per subject. All subjects were asked not to eat and drink 1hr before commencement of the study. The first part of the experiment included testing with the Sniffin' Sticks test (a 16-item odor identification test) to ascertain normal sense of smell. Nasal endoscopy was performed to rule out any abnormal nasal anatomy. Then the NEOFFI and the IOQ was filled in.

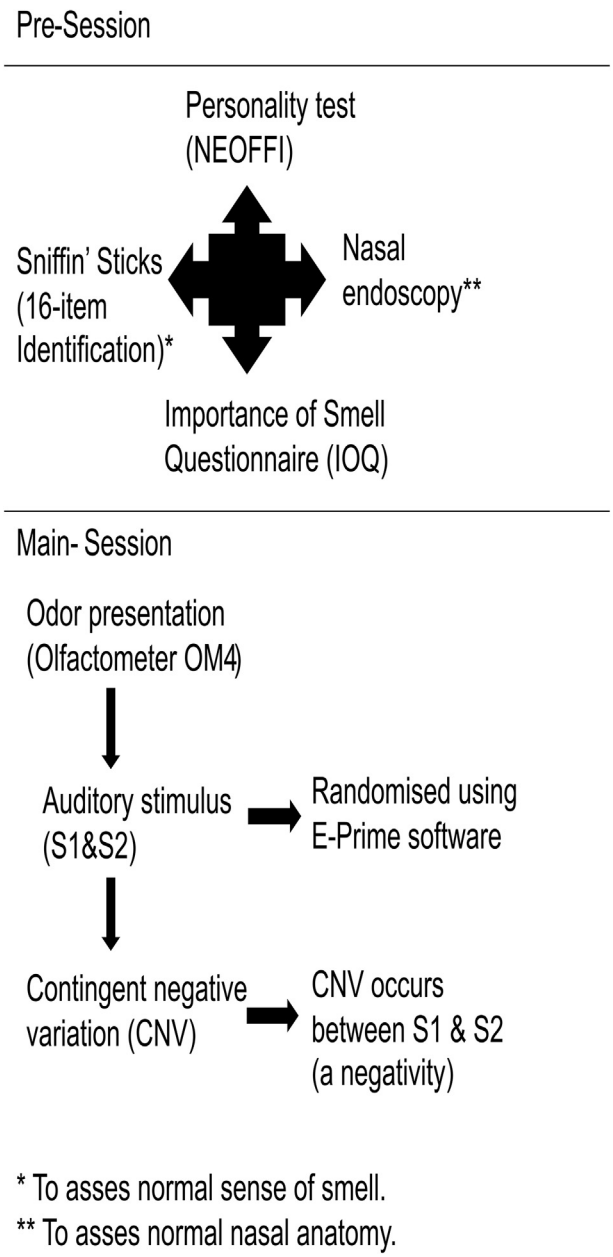

Fig. 1. Experimental workflow. Experiments were divided into two sessions; Pre-Session and Main Session. During Pre-sessions participants filled in questionnaires like the Importance of Smell Questionnaire (IOQ) and a personality test (NEOFFI). They also received nasal endoscopy and the Sniffin Sticks 16 item odor identification test. During Main-sessions the EEG-related CNV was recorded, in response to auditory stimuli generated by E-Prime software.

The pre-session was followed by a main session where precise bilateral stimulation of the olfactory system was achieved using a computer-controlled olfactometer (total airflow 2 l/min) (Sommer et al., 2012). We used odors that are typically rated as pleasant but cover different areas of the olfactory space, namely peppermint oil (20% dilution in propylene glycol: Sigma

Aldrich, Deisenhofer Germany: order number 398039), vanilla (10% dilution in propylene glycol), musk (undiluted), and orange (10% in propylene glycol) (all odors from Takasago, Tokyo, Japan; order numbers: peppermint ICC#016019; vanilla ICC#022007; musk ICC#013113; orange ICC#015026). During pilot tests, based on ratings from 10 experienced individual's odor intensities were adjusted to be approximately equal. Odor-less air was used for control (Air). After the session subjects were asked to rate the odor intensity, pleasantness and calmness associated with the odors on scales of 0 to 100, with 0 being the lowest intensity /very unpleasant/very calm and 100 being the highest intensity /very pleasant/highest degree of being excited.

EEG-segments of 5120 ms length (including a pre-trigger period of 500 ms before the first acoustic stimulus) were recorded at a sampling frequency of 250 Hz using a band-pass filter of 0.2–30 Hz (16-channel amplifier; SIR: Röttenbach, Germany) from positions Fp2 (frontopolar, recording of vertical eye movements), and the midline positions Fz (frontal), Cz (central), and Pz (posterior), referenced against linked earlobes (A1 + A2). Subjects sat on a comfortable chair with headphones (for auditory stimulation) and nasal cannula (for olfactory stimulation). Pairs of acoustic stimuli were presented via headphones. The first tone had a lower pitch (~500 Hz) (S1), the second was either the same deep or a higher pitch (~700 Hz) (S2). The interstimulus interval (ISI) between S1 and S2 was 2.4 s. The interval between pairs of tones (S1S2) was 9.6 s. The ISI of 2.4 s was inspired by the previous study where a longer ISI was recommended to correctly detect the CNV (Lorig et al., 1995). Subjects were instructed to click the mouse button for the high tone. Their reaction time (RT) was recorded. This was achieved using E Prime 3.0 (Psychology Software Tools, Inc. [E-Prime 3.0] (2016) https://www.pstnet.com). The main experiment was comprised of three sessions with 50 pairs of auditory stimuli each (150 stimuli in total; 30 min in total). During each of the three sessions the five odors were presented in a randomized fashion continuously during the session, where each session lasted 10 mins. The sequence of odors was kept the same for the three sessions. The order of odor presentation was randomized across subjects. Odor's ratings are presented in Table 1, which were rated by the subjects after the session. CNV and ERP amplitudes are presented in Table 2–4, for background odors (intranasal odors given during the CNV recordings).

Signal analysis and data analysis

The data was heuristically evaluated with EP Evaluate (Kobal, Erlangen, Germany) based on LabVIEW 6.1 (National Instruments, Austin, TX, USA). For the CNV, data was additionally filtered offline with a low pass filter of 15 Hz. The starting and ending points of the CNV were measured using markers placed on the curve, with the first marker placed at the EEG curve following the acoustic ERP (~200 ms after onset of stimulus 1), and the second marker placed at the end of the negativity, at occurrence of stimulus two (~2400 ms after onset of stimulus 1). In order to examine how focused the participants were during the task, we recorded the reaction times (RT). We also analyzed auditory event related potential (ERP) data. ERP consist largely, of negative N1 component, which is followed by positive component P2. We were interested in amplitudes between P2 and N1 (N1P2) (Fig. 2).

Statistical analysis

The data were statistically evaluated with the program package SPSS (SPSS Statistics for Windows, vs. 27; IBM, Armonk, NY, USA). We found that the data were normally distributed by means of Shapiro-Wilk test, where we found $p = 0.12$. We also ran test for equal variance (Levene's test) and found no statistically significant difference in variance between our groups (YOUNG and OLD) for CNV measures at different recording site. We used mixed-model repeated measures ANOVA (full-factorial) for analyzing within subject variance using two factors, ''odor" (peppermint, vanilla, musk, orange, and air) and ''recording site" (Fz, Cz, and Pz). We also employed multivariate analysis of variance (MANOVA) to compare NEOFFI and IOQ scores among groups, YOUNG and OLD. Post-hoc analysis using Bonferroni corrections were used where necessary. Level of significance was ascertained at $p < 0.05$.

RESULTS

Sixty-two subjects were included for the final analysis. The mean age of the younger population group was 23.1 ± 3.1 years ($N = 30$, $f = 12$, $m = 18$) whereas the older population had a mean ± SD age of 52.3 ± 11.6 years ($N = 32$, $f = 15$, $m = 17$).

Table 1. Odor rating scores in terms of pleasantness, calmness and intensity for the younger and the older groups and the five different stimuli (Mean ± SD; all scores in arbitrary units)

Younger population ($N = 30$, $f = 12$, $m = 18$)	Orange	Peppermint	Musk	Vanilla	Air
Pleasantness	75.2 ± 23.7	65.6 ± 20.6	59.9 ± 18.1	80.3 ± 14.7	58.0 ± 15.5
Calmness	55.6 ± 25.7	69.4 ± 27.3	40.7 ± 27.3	57.5 ± 25.5	17.9 ± 22.1
Intensity	56.3 ± 25.1	51.9 ± 26.4	48.2 ± 23.1	73.5 ± 18.7	43.2 ± 24.9
Older population ($N = 32$, $f = 15$, $m = 17$)	Orange	Peppermint	Musk	Vanilla	Air
Pleasantness	70.0 ± 24.1	67.4 ± 24.9	68.2 ± 23.4	78.6 ± 20.6	51.0 ± 22.0
Calmness	60.4 ± 29.8	73.8 ± 23.8	39.7 ± 25.1	67.5 ± 17.7	17.0 ± 16.8
Intensity	55.2 ± 28.8	57.7 ± 27.7	56.3 ± 27.4	65.7 ± 21.8	39.1 ± 25.2

Table 2. Mean amplitudes (Mean ± SD; in μV) of contingent negative variation (CNV) for younger and older group at recording sites Fz, frontal, Cz, central and Pz, posterior for five different stimuli. *, **denotes younger population had significantly higher CNV amplitudes for vanilla odor at position Fz ($p = 0.03$) and Cz ($p = 0.03$), respectively

Groups	Recording site	Orange background odor	Peppermint background odor	Musk background odor	Vanilla background odor	Air
YOUNG	Position Fz	12.4 ± 10.8	13.9 ± 10.8	12.9 ± 9.4	15.1 ± 11.5*	16.4 ± 16.1
	Position Cz	15.2 ± 11.3	14.3 ± 8.5	13.8 ± 8.9	14.8 ± 9.2**	14.9 ± 11.0
	Position Pz	10.0 ± 5.7	11.6 ± 10.6	9.7 ± 7.2	8.2 ± 6.7	13.1 ± 14.4
OLD	Position Fz	12.9 ± 9.4	12.8 ± 9.3	15.0 ± 10.0	8.2 ± 9.0	15.1 ± 15.6
	Position Cz	11.8 ± 6.9	16.8 ± 18.4	14.1 ± 9.5	9.7 ± 5.4	10.6 ± 11.6
	Position Pz	8.6 ± 7.9	12.1 ± 12.0	9.8 ± 8.9	6.7 ± 3.4	11.8 ± 11.3

Table 3. Mean amplitudes (Mean ± SD; in μV) of contingent negative variation (CNV) at recording sites Fz, frontal, Cz, central and Pz, posterior for the five different stimuli. Averages are presented separately for the 5 stimuli

	CNV amplitudes [μV]				
Recording site	Orange background odor	Peppermint background odor	Musk background odor	Vanilla background odor	Air
Position Fz	10.8 ± 6.0	21.8 ± 22.3	9.8 ± 7.6	13.4 ± 4.6	11.8 ± 8.9
Position Cz	9.4 ± 3.8	16.3 ± 15.1	14.0 ± 6.4	10.3 ± 4.7	17.3 ± 10.2
Position Pz	6.0 ± 3.2	16.8 ± 23.8	8.0 ± 5.4	7.6 ± 5.2	9.0 ± 4.0

Table 4. Mean amplitudes (Mean ± SD; in μV) of the auditory stimulus S1 at recording sites Fz, frontal, Cz, central and Pz, posterior for the five different stimuli, for the peak-to-peak amplitudes N1P2

	Amplitudes N1P2 [μV] to auditory stimulus S1				
Recording site	Orange background odor	Peppermint background odor	Musk background odor	Vanilla background odor	Air
Position Fz	22.6 ± 34.8	16.7 ± 7.7	17.4 ± 10.2	16.8 ± 7.4	17.9 ± 15.7
Position Cz	19.6 ± 18.1	16.4 ± 6.7	17.4 ± 9.3	19.0 ± 7.0	16.8 ± 7.9
Position Pz	16.3 ± 13.6	13.6 ± 7.6	15.1 ± 8.5	14.3 ± 7.0	14.3 ± 7.9

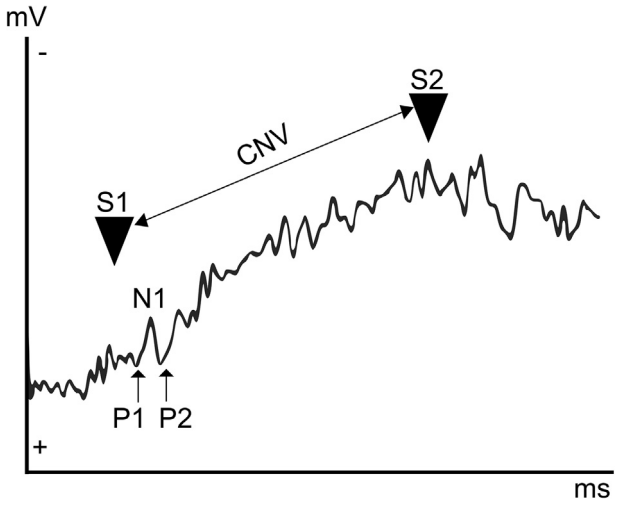

Fig. 2. Schematic drawing of the generation of CNV. CNV is elicited by an auditory stimulus, S1, which also generates an auditory event-related potential the peaks of which are measured as positivities (P1, P2) and a negativity (N1). The subjects' task is to push a button as soon as they hear S2 which elicits another auditory event-related potential. After that the S2-elcitied negativity in the EEG returns to baseline.

Ratings of odors

Intensities differed between odors ($F_{4,\ 244} = 50.4$, $p < 0.001$) which was largely due to the "air" conditions. Air was rated lower than all other odorous stimuli ($p < 0.001$). However, also musk was rated lower than other odors with the exception of air ($p < 0.01$). There were no significant effects of factors "age group" or "sex". Odor **pleasantness** was also significantly different between stimuli with air being close to neutral ($p < 0.05$). In contrast, vanillin was perceived as more pleasant compared to all other stimuli ($p < 0.01$) except for orange ($p = 0.55$). As with odor intensities there were no significant effects of factors "age group" or "sex". Finally, ratings for **calmness** different significantly between stimuli, showing a similar pattern as ratings for pleasantness. Air was rated as least associated with calmness ($p < 0.05$; no difference between air and musk) while vanillin was rated as most strongly associated with calmness ($p < 0.05$). While the factor "age" had no significant effect, there was an interaction between factors "odors and "sex" ($F_{4,\ 232} = 3.49$, $p = 0.011$). Post-hoc analysis showed that men rated peppermint high on calmness compared to women ($p = 0.04$) whereas it was the other way around for musk ($p = 0.007$) (Table 1).

The ratings of odor characteristics were found to be correlated. **Intensity** of vanillin was higher with increasing pleasantness ($r = 0.28$, $p = 0.028$) and calmness ($r = 0.41$, $p = 0.001$). Increased **pleasantness** was associated with increased calmness

for orange ($r = 0.59$), musk ($r = 0.65$), vanillin ($r = 0.61$), and air ($r = 0.51$) (all p's $p < 0.001$).

Reaction time

RT was no significantly different between the odor conditions ($F_{4,\ 224} = 1.23$, $p = 0.30$). For the condition's peppermint and vanillin, respectively, RT was positively correlated with age (peppermint: $r = 0.29$, $p = 0.028$; vanillin: ($r = 0.26$, $p = 0.049$)

CNv

CNV was seen in all participants. A total of 43 of 62 subjects (69 %) had consistent CNV for all odor conditions and at all recording positions.

In terms of the **topographical distribution** of the CNV amplitudes an analysis of variance for repeated measures (using within-subject-factors ''odor" [vanillin, musk, peppermint, orange, air] and ''channel" [Fz, Cz, Pz]) suggested that CNV amplitudes were larger for fontal and central electrode positions compared to the posterior position (factor ''channel": $F_{2,\ 80} = 8.61$, $p = 0.001$). Post-hoc testing revealed that amplitudes at Fz and Cz were higher compared to Pz ($p \leq 0.002$) (Table 2). The background **odors** did not produce significant differences in CNV (factor ''odor": $F_{4,\ 160} = 0.88$, $p = 0.48$). Also, neither ''**age**" nor ''**sex**" had significant effects in this analysis (factor ''age group": $F_{1,\ 40} = 0.57$, $p = 0.46$; factor ''sex": $F_{1,\ 40} = 0.57$, $p = 0.46$; Table 3).

Correlations: For peppermint **RT** was the faster the larger CNV amplitude at position Fz ($r = 0.46$, $p = 0.001$). Similar observations were observed for musk for amplitudes at positions Fz ($r = 0.42$, $p = 0.002$) and Pz ($r = 0.30$, $p = 0.03$). With regard to odor **pleasantness** a positive correlation was observed for orange at positions Fz ($r = 0.28$, $p = 0.048$) and Cz ($r = 0.40$, $p = 0.004$). Ratings for association of odors with **calmness** were found for orange (position Pz: $r = 0.31$, $p = 0.028$) and air (position Pz: $r = 0.29$, $p < 0.042$). There were no significant correlations between odor **intensity** and CNV amplitudes. CNV amplitudes were found to be positively related to **NEOFFI conscientiousness** score (musk odor, position Cz: $r = 0.32$, $p = 0.02$), **NEOFFI tolerance** score (air, position Pz: $r = -0.28$, $p = 0.045$), and negatively with **NEOFFI extraversion** score (peppermint odor, position Cz: $r = -0.30$, $p = 0.031$). CNV amplitudes were also found to be related to **IOQ consequence** score (orange odor, position Pz: $r = -0.30$, $p = 0.033$; peppermint odor, position Fz: $r = +0.35$, $p = 0.012$). However, we did not find any significant difference among the groups (YOUNG and OLD) when it comes to NEOFFI and IOQ scores, as summarized in Table 5.

DISCUSSION

The results of the present study suggest (Lorig et al., 1995) younger and older subjects irrespective of sex consistently demonstrate CNV Lorig and Roberts (1990) with larger fronto-central amplitudes, as described in the literature (Lorig et al., 1995), but (Manley, 1993) that there is no major difference between CNV amplitudes in relation to background odors. However, (Khan et al., 2007) for some odor conditions CNV amplitudes exhibit a weak relation to odor pleasantness and associations with calmness.

CNV amplitudes were largest at frontal and central electrodes which coincides with previous observations by Lorig et al. (1995). Still, in the present study we did not find conclusive modulatory effects of odors on the CNV. This remains contrary to the earlier findings by Torii et al. (1988) who reported that the early component of CNV is larger when a jasmine odor was presented and is lower with lavender odor. Lorig and Roberts (1990) replicated this study and found that subjective expectancies related to odors play an important role in modulating the effects of CNV. The reasons for such discrepancies may be attributed to the fact that no study till date had a really large sample size. In fact, some the study by Lorig and Roberts (1990) had a sample size of 15. Hence, careful interpretations are required when comparing previous work with the present study mostly because this of the different sample sizes.

The present study did not reveal major differences in CNV amplitude between the odor conditions. However, in some of the conditions (orange odor, air) we observed a positive correlation between calmness/pleasantness and CNV amplitudes which suggested that the CNV amplitude could serve as a surrogate marker of the perception of pleasantness (Torii et al., 1988; Lignell et al., 2008). Still, it has to be noted that this effect did not appear to be stable but was only detected with relatively weak correlative analyses.

CNV amplitudes also appeared to be related to certain personality traits, with a positive relation between CNV amplitudes and **conscientiousness** and a negative relation with **tolerance** and **extraversion**. Considering the additional correlation between CNV amplitudes and the **consequence** score which differed in sign in relation to the odor used, these results confirm that CNV is related to the subject's expectations (Satoh and Sugawara, 2003) and hence, that it may be sensitive to the modulation through odors. However, as indicated by the present results CNV amplitudes apparently are not discriminative between effects of odors which differ to some, but not an extensive degree in terms of pleasantness.

The relation between CNV amplitudes and alertness is also indicated by the positive correlation between CNV amplitude and RT (Loveless and Sanford, 1975). Still, this relation was no consistent for all odor conditions, but only for peppermint and musk - which were rated differently in terms of their association with calmness ratings (Satoh and Sugawara, 2003).

An interesting finding was that odorless air received ratings in terms of odor intensity. These ratings were low, and significantly lower than intensity ratings of all other odors. Similar observations have been made by others, e.g. (Flohr et al., 2017). However, these reports indicate that ratings of odors can be erratic which in turn

Table 5. Means scores (mean ± SD) from the NEOFFI and IOQ questionnaires for younger and older group, separately for the various subcategories of the respective questionnaires

NEOFFI Scores (mean ± SD)					
Age Group	Neuroticism	Extraversion	Openness to experience	Agreeableness	Conscientiousness
YOUNG	18.2 ± 7.5	29.0 ± 6.5	30.9 ± 5.8	31.0 ± 6.2	35.6 ± 5.9
OLD	19.1 ± 7.5	27.8 ± 8.4	29.6 ± 6.6	32.5 ± 5.1	36.2 ± 5.0
IOQ Scores (mean ± SD)					
Age Group	Association	Application	Consequence		
YOUNG	14.8 ± 2.6	15.3 ± 3.1	14.1 ± 2.3		
OLD	14.8 ± 3.6	13.3 ± 4.5	14.2 ± 3.0		

emphasizes the interest in methods that would allow a more "objective" assessment of the perception of odors, e.g., functional magnetic resonance imaging or EEG-related analyses using different paradigms (De Araujo et al., 2005; Sorokowska et al., 2016; Ruser et al., 2021).

In conclusion, the present data confirm that CNV is related to stimulus expectation and alertness and is also subject to modulation in relation to the perception of odors. Hence, the results suggest the usefulness of CNV as an electrophysiological equivalent of cognition. However, we did not find any odor modulating effect on CNV. Given the sample size and solid study design of the study, we can conclude that odors have little or no modulating effect on CNV.

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Publication 2 (Second study) Subtle Differences in Brain Architecture in Patients with Congenital Anosmia

Abstract of Publication 2

Objective: People suffering from congenital anosmia show normal brain architecture although they do not have functional sense of smell. Some studies in this regard point to the changes in secondary olfactory cortex, orbitofrontal cortex (OFC), in terms of gray matter volume increase. However, diffusion tensor imaging has not been explored so far.

Methods: We included 13 congenital anosmia subjects together with 15 controls and looked into various diffusion parameters like FA.

Results and Conclusions: Increased FA in bilateral OFC confirms the earlier studies reporting increased gray matter thickness. However, it is quite difficult to interpret FA in terms of gray matter volume. Increased FA has been seen with recovery after traumatic brain injury. Such changes in OFC point to the plastic nature of the brain.

Brain Topography
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BRIEF COMMUNICATION

Subtle Differences in Brain Architecture in Patients with Congenital Anosmia

Divesh Thaploo[1] · Charalampos Georgiopoulos[1,2] · Antje Haehner[1] · Thomas Hummel[1]

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Abstract
People suffering from congenital anosmia show normal brain architecture although they do not have functional sense of smell. Some studies in this regard point to the changes in secondary olfactory cortex, orbitofrontal cortex (OFC), in terms of gray matter volume increase. However, diffusion tensor imaging has not been explored so far. We included 13 congenital anosmia subjects together with 15 controls and looked into various diffusion parameters like FA. Increased FA in bilateral OFC confirms the earlier studies reporting increased gray matter thickness. However, it is quite difficult to interpret FA in terms of gray matter volume. Increased FA has been seen with recovery after traumatic brain injury. Such changes in OFC point to the plastic nature of the brain.

Keywords Congenital anosmia · Orbitofrontal cortex · Diffusion tensor imaging · Plasticity

Introduction

Inability to smell from birth, also known as congenital anosmia (CA), is typically associated with absence of olfactory bulb. In a recent study, the authors were astonished by the fact that two congenital anosmic females performed at par with healthy controls in terms of standard olfactory tests despite having no clear olfactory bulbs (Weiss et al. 2020). However, when it comes to odor processing, people with normal or higher sense of smell show a relationship between olfactory performance, measured by odor threshold, identification and discrimination and orbitofrontal cortex (OFC) which is highly significant in the formation of olfactory precepts (Seubert et al. 2013). Using regression models, the authors concluded that gray matter differences in OFC were responsible for variances in odor discrimination but little variances in threshold scores. In CA an increase in gray matter thickness was found to be present bilaterally in OFC and the authors concluded that it may be due to lack of synaptic pruning due to absence of peripheral sensory input (Frasnelli et al. 2013). A recent review related to brain structural changes in congenital or acquired anosmia also indicated an increased gray matter thickness within the OFC in CA while it was reduced in acquired anosmia (Manan et al. 2022). As indicate above, this increase of the gray matter in CA may be explained by the lack of input to OFC from primary olfactory areas in CA, changing the input-dependent development of the brain architecture.

To date, no study has focused on individuals with CA in terms of diffusion tensor imaging (DTI). DTI is a robust tool to investigate structural integrity where one of the measures is fractional anisotropy (FA). Higher FA values indicate more axon myelination (Osuka et al. 2012). FA has been found to be a marker of improved function in various neurodegenerative diseases and recovery from traumatic brain injury (Alba-Ferrara and de Erausquin 2013; Wallace et al. 2018). Increased cerebral myelination has been associated with increased gray matter thickness and FA, both sharing a linear correlation (Kochunov et al. 2011). However, the effect has yet not been clearly understood. The main purpose of the study was to investigate whether FA can explain the differences noted previously in OFC in CA and compare them with healthy controls.

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✉ Divesh Thaploo
taploodivesh4@gmail.com

[1] Smell & Taste Clinic, Department of Otorhinolaryngology, TU Dresden, Haus 5, Fetscherstraße 74, 01307 Dresden, Germany

[2] Department of Radiology in Linköping, and Department of Health, Medicine and Caring Sciences, Linköping University, Linköping, Sweden

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Methods

We present an investigation in 13 CA participants and 15 healthy controls using a ROI-based approach. Diffusion tensor imaging was performed using 3 T MRI scanner (Verio; Siemens Healthineers, Erlangen, Germany). An eight-channel receiver head coil was used for image data acquisition. DTI was acquired as 2D fast spin echo planar imaging with following specifications; TR = 71 ms, TE = 6 ms, Slice thickness = 2 mm, FoV = 110 × 110, repetitions = 1, flip angle = 180°. Diffusion scans were acquired at b = 0 and b = 800 with number of diffusion directions = 20. Following written informed consent, participants underwent olfactory testing with the Sniffin' Sticks battery (odor threshold, discrimination, and identification: TDI score) (Oleszkiewicz et al. 2019). Masks for piriform cortex and orbitofrontal cortex (OFC) were adapted and thresholded from two published studies (Fjaeldstad et al. 2017; Seubert et al. 2013) using FSL edit mode (FMRIB software library v6.0.2) (Jenkinson et al. 2012) to include white matter areas and manual removal of any underline gray matter, if required. These ROIs were visually inspected by expert neuroradiologists, who also helped in normalisation and outline of the ROIs. We also analysed the FA values in piriform cortex (PFC) using the same approach. Voxelwise statistical analysis of the FA data was carried out using TBSS (Tract-Based Spatial Statistics (Smith et al. 2006)), part of FSL. TBSS projects all subjects' FA data onto a mean FA tract skeleton, before applying voxelwise cross-subject statistics. Statistical analysis was carried out using SPSSv27 (Armonk, NY, USA: IBM Corp). We used Mann–Whitney U test, a non-parametric test given the sample of the study, where $r < 0.3$ represents a small effect, r between 0.3 and 0.5 medium effect and $r > 0.5$ a large effect ($r = z/\sqrt{n}$; z: standardised test statistic, n: number of samples).

Results

Thirteen CA subjects (mean age 30.6 ± 12.4 years) and 15 healthy controls (38.6 ± 11.3 years) were included in the study. The distribution of age was similar across the groups which was revealed by independent samples Mann–Whitney U test ($r = 0.36$, $p = 0.052$). On testing, CA subjects had a significantly lower TDI score ($r = 0.85$, $p = 0.001$) (12.69 ± 2.9) as compared to healthy controls (34.1 ± 3.0). Mann–Whitney U test revealed significant changes in FA values within the OFC in each hemisphere between the two groups. FA values within left and right OFC were higher ($r = 0.58$, $p = 0.002$ and $r = 0.44$, $p = 0.019$, respectively) in CA group (FA in left OFC, 0.49 ± 0.02, right OFC, 0.44 ± 0.01) as compared to healthy controls (FA in left OFC, 0.44 ± 0.01, right OFC, 0.39 ± 0.01) A graphical representation can be seen in Fig. 1. As pertaining to the analysis for PFC, we did not find significant differences between the groups ($r = 0.20$, $p = 0.37$ for left PFC and $r = 0.17$, $p = 0.38$ for right PFC).

Discussion

FA values in bilateral OFC were significantly higher in CA as compared to healthy controls. The results of the present study partly confirms an earlier study, where the authors reported higher cortical thickness bilaterally within OFC in CA subjects in terms of an increase in gray matter thickness (Frasnelli et al. 2013). Higher FA values and the increase in cortical thickness within the OFC, which is a secondary olfactory area, suggests the plastic nature of the brain (Sakai 2020). However, the exact implication of FA for gray matter thickness is still unknown. Nonetheless, some studies have observed that increased grey matter volume and higher FA may be related to neuroplasticity (Hsin et al. 2017). However, when we look into the FA values between groups, the differences may be subtle but, nonetheless, they are statistically significant. Some studies have shown absence of differences between both groups with no morphological alterations in primary olfactory cortex (Peter et al. 2020). The authors concluded that the lack of lifelong olfactory experience had no major effect on the primary olfactory cortex. However, there were some changes in OFC which may be the result of developmental processes and also due to the multimodal nature of the OFC. Also, no gray matter alterations in primary olfactory cortex, which includes the

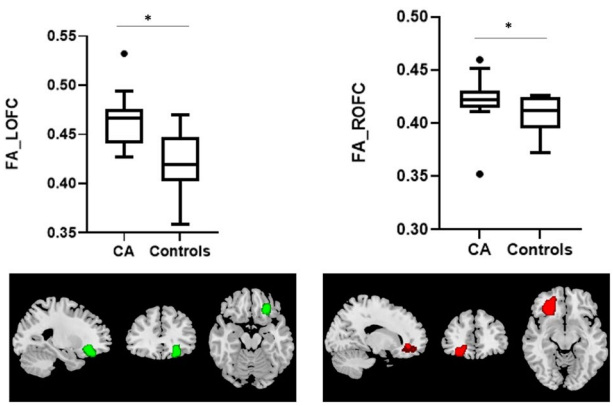

Fig. 1 The box plots for FA values in LOFC and ROFC. * denotes significant difference between two groups ($p < 0.05$). *LOFC* left orbitofrontal cortex, *ROFC* right orbitofrontal cortex, *CA* congenital anosmia subjects. Green and red colour provide the location of left and right orbitofrontal cortex, respectively in standard space

piriform cortex, have been seen in rodents. There, postnatal removal of olfactory bulb, severing inputs to primary olfactory cortex, produced little or no alterations in the thickness of the piriform cortex (Friedman and Price 1986; Westrum and Bakay 1986). A study by (Karstensen et al. 2018) on CA patients points to the loss of grey matter volume in medial OFC. However, inclusion of hyposmic patients in the CA group by the authors, could be responsible for such reduced volume in medial OFC as was observed by Yao and colleagues, where patients with hyposmia show atrophy in right orbitofrontal cortex (Yao et al. 2018). Based on the existing literature, and the present findings, we conclude that people with CA have higher FA values in OFC pointing towards the neuroplastic nature of the brain.

Conclusion

In congenital anosmia the increased FA in OFC and no changes in piriform cortex points to the plastic nature of the brain.

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Data Availability Not applicable.

Declarations

Conflict of interest All authors declare no conflict of interest.

Ethical Approval Study was conducted in compliance with local ethics committee standards of TU Dresden.

Consent to Participate All subjects gave written consent before commencing the study.

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Publication 3 (Third study) Tractography indicates lateralized differences between trigeminal and olfactory pathways

Abstract of Publication 3

Objective: Odorous sensations are based on trigeminal and olfactory perceptions. Both trigeminal and olfactory stimuli generate overlapping as well as distinctive activations in the olfactory cortex including the piriform cortex. Orbitofrontal cortex (OFC), an integrative center for all senses, is directly activated in the presence of olfactory stimulations. In contrast, the thalamus, a very important midbrain structure, is not directly activated in the presence of odors, but rather acts as a relay for portions of olfactory information between primary olfactory cortex and higher-order processing centres. The aims of the study were (1) to examine the number of streamlines between the piriform cortex and the OFC and also between the piriform cortex and the thalamus and (2) to explore potential correlations between these streamlines and trigeminal and olfactory chemosensory perceptions.

Methods: Thirty-eight healthy subjects were recruited for the study and underwent diffusion MRI using a 3T MRI scanner with 67 diffusion directions. ROIs were adapted from two studies looking into olfaction in terms of functional and structural properties of the olfactory system. The "waytotal number" was used which corresponds to number of streamlines between two regions of interests.

Results: We found the number of streamlines between the piriform cortex and the thalamus to be higher in the left hemisphere, whereas the number of streamlines between the piriform cortex and the OFC were higher in the right hemisphere. We also found streamlines between the piriform cortex and the thalamus to be positively correlated with the intensity of irritating (trigeminal) odors. On the other hand, streamlines between the piriform cortex and the OFC were correlated with the threshold scores for these trigeminal odors.

Conclusions: This is the first studying the correlations between streamlines and olfactory scores using tractography. Results suggest that different chemosensory stimuli are processed through different networks in the chemosensory system involving the thalamus.

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Tractography indicates lateralized differences between trigeminal and olfactory pathways

Divesh Thaploo[a,*], Akshita Joshi[a], Charalampos Georgiopoulos[a,b], Jonathan Warr[c], Thomas Hummel[a]

[a] Smell and Taste Clinic, Department of Otorhinolaryngology, TU Dresden, Haus 5, Fetscherstraße 74, Dresden 01307, Germany
[b] Department of Radiology in Linköping, and Department of Health, Medicine and Caring Sciences, Linköping University, Linköping, Sweden
[c] Takasago Europe, Paris, France

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ABSTRACT

Odorous sensations are based on trigeminal and olfactory perceptions. Both trigeminal and olfactory stimuli generate overlapping as well as distinctive activations in the olfactory cortex including the piriform cortex. Orbitofrontal cortex (OFC), an integrative center for all senses, is directly activated in the presence of olfactory stimulations. In contrast, the thalamus, a very important midbrain structure, is not directly activated in the presence of odors, but rather acts as a relay for portions of olfactory information between primary olfactory cortex and higher-order processing centers. The aims of the study were (1) to examine the number of streamlines between the piriform cortex and the OFC and also between the piriform cortex and the thalamus and (2) to explore potential correlations between these streamlines and trigeminal and olfactory chemosensory perceptions. Thirty-eight healthy subjects were recruited for the study and underwent diffusion MRI using a 3T MRI scanner with 67 diffusion directions. ROIs were adapted from two studies looking into olfaction in terms of functional and structural properties of the olfactory system. The "waytotal number" was used which corresponds to number of streamlines between two regions of interests. We found the number of streamlines between the piriform cortex and the thalamus to be higher in the left hemisphere, whereas the number of streamlines between the piriform cortex and the OFC were higher in the right hemisphere. We also found streamlines between the piriform cortex and the thalamus to be positively correlated with the intensity of irritating (trigeminal) odors. On the other hand, streamlines between the piriform cortex and the OFC were correlated with the threshold scores for these trigeminal odors. This is the first studying the correlations between streamlines and olfactory scores using tractography. Results suggest that different chemosensory stimuli are processed through different networks in the chemosensory system involving the thalamus.

1. Introduction

The sense of smell is one of the many senses that makes us aware of our environment. Functionally, this sense is bimodal with the trigeminal and olfactory systems working together in concert (Cain, 1974; Hummel and Livermore, 2002). Olfactory stimulants predominantly activate the piriform cortex, insular cortex, amygdala whereas chemosensory trigeminal stimuli, in addition to these areas, also activate the thalamus and substantia nigra (Joshi et al., 2021; Albrecht et al., 2010). The olfactory system is related to complex brain functions like emotions or memory (Soudry et al., 2011).

These brain functions are mediated by white matter connections between the many "olfactory areas" and higher cognitive processing centers. Such white matter fiber streamlines can be visualized using diffusion MRI (DMRI). Fiber tractography, which is derived from DMRI, is an *in-vivo* approach to visualize white matter fibers and has been used in various contexts, e.g., central and peripheral nervous system visualization, or whole brain white matter reconstruction (Basser et al., 2000; Sarwar et al., 2019). DMRI has also been used to better understand the olfactory system. One of the first studies presented the olfactory tract (OT) *in-vivo* using diffusion tensor fiber tractography (Skorpil et al., 2011). The authors visualized in five healthy controls a small white matter tract originating from the olfactory bulb, connecting to inferior surfaces of the frontal lobe. No such streamlines could be found in a patient with congenital anosmia which was seen as validation of the methodology. More recently, using a constrained spherical deconvolution (CSD) diffusion model Milardi et al. (2017) could visualize the OT directly projecting to the piriform cortex, the entorhinal cortex and the amygdala and furthermore to the orbitofrontal cortex.

* Corresponding author.
E-mail address: taploodivesh4@gmail.com (D. Thaploo).

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Fiber tracking within the olfactory system is complicated as the system is intimately connected with other brain regions, e.g., thalamus, cerebellum, and insula (Soudry et al., 2011). Another complication is that some regions that are directly involved in olfaction, such as piriform cortex or the entorhinal cortex, are structurally difficult to delineate. The piriform cortex, part of the olfactory cortex, has many projections to other brain regions such as the amygdala and hippocampus, playing a major role in converting sensory input into specific behavioral output (Chen et al., 2014; Diodato et al., 2016). On the other hand, the orbitofrontal cortex (OFC), a secondary olfactory area, is not solely dedicated to olfactory processing but is, for example, strongly involved in sensory integration (Wang et al., 2020). Whilst there is a consensus about olfactive information processing between the piriform cortex and OFC, there is less agreement about the role of the thalamus. Some authors (Shepherd, 2005) consider that the exact role of the thalamus in olfaction is not clear and that there seems to be no direct involvement of the thalamus in the connections between the piriform cortex and the OFC. In contrast, Courtiol and Wilson concluded that the thalamus serves as a crucial center for olfactory processing by forming a reciprocal connection with the OFC via the piriform cortex (Courtiol and Wilson, 2015).

In order to assess the role of the connections between the piriform cortex, thalamus and OFC, we aimed to examine white matter streamlines within the olfactory system using fiber tractography. Additionally, we explored how the processing of olfactory versus trigeminal odors (Joshi et al., 2021) impacted potential relations between these streamlines.

2. Methods

2.1. Participants

Thirty-eight healthy subjects (20 males, 18 females) were recruited to take part in the study after having provided written informed consent. The experiments were conducted according to the Helsinki declaration. The ethics committee at the medical faculty of the Technical University of Dresden approved the study design (approval number EK558122019). A detailed, structured medical history (Hummel and Welge-Lüssen, 2006) was conducted including questions regarding drinking habits, smoking, medications, or current disorders. The sample size is always debatable in functional or structural studies. Still, considering previous work (Joshi et al., 2021) a sample above 30 is thought to provide reliable results.

2.2. Olfactory testing

A normal sense of smell was ascertained using the “Sniffin’ Sticks” odor identification test with maximum score of 16. This test is based on a forced choice paradigm where subjects have to identify 16 odors at suprathreshold concentrations using flash cards with four verbal descriptors each (Oleszkiewicz et al., 2019). For olfactory activation in the MR scanner, we used four odors (provided by Takasago, Paris, France), two more trigeminally active stimuli and two more olfactory active stimuli. The trigeminal odors were peppermint (order number ABX321352) and spearmint (ABX321351A), the olfactory stimuli were cherry (ABX321603) and strawberry (ABX321354A). Prior to the administration in the MR environment each of the pure, undiluted odors was rated by the participants for their intensity and pleasantness using visual analogue scales (0–10, with 0 meaning no intensity perceived / extremely unpleasant and 10 meaning extremely intense / extremely pleasant). Threshold scores (1 to 8, with 1 meaning high threshold [relatively insensitive] and 8 meaning low threshold [very sensitive]) for each odor were also obtained from each participant using a 3-alternative forced choice task with odors presented in glass bottles using a staircase design (Hummel et al., 1997) (4 ml odor in 50 ml volume bottles with an opening of 4 cm diameter). Participants had to discriminate the odor-containing bottle from two others containing the solvent propylene glycol (Sigma-Aldrich, Deisenhofen, Germany, order number 398039). All tests were performed within a forced choice design.

The two trigeminal odors (peppermint and spearmint) did not differ statistically (t-test) in terms of threshold ($p = 0.83$), or intensity ($p = 0.45$) but differed in terms of pleasantness ($p = 0.02$). Still, peppermint and spearmint were categorized and combined together as “trigeminal odors” activating both the olfactory and trigeminal systems (Han et al., 2020; Krone et al., 2001; Joshi et al., 2021). Their trigeminal nature was characterized by lateralization tests where 20 randomized odor pairs (in two squeeze bottles) are presented to each nostrils where one bottle contained no odor whereas the other did contain an odor (Frasnelli et al., 2011). The two olfactory odors did not differ in intensity ($p = 0.09$), pleasantness ($p = 0.93$) and threshold ($p = 0.53$) and were categorized and combined together as “olfactory odors” with little or no activation of the trigeminal system (Pellegrino et al., 2017).

2.3. Image acquisition

All image data was acquired on a 3T Prisma (Siemens Healthcare, Erlangen, Germany) MRI scanner, using a 32-channel head coil. A 3D magnetization prepared gradient echo T1 image was acquired (TR = 2000 ms, TE = 1.95 s, FoV = 256 mm × 256 mm, slice thickness = 1.7 mm and voxel size of 1 × 1 × 1 mm). A diffusion weighted, generalized auto-calibrating partial parallel acquisition (GRAPPA) sequence image (TR = 6070 ms, TE = 100.8 ms, multiband acceleration factor = 2, 67 diffusion directions, b-value = 1000 s/mm^2, slice thickness = 1.7 mm, voxel size = 1.7 × 1.7 × 1.7 mm) was acquired. b0 images, no diffusion images, were also acquired in 6 directions which resulted in longer TR = 12,110 ms and did not affect tensor fitting.

2.4. Image analysis

All image analysis was carried out in FSL, a FMRIB software from the Oxford center for functional magnetic resonance imaging of the brain version 6.0.2(Jenkinson et al., 2012) more specifically, FDT (FSL diffusion toolbox) was used. A standard pipeline was used for pre-processing of the diffusion data which included head motion, eddy current correction using eddy from FDT, and followed by removal of non-brain tissue using bet. Using Quality assessment of diffusion (QUAD) we assessed quality at subject level and Study-wise quality assessment of diffusion (SQUAD) for group level, where a cut-off value for mean displacement was set at > 2 mm in terms of absolute motion and > 0.3 mm in terms of relative motion for the whole study population (Bastiani et al., 2019). No dataset had to be discarded. BedpostX was used to map out diffusion parameters at each voxel (Behrens et al., 2007).

2.5. Fiber tracking

Tractography was performed using PROBTRACKX probabilistic tracking in FDT diffusion toolbox (Behrens et al., 2007). For seed space, a single mask option was chosen with piriform cortex as the mask. Further, since the mask was not defined in diffusion space, appropriate transforms (from structural space to diffusion space) were selected as tracking is performed in diffusion space. In optional targets, waypoint mask, exclusion mask and termination mask were chosen, where the midline mask was selected as an exclusion mask (Lancaster et al., 2000). By keeping the waypoint and termination mask as the same, we make sure that all streamlines that (1) start from the piriform cortex pass through the orbitofrontal cortex and also end there or all streamlines that (2) start from piriform cortex pass through the thalamus and also end there will be counted.

ROIs were used from already published data (Fjaeldstad et al., 2017; Seubert et al., 2013). They were mapped to each subject in two steps; since the ROIs were defined in structural space or rather MNI space,

Table 1
Psychophysical data. Intensity and pleasantness ratings ranged from 0 to 20 because here the scores for olfactory and trigeminal odors were combined as mentioned in method section. Threshold scores ranged between 1 and 16. All values are presented as mean ± SD, for olfactory and trigeminal odors.

Age: 26.1 ± 3.0 years, 20 male, 18 female	Olfactory odors (Strawberry and cherry)	Trigeminal odors (Spearmint and peppermint)
Intensity (0–20)	15.0 ± 3.0	13.1 ± 3.5
Pleasantness (0–20)	10.5 ± 4.5	12.8 ± 3.1
Threshold (1–16)	12.9 ± 1.4	13.6 ± 1.6

firstly FLIRT (Jenkinson and Smith, 2001) was used to obtain a linear transformation matrix between diffusion and structural space and then FNIRT (Simpson et al., 2015) was used to obtain non-linear transformation to store results in standard space. ROIs from Seubert et al. were derived using ALE from 40 different functional olfactory studies. These authors also mentioned that they initially had insular cortex included in the mask; it was removed in order to increase functional homogeneity of the OFC and piriform cortex mask. ROIs from Fjaeldstad et al. were superimposed. The ROIs from Fjaeldstad have been derived using functional and structural connectivity patterns from 16 healthy participants and include others olfactory related regions as well. Superimposing here means to overlay masks from Seubert et al. over the mask from Fjaeldstad et al., considering the latter as standard and keeping the congruent areas. We used this approach for reducing false positives in tracking which might have occurred if we had used a larger mask instead. Each mask was then reviewed by an expert neuroradiologist (CG). The mask for the OFC included only the central part. Thalamus, as a mask, was chosen from Harvard-Oxford subcortical structural atlas (Frazier et al., 2005). This atlas does not divide the thalamus based on its nuclei because this atlas is largely taken from functional studies. We also thresholded the mask. All ROIs were thresholded using fslmaths (Lancaster et al., 2000). For the purpose of calculating the streamlines between ROIs, the waytotal number was used. The waytotal number was derived as one of the metrics while running probtrackX, and it infers the total number of streamlines for all seed voxels (piriform cortex) which have not been rejected. The volume of the tract was not computed. Simple tracking was performed from piriform cortex to orbitofrontal cortex or Thalamus and the waytotal number was used to determine how many streamlines made it to the termination mask.

2.6. Statistics

We used non-parametric Kruskal-Wallis ANOVA independent samples test with Mann-Whitney U test for pairwise comparisons using Bonferroni corrections. We ran the test for side (left and right) and track pathway. We also ran partial correlations between track pathways and identification scores, threshold scores, intensity, and pleasantness ratings. A p-value < 0.05 was considered to be significant. All statistical analysis was performed using SPSS v27.0 (IBM Corporation, Armonk, NY, USA).

3. Results

3.1. Psychophysical data

The mean age for the whole population was 26.1 ± 3.0 years (Table 1). Participants had an average identification score of 13.6 ± 1.4 (mean ±SD) indicating normal olfactory function. Psychophysical data including intensity, pleasantness and threshold scores are summarized in Table 1. No gender-related differences were found.

3.2. Tracking

3.2.1. Comparison between streamlines piriform-OFC vs. piriform-thalamus

In the left hemisphere streamlines between piriform cortex and thalamus were significantly more in comparison to streamlines between piriform cortex and OFC (X^2 (df 1) = 8.52, $p = 0.004$). However, in the right hemisphere, streamlines between piriform cortex and OFC were more in comparison to streamlines between piriform cortex and thalamus (X^2 (df 1) = 6.5, $p = 0.01$) (Fig. 1).

3.2.2. Comparison between hemispheres

Lateralized differences reveal that streamlines were higher between piriform cortex and OFC in the right hemisphere (X^2 (df 1) = 11.8, $p = 0.001$). However, no lateralized differences in streamlines between piriform cortex and thalamus could be found (X^2(df 1) = 3.5, $p = 0.06$) The graphical representation of the outputs from the tractography analysis are summarized in Figure 2.

3.3. Correlations between results from tractography and stimulus intensity/thresholds

A partial correlation analysis, controlling for age, revealed that streamlines between both left and right piriform cortex to thalamus exhibited positive correlations with intensity ratings for trigeminal odors ($r = 0.40$, $p = 0.01$, and $r = 0.33$, $p = 0.03$ respectively). A positive correlation between the number of streamlines (piriform cortex to OFC) and threshold score for trigeminal odors ($r = 0.40$, $p = 0.01$) were also seen. However, no such correlation was seen for olfactory odors with any of the streamlines (Fig. 3 and Table 2).

4. Discussion

Using probabilistic tractography *in vivo*, the probability map of the olfactory system in humans was illustrated where white matter streamlines could be visualized from the piriform cortex to the OFC and also from the piriform cortex to the thalamus. The main findings were (1) a significantly higher number of streamlines between the piriform cortex and the thalamus in the left hemisphere and (2) a higher number of streamlines between the piriform cortex and the OFC in the right hemisphere. Moreover, (3) the streamlines between the piriform cortex and the OFC were more prominent in the right hemisphere. Last, but not least, (4) we found that the number of streamlines between the piriform cortex and the thalamus positively correlated with the perceived intensity and streamlines between the piriform cortex and the OFC with threshold of trigeminal odors.

The present tractography analysis showed the presence of significantly higher streamlines in the right hemisphere. This is in line with previous research (Zatorre et al., 1992; Royet et al., 2001) where authors noted higher neural activity in the right OFC in response to passive odor stimulation. Positron emission tomography based studies (Royet et al., 2001, 1999) found that odor judgements based on familiarity activate the right OFC, whereas hedonic judgements activate the left OFC. In contrast, a study based on the above mentioned assumptions showed that odor familiarity specifically invokes piriform cortex and brain areas like, entorhinal cortex, amygdala, inferior gyrus, possibly through a thalamic relay, among other possibilities (Plailly et al., 2005).

The role of the thalamus is debated when it comes to olfaction. The mediodorsal thalamic nuclei (MDT) act as an important relay in olfaction, and that piriform cortex has been shown to have excitatory projections to MDT nuclei in rats (Courtiol and Wilson, 2015; Cornwall and Phillipson, 1988). A comprehensive review of the literature suggests that

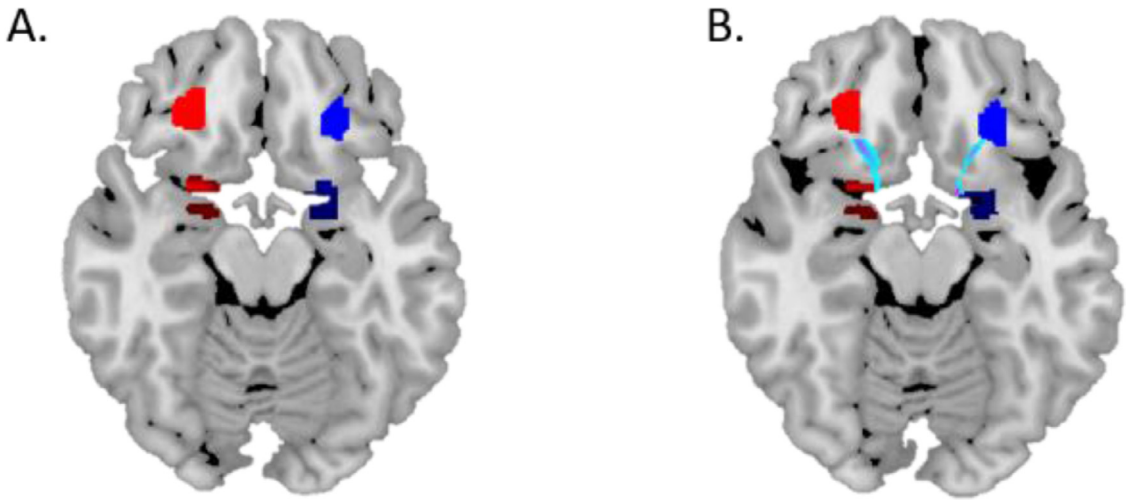

Fig. 1. Represents the ROIs for olfactory cortex and the tractography outputs. A represents the olfactory cortex masks, namely left piriform cortex and OFC (blue) and right piriform cortex and OFC (red). B represents streamlines (light-blue) between piriform cortex and OFC using probabilistic tractography. ROIs; region of interests, OFC; orbitofrontal cortex.

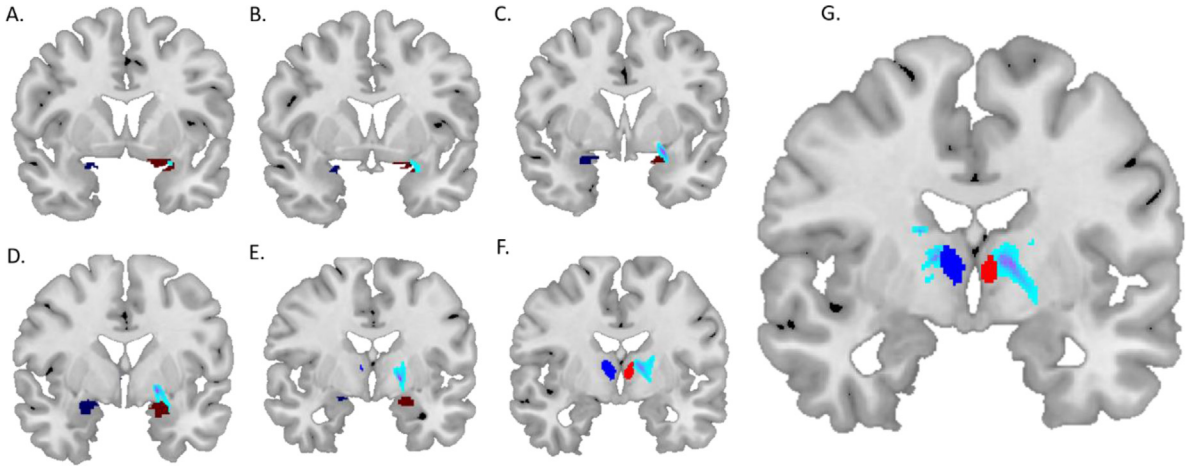

Fig. 2. Represents the track convergence from piriform cortex to thalamus represented from A to F.G represents bilateral streamlines (light-blue) between piriform cortex and thalamus using probabilistic tractography. Left thalamus is represented by red color and right thalamus by blue color.

Table 2
Tractography results in terms of number of streamlines. Brain hemisphere represents which side tracking was performed and tracking direction represents the ROIs used as initiation and termination masks.

Side (Brain hemisphere)	Tracking direction	Number of tracts (Mean ± SD)	p-value (for significance p<0.05)
Left	Piriform cortex OFC	1389 ± 2807.7	0.004
	Piriform cortex — Thalamus	4215 ± 6379.6	0.001
Right	Piriform cortex — OFC	7356 ± 11421.8	0.01
	Piriform cortex Thalamus	2420 ± 5188.4	

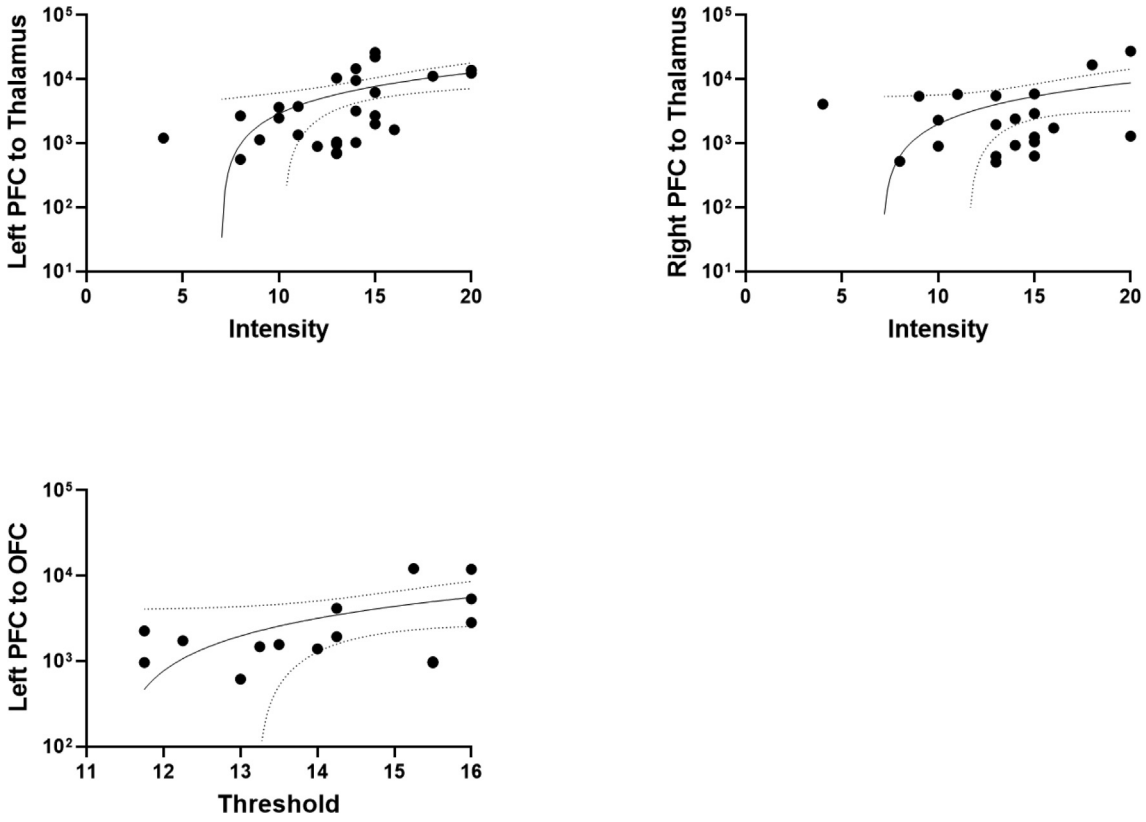

Fig. 3. Partial correlation analysis. The top row represents positive correlations between intensity score for trigeminal odors and left and right streamlines between PFC and Thalamus, respectively. The bottom row represents positive correlations between threshold scores for trigeminal odors and left streamlines between PFC and OFC path. PFC, piriform cortex, OFC, orbitofrontal cortex.

the orbitofrontal cortex has close relations with the thalamus. However, when it comes to connections between the piriform cortex and the thalamus, not much is known (Soudry et al., 2011). It may be that the thalamus functions as a relay center providing a link between olfactory processing streams from various brain areas, similar to the amygdala, the nucleus accumbens, the anterior cingulate or the somatosensory system (Courtiol and Wilson, 2015) or as in rats, that there is a direct connection between the piriform cortex and the thalamus. Hence, for example valence of odor may be partly generated through these multi-synaptic connections between the thalamus and olfactory eloquent structures like the piriform cortex. However, in the present study we did not find any such correlations for olfactory odors (cherry & strawberry). In contrast, trigeminal odors (peppermint & spearmint) being somatosensory stimuli have been shown to produce robust activations in the thalamus (Frasnelli et al., 2011; Hummel and Frasnelli, 2019; Pellegrino et al., 2017). This duality of the stimulation may also explain the observed correlation between streamlines between the piriform cortex and the thalamus and intensity ratings for trigeminal odors but not for olfactory odors.

Correlation analysis revealed that the trigeminal odor threshold scores had positive correlations with the streamlines between piriform cortex and OFC whereas intensity scores for trigeminal odors had positive correlations with streamlines between the piriform cortex and the thalamus. Odor threshold scores are based on a simple 3-alternative forced choice olfactory task which does not involve major cognitive functions. This is consistent with previous studies where authors found performance in threshold tests to be unrelated to cognitive factors (Hedner et al., 2010). This is also in accordance with the previous literature which suggests strong involvement of the OFC in odor perception (Zald et al., 2002). Olfactory tasks with higher cognitive load like intensity ratings, verbal identification tasks, or pleasantness ratings activate secondary olfactory areas, like amygdala, hippocampus, anterior cingulate cortex and thalamus (Soudry et al., 2011). In the present study intensity scores were found to be positively correlated to streamlines between the piriform cortex and the thalamus possibly indicating that the processing of this information requires a higher degree of networking. The bimodal nature of the “trigeminal odors” may lead to a higher degree of memorization, because of their activation of two sensory system (Joshi et al., 2021; Pellegrino et al., 2017; Livermore et al., 1992). A practical consequence of that may be, for example, the high identification rates typically found for the bimodal trigeminal/olfactory peppermint in odor identification tests compared to the lower rates of identification typically found for the less trigeminally active stimuli cinnamon or pineapple (Hummel et al., 1997; Doty et al., 1984). Probabilistic tractography can be hampered by false-positives (Sarwar et al., 2019). A recent review pointed out that tractography may be more accurate with combinatorial strategies (Schilling et al., 2019). Although the present tractography findings may not be the exact representation of the overall streamlines between ROIs, the results were consistent within the presently investigated sample.

Moreover, a careful selection of ROIs, by taking into account the presence of white matter areas near them, makes it a powerful data-driven approach.

5. Conclusion

Using probabilistic tractography more streamlines were found between the piriform cortex and the thalamus in the left hemisphere suggesting a direct connection between the two ROIs. A positive correlation with intensity ratings for trigeminal odors appeared to reflect the role of the thalamus in mediating attention towards trigeminal properties of bimodal odors. There were also more streamlines between the piri-

form cortex and the OFC in the right hemisphere which largely confirms that the two structures are well connected. The positive correlation with threshold scores for trigeminal odors suggested the involvement of primary and secondary cortices in simple olfactory tasks. It would be interesting to see how such an analysis could be utilized in patients with olfactory dysfunction.

Data availability statement

The data that support the findings of this study are not publicly available. However, the authors will share them by request from any qualified investigator after completion of a data sharing agreement.

Declaration of Competing Interest

The authors declare no competing financial interests.

Credit authorship contribution statement

Divesh Thaploo: Formal analysis. **Akshita Joshi:** Formal analysis, Writing – review & editing. **Charalampos Georgiopoulos:** Formal analysis, Writing – review & editing. **Jonathan Warr:** Visualization, Project administration, Writing – review & editing. **Thomas Hummel:** Writing – review & editing, Methodology, Project administration, Supervision.

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Discussion and Outlook

In our first study (Publication 1), younger and older subjects irrespective of sex consistently demonstrate CNV (Lorig and Roberts, 1990). CNV amplitudes were largest at frontal and central electrodes which coincides with previous observations by Lorig et al. (1995). Still, in the study we did not find conclusive modulatory effects of odors on the CNV. This remains contrary to the earlier findings by Torii et al. (1988) who reported that the early component of CNV is larger when a jasmine odor was presented and is lower with lavender odor. Lorig and Roberts (1990) replicated this study and found that subjective expectancies related to odors play an important role in modulating the effects of CNV. The reasons for such discrepancies may be attributed to the fact that no study till date had a really large sample size. In fact, some the study by Lorig and Roberts (1990) had a sample size of 15. Hence, careful interpretations are required when comparing previous work with the present study mostly because this of the different sample sizes. The study did not reveal major differences in CNV amplitude between the odor conditions. However, in some of the conditions (orange odor, air) we observed a positive correlation between calmness/pleasantness and CNV amplitudes which suggested that the CNV amplitude could serve as a surrogate marker of the perception of pleasantness (Torii et al., 1988; Lignell et al., 2008). Still, it has to be noted that this effect did not appear to be stable but was only detected with relatively weak correlative analyses. CNV amplitudes also appeared to be related to certain personality traits, with a positive relation between CNV amplitudes and conscientiousness and a negative relation with tolerance and extraversion. Considering the additional correlation between CNV amplitudes and the consequence score which differed in sign in relation to the odor used, these results confirm that CNV is related to the subject's expectations (Satoh and Sugawara, 2003) and hence, that it is sensitive to the modulation through odors. However, as indicated by the present results CNV amplitudes apparently do not discriminate between effects of odors which differ to some, but not an extensive degree in terms of pleasantness. The relation between CNV amplitudes and alertness is also indicated by the positive correlation between CNV amplitude and RT (Loveless and Sanford, 1975). Still, this relation was not consistent for all odor conditions, but only for peppermint and musk - which were rated differently in terms of their association with calmness (Satoh and Sugawara, 2003).

An interesting finding was that odorless air received ratings in terms of odor intensity. These ratings were low, and significantly lower than intensity ratings of all other odors. Similar observations have been made by others, e.g. (Flohr et al., 2017). However, these reports indicate that ratings of odors can be erratic which in turn emphasizes the interest in methods that would allow a more ‘‘objective” assessment of the perception of odors, e.g., functional magnetic resonance imaging or EEG related analyses using different paradigms (De Araujo et

al., 2005; Sorokowska et al., 2016; Ruser et al., 2021). In conclusion, the data confirm that CNV is related to stimulus expectation and alertness and is also subject to modulation in relation to the perception of odors. Hence, the results suggest the usefulness of CNV as an electrophysiological equivalent of cognition. However, we did not find any odor modulating effect on CNV. Given the sample size and solid study design of the study, we can conclude that odors have little modulating effect on CNV.

In our second study (Publication 2), FA values in bilateral OFC were significantly higher in CA as compared to healthy controls. The results of the study partly confirm an earlier study, where the authors reported higher cortical thickness bilaterally within OFC in CA subjects in terms of an increase in gray matter thickness (Frasnelli et al. 2013). Higher FA values and the increase in cortical thickness within the OFC, which is a secondary olfactory area, suggests the plastic nature of the brain (Sakai 2020). However, the exact implication of FA for gray matter thickness is still unknown. Nonetheless, some studies have observed that increased grey matter volume and higher FA may be related to neuroplasticity (Hsin et al. 2017). However, when we look into the FA values between groups, the differences may be subtle but, nonetheless, they are statistically significant. Some studies have shown absence of differences between both groups with no morphological alterations in primary olfactory cortex (Peter et al. 2020). The authors concluded that the lack of lifelong olfactory experience had no major effect on the primary olfactory cortex. However, there were some changes in OFC which may be the result of developmental processes and also due to the multimodal nature of the OFC. Also, no gray matter alterations in primary olfactory cortex, which includes the piriform cortex, have been seen in rodents. There, postnatal removal of olfactory bulb, severing inputs to primary olfactory cortex, produced little or no alterations in the thickness of the piriform cortex (Friedman and Price 1986; Westrum and Bakay 1986). A study by (Karstensen et al. 2018) on CA patients points to the loss of grey matter volume in medial OFC. However, inclusion of hyposmic patients in the CA group by the authors, could be responsible for such reduced volume in medial OFC as was observed by Yao and colleagues, where patients with hyposmia show atrophy in right orbitofrontal cortex (Yao et al. 2018). Based on the existing literature, and the findings, we conclude that people with CA have higher FA values in OFC pointing towards the neuroplastic nature of the brain. In congenital anosmia the increased FA in OFC and no changes in piriform cortex points to the plastic nature of the brain.

In our third study (Publication 3), using probabilistic tractography in vivo, the probability map of the olfactory system in humans where white matter stream- lines could be visualized from the piriform cortex to the OFC and also from the piriform cortex to the thalamus. The main findings were (1) a significantly higher number of streamlines between the piriform cortex and the

thalamus in the left hemisphere and (2) a higher number of streamlines between the piriform cortex and the OFC in the right hemi- sphere. Moreover, (3) the streamlines between the piriform cortex and the OFC were more prominent in the right hemisphere. Last, but not least, (4) we found that the number of streamlines between the piriform cortex and the thalamus positively correlated with the perceived intensity. The tractography analysis showed the presence of significantly higher streamlines in the right hemisphere. This is in line with previous research (Zatorre et al., 1992; Royet et al., 2001) where authors noted higher neural activity in the right OFC in response to passive odor stimulation. Early positron emission tomography-based studies (Royet et al., 2001, 1999) found that odor judgements based on familiarity activate the right OFC, whereas hedonic judgements activate the left OFC. In contrast, a study based on the above-mentioned assumptions showed that odor familiarity specifically invokes piriform cortex and brain areas like, entorhinal cortex, amygdala, inferior gyrus, possibly through a thalamic relay (Plailly et al., 2005).

The role of the thalamus is debated when it comes to olfaction. The mediodorsal thalamic nuclei (MDT) act as an important relay in olfaction, and that piriform cortex has been shown to have excitatory projections to MDT nuclei in rats (Courtiol and Wilson, 2015; Cornwall and Phillipson, 1988). A comprehensive review of the literature suggests that the orbitofrontal cortex has close relations with the thalamus. However, when it comes to connections between the piriform cortex and the thalamus, not much is known (Soudry et al., 2011). It may be that the thalamus functions as a relay center providing a link between olfactory processing streams from various brain areas, similar to the amygdala, the nucleus accumbens, the anterior cingulate or the somatosensory system (Courtiol and Wilson, 2015) or as in rats, that there is a direct connection between the piriform cortex and the thalamus. Hence, for example valence of odor may be partly generated through these multi-synaptic connections between the thalamus and olfactory eloquent structures like the piriform cortex. However, in the present study we did not find any such correlations for olfactory odors (cherry & strawberry). In contrast, trigeminal odors (peppermint & spearmint) being somatosensory stimuli have been shown to produce robust activations in the thalamus (Frasnelli et al., 2011; Hummel and Frasnelli, 2019; Pellegrino et al., 2017). This duality of the stimulation may also explain the observed correlation between streamlines between the piriform cortex and the thalamus and intensity ratings for trigeminal odors but not for olfactory odors.

Correlation analysis revealed that the trigeminal odor threshold scores had positive correlations with the streamlines between piriform cortex and OFC whereas intensity scores for trigeminal odors had positive correlations with streamlines between the piriform cortex and the thalamus. Odor threshold scores are based on a simple 3-alternative forced choice olfactory task which

does not involve major cognitive functions. This is consistent with previous studies where authors found performance in threshold tests to be unrelated to cognitive factors (Hedner et al., 2010). This is also in accordance with the previous literature which suggests strong involvement of the OFC in odor perception (Zald et al., 2002). Olfactory tasks with higher cognitive load like intensity ratings, verbal identification tasks, or pleasantness ratings activate secondary olfactory areas, like amygdala, hippocampus, anterior cingulate cortex and thalamus (Soudry et al., 2011). In the present study intensity scores were found to be positively correlated to streamlines be- tween the piriform cortex and the thalamus possibly indicating that the processing of this information requires a higher degree of networking. The bimodal nature of the "trigeminal odors" may lead to a higher degree of memorization, because of their activation of two sensory system (Joshi et al., 2021; Pellegrino et al., 2017; Livermore et al., 1992). A practical consequence of that may be, for example, the high identification rates typically found for the bimodal trigeminal/olfactory pepper- mint in odor identification tests compared to the lower rates of identification typically found for the less trigeminally active stimuli cinnamon or pineapple (Hummel et al., 1997; Doty et al., 1984). Probabilistic tractography can be hampered by false-positives (Sarwar et al., 2019). A recent review pointed out that tractography may be more accurate with combinatorial strategies (Schilling et al., 2019).

Although the present tractography findings may not be the exact representation of the overall streamlines between ROIs, the results were consistent within the presently investigated sample. Moreover, a careful selection of ROIs, by taking into account the presence of white matter areas near them, makes it a powerful data- driven approach. Using probabilistic tractography more streamlines were found be- tween the piriform cortex and the thalamus in the left hemisphere suggesting a direct connection between the two ROIs. A positive correlation with intensity ratings for trigeminal odors appeared to reflect the role of the thalamus in mediating attention towards trigeminal properties of bimodal odors. There were also more streamlines between the piriform cortex and the OFC in the right hemisphere which largely confirms that the two structures are well connected. The positive correlation with threshold scores for trigeminal odors suggested the involvement of primary and secondary cortices in simple olfactory tasks. It would be interesting to see how such an analysis could be utilized in patients with olfactory dysfunction.

Summary in German

Hintergrund

Die Forschung auf dem Gebiet des Geruchssinns ist vielfältig, wobei sich Studien u. a. mit Semantik, Gehirnaktivierung oder verzerrtem Geruch beschäftigen. Störungen des Geruchssinns können zu einer verminderten Lebensqualität, schlechter Ernährung, sexuellen und/oder psychischen Funktionsstörungen führen. Vor allem bei der Untersuchung der Auswirkungen von Riechstörungen ist es nicht nur wichtig, eine subjektive Bewertung des Riechvermögens einzubeziehen, sondern es sollten auch immer objektivierende Messungen vorgenommen werden. Der Einsatz von EEG und fMRI ist recht gut untersucht worden. Allerdings spielt der Analyseansatz eine entscheidende Rolle bei der Interpretation der Daten. Ich habe mich in meiner Arbeit auf die Verwendung neuerer oder aktualisierter Verarbeitungsmöglichkeiten konzentriert, um das Riechvermögen besser zu verstehen.

Hypothese/Fragestellung

In Publikation 1 stellten wir die Hypothese auf, dass die kontingente negative Variation (CNV) bei jüngeren und älteren Erwachsenen durch Geruchsvorlieben moduliert werden kann. Obwohl die Geruchsempfindlichkeit mit der Wahrnehmung von Gerüchen zusammenhängt, ist es schwierig, die geruchsinduzierte Empfindlichkeit zu quantifizieren. Ziel der Studie war es, den Zusammenhang zwischen der Valenz von Gerüchen und der CNV zu untersuchen. Wir verwendeten Gerüche, die typischerweise als angenehm empfunden werden, aber dennoch verschiedene Bereiche des „Geruchsraums“ abdecken, nämlich Pfefferminze, Vanille, Moschus und Orange. Da jüngere Probanden im Allgemeinen einen besseren Geruchssinn haben als die ältere Bevölkerung, stellten wir die Hypothese auf, dass sich dies in der Modulation der CNV widerspiegeln würde, die bei jüngeren Probanden stärker ausgeprägt sein sollte als bei älteren.

In Publikation 2 konzentrierten wir uns auf Personen mit kongenitaler Anosmie (CA) im Hinblick auf die Diffusions-Tensor-Bildgebung (DTI). DTI ist ein robustes Instrument zur Untersuchung der strukturellen Integrität des Gehirns, wobei eine der Messgrößen die fraktionale Anisotropie (FA) ist. Höhere FA-Werte weisen auf eine stärkere Myelinisierung der Axone hin. FA hat sich als Marker für eine verbesserte Funktion bei verschiedenen neurodegenerativen Erkrankungen und bei der Genesung nach traumatischen Hirnverletzungen erwiesen. Eine verstärkte zerebrale Myelinisierung wurde mit einer Zunahme der Dicke der grauen Substanz und der FA in Verbindung gebracht, die eine lineare Korrelation aufweisen. Das Hauptziel der Studie war es, zu untersuchen, ob die FA die zuvor festgestellten Unterschiede im orbitofrontalen Kortex (OFC) bei CA zeigen kann. Die Ergebnisse bei Teilnehmern mit CA sollte mit riech-gesunden Kontrollpersonen verglichen werden.

In Publikation 3 untersuchten wir die Verarbeitung von trigeminalen und olfaktorischen Wahrnehmungen. Sowohl trigeminale als auch olfaktorische Reize erzeugen getrennte aber größtenteils überlappende Aktivierungen im olfaktorischen Kortex, wie auch im piriformen Kortex. Der OFC, ein integratives Zentrum für alle Sinne, wird durch Geruchsreize direkt aktiviert. Im Gegensatz dazu wird der Thalamus, eine sehr wichtige Mittelhirnstruktur, in Gegenwart von Gerüchen nicht direkt aktiviert, sondern fungiert eher als Relais für Teile der Geruchsinformationen zwischen dem primären olfaktorischen Kortex und den Verarbeitungszentren höherer Ordnung. Ziel der Studie war es, (1) die Anzahl der Stromlinien zwischen dem piriformen Kortex und dem OFC sowie zwischen dem piriformen Kortex und dem Thalamus zu untersuchen und (2) mögliche Korrelationen zwischen diesen Vernbindungen und trigeminalen und olfaktorischen chemosensorischen Wahrnehmungen zu untersuchen.

Materialien und Methoden

Für die Veröffentlichung 1 haben wir achtzig Probanden untersucht. Für die endgültige Analyse wurden jedoch nur 62 Probanden verwendet. Wir teilten die Probanden in zwei Gruppen ein, JUNG (n=30) und ALT (n=32). Das Experiment wurde an zwei aufeinanderfolgenden Tagen durchgeführt. Am ersten Tag wurde ein Vortest durchgeführt, der u.a. Fragebögen zur Gesundheit und zur Persönlichkeit umfasste. Am zweiten Tag wurden bei beiden Gruppen EEG-Aufzeichnungen durchgeführt, während ihnen Pfefferminz-, Vanille-, Moschus- und Orangengeruch präsentiert wurde. Nach der olfaktorischen Stimulation wurden die Probanden nach der Intensität und der Annehmlichkeit befragt.

In Publikation 2 führten wir bei 13 CA- und 15 gesunden Kontrollpersonen eine auf der Region of Interest (ROI) basierende Analyse durch. Die diffusionsgewichtete Bildgebung wurde mit einem 3T-Scanner durchgeführt. Wir verwendeten bilaterale Masken für den piriformen Kortex und den orbitofrontalen Kortex aus früheren Studien. Mit TBSS verglichen wir die FA-Werte in beiden Gruppen für jede ROI.

In Publikation 3 wurden 38 gesunde Probanden mit normalem Geruchssinn (20 Männer, 18 Frauen) für die Studie rekrutiert. Wir führten DWI mit einem 3T-MRT Gerät durch. Wir führten eine probabilistische Traktographie zwischen dem piriformen Kortex, dem OFC und dem Thalamus durch, was Stromlinien ergab. Zusätzlich wurden den Probanden im Rahmen desselben Experiments zwei trigeminale und zwei olfaktorische Gerüche präsentiert. Die Stromlinien der weißen Substanz wurden mit den Bewertungen der Intensität und der Annehmlichkeit korreliert.

Ergebnisse

In Publikation 1 wiesen von 62 Probanden 43 eine konsistente CNV für alle Aufnahmeorte und Gerüche auf. Die topographische Verteilung der CNV-Amplituden und eine Varianzanalyse für wiederholte Messungen (unter Verwendung der Faktoren "Geruch" [Vanillin, Moschus, Pfefferminze, Orange, Luft] und "Kanal" [Fz, Cz, Pz]) ergaben, dass die CNV-Amplituden an den frontalen und zentralen Elektrodenpositionen größer waren als an der parietalen Position (Faktor "Kanal": $F2, 80 = 8,61$, $p = 0,001$). Post-hoc-Tests ergaben, dass die Amplituden bei Fz und Cz höher waren als bei Pz ($p < 0,002$).

In Publikation 2 zeigte der Mann-Whitney-U-Test signifikante Veränderungen der FA-Werte innerhalb des OFC in jeder Hemisphäre zwischen den beiden Gruppen. Die FA-Werte in der linken und rechten OFC waren höher ($r = 0,58$, $p = 0,002$ bzw. $r = 0,44$, $p = 0,019$) in der CA-Gruppe (FA im linken OFC, 0,49 ± 0,02, rechten OFC, 0,44 ± 0,01) im Vergleich zu den gesunden Kontrollen (FA im linken OFC, 0,44 ± 0,01, rechten OFC, 0,39 ± 0,01). Was die Analyse des PFC betrifft, so fanden wir keine signifikanten Unterschiede zwischen den Gruppen ($r = 0,20$, $p = 0,37$ für den linken PFC und $r = 0,17$, $p = 0,38$ für den rechten PFC).

In Publikation 3 konnten wir zeigenn, dass es in der linken Hemisphäre mehr Stromlinien zwischen piriformem Kortex und Thalamus als zwischen piriformem Kortex und OFC gibt (χ^2 (df 1) = 8,52, $p = 0,004$). Im Gegensatz dazu wurden in der rechten Hemisphäre mehr Stromlinien zwischen dem piriformen Kortex und dem OFC beobachtet (χ^2 (df 1) = 6,5, $p = 0,01$). Eine partielle Korrelationsanalyse unter Kontrolle des Alters ergab, dass die Stromlinien zwischen linkem und rechtem piriformem Kortex und Thalamus positiv mit den Intensitätsbewertungen für trigeminale Gerüche korrelierten ($r = 0,40$, $p = 0,01$, bzw. $r = 0,33$, $p = 0,03$). Es wurde auch eine positive Korrelation zwischen der Anzahl der Stromlinien (piriformer Kortex zu OFC) und der Schwellenbewertung für trigeminale Gerüche ($r = 0,40$, $p = 0,01$) festgestellt. Für olfaktorische Gerüche wurde jedoch keine derartige Korrelation mit einer der Stromlinien festgestellt.

Schlußfolgerungen

Wir fanden keine Hinweise darauf, dass CNV eindeutig und stark durch Geruchsreize moduliert wird (Publiaktion 1) - obwohl die Mehrheit der Stichprobe eine CNV zeigte. Diese Ergebnisse deuten darauf hin, dass die CNV einen sehr engen Anwendungsbereich hat, der eher im experimentellen als im klinischen Bereich liegt. Im Gegensatz dazu werden Techniken wie Traktographie und TBSS in der klinischen Praxis nicht eingesetzt. Sie können jedoch einzigartige Einblicke in die olfaktorische Dysfunktion bieten. In Publikation 2 wurde bei

anosmischen Patienten mit TBSS eine erhöhte Wasserdiffusion in Korrelation zur Riechfunktion festgestellt, was auf eine verstärkte Myelinisierung im OFC schließen lässt. Frühere Untersuchungen hatten bereits gezeigt, dass das Volumen der grauen Substanz im OFC bei angeborenen Anosmikern zunimmt. In ähnlicher Weise wurden in Publikation 3 mit Hilfe der probabilistischen Traktographie Stromlinien der weißen Substanz zwischen primären und sekundären Riechbereichen festgestellt und Korrelationen zur Geruchsschwelle und Geruchsidentifikation gefunden. Die Korrelation von Stromlinien mit der Riechfunktion eröffnet neue Wege bei der klinischen Interpretation von Riechverlusten und begründet weitere Forschungen. Die vorliegenden Studien deuten darauf hin, dass Konnektivitätsstudien auf der Grundlage der MRT zu einem besseren Verständnis der olfaktorischen Dysfunktion beitragen können. Es ist zu erwarten, dass sie nach und nach Eingang in die klinische Routine finden werden.

Summary in English

Background

Research in olfaction is been quite diverse, for example with studies on semantics, brain activations, or distorted smells. Olfactory dysfunction can lead to reduced quality of life, poor dietary habits, sexual and/or mental dysfunctions. Especially in terms of the investigation of olfactory loss it is not only important to assess olfactory function with ratings subjectively assess but more objective measures should be considered. Use of EEG and fMRI has been quite well studied. I have focused my thesis on the use of newer or updated use of existing processing pipelines in order to understand olfaction in a better way.

Hypothesis/Question

In Publication 1, we hypothesised that odor pleasantness can be modulated using contingent negative variation (CNV) among younger and older adults. Although odor pleasantness is intimately related to the perception of odors it is difficult to quantify odor induced pleasantness. Aim of the present study was to investigate the association between the valence of odors and CNV. We used odors that are typically rated as pleasant but still cover different areas of the "olfactory space", namely peppermint, vanilla, musk, and orange. Because younger subjects generally have a better sense of smell compared to the older population, we hypothesized that this would be reflected in the modulation of CNV which should be more pronounced in younger compared to older subjects.

In Publication 2, we focused on individuals with congenital anosmia (CA) in terms of diffusion tensor imaging (DTI). DTI is a robust tool to investigate structural integrity where one of the measures is fractional anisotropy (FA). Higher FA values indicate more axon myelination. FA has been found to be a marker of improved function in various neurodegenerative diseases and recovery from traumatic brain injury. Increased cerebral myelination has been associated with increased gray matter thickness and FA, both sharing a linear correlation. However, the effect has yet not been clearly understood. The main purpose of the study was to investigate whether FA can explain the differences noted previously in orbitofrontal cortex OFC in CA and compare them with healthy controls.

In Publication 3, Odorous sensations are based on trigeminal and olfactory perceptions. Both trigeminal and olfactory stimuli generate overlapping as well as distinctive activations in the olfactory cortex including the piriform cortex. Orbitofrontal cortex (OFC), an integrative center for all senses, is directly activated in the presence of olfactory stimulations. In contrast, the thalamus, a very important midbrain structure, is not directly activated in the presence of odors,

but rather acts as a relay for portions of olfactory information between primary olfactory cortex and higher-order processing centres. The aims of the study were (1) to examine the number of streamlines be- tween the piriform cortex and the OFC and also between the piriform cortex and the thalamus and (2) to explore potential correlations between these streamlines and trigeminal and olfactory chemosensory perceptions.

Materials and Methods

In publication 1, we recruited eighty subjects. But for final analysis 62 subjects were used. we divided the subjects into two groups, YOUNG (n=30) and OLD(n=32). The experiment was conducted on two consecutive days. On day one, pre-testing was done which included health screening questionaries, personality related questionnaires, among others. On day two, EEG recordings on both the groups while they were presented with peppermint, vanilla, musk and orange odors was performed. After reach olfactory stimulation, they were asked about intensity and pleasantness.

In publication 2, we performed region of interest (ROI) based analysis on 13 CA and 15 healthy controls. Diffusion weighted imaging was performed on a 3T scanner. We chose bilateral piriform cortex and orbitofrontal cortex masks from previously published studies and thresholded them in order to include concurrent areas. Using TBSS, we compared FA values in both the groups for each ROI.

In publication 3, thirty-eight healthy subjects with normal sense of smell (20 males, 18 females) were recruited for the study. We performed DWI using 3T scanner. We performed probabilistic tractography between piriform cortex, OFC and thalamus which resulted in streamlines. Additionally, subjects were presented with two trigeminal and two olfactory odors, which was part of the same experiment. We used the ratings, intensity and pleasantness, for correlating with the white matter streamlines.

Results

In publication 1, among 62 subjects, 43 had consistent CNV for all recording sites and odors. Topographical distributions of CNV amplitudes an analysis of variance for repeated measures (using within-subject-factors ‘‘odor” [vanillin, musk, peppermint, orange, air] and ‘‘channel” [Fz, Cz, Pz]) suggested that CNV amplitudes were larger for fontal and central electrode positions compared to the posterior position (factor ‘‘channel”: $F2, 80 = 8.61$, $p = 0.001$). Post-hoc testing revealed that amplitudes at Fz and Cz were higher compared to Pz ($p < 0.002$).

In publication 2, Mann–Whitney U test revealed significant changes in FA values within the OFC in each hemisphere between the two groups. FA values within left and right OFC were higher ($r = 0.58$, $p = 0.002$ and $r = 0.44$, $p = 0.019$, respectively) in CA group (FA in left OFC, 0.49 ± 0.02, right OFC, 0.44 ± 0.01) as compared to healthy controls (FA in left OFC, 0.44 ± 0.01, right OFC, 0.39 ± 0.01). As pertaining to the analysis for PFC, we did not find significant differences between the groups ($r = 0.20$, $p = 0.37$ for left PFC and $r = 0.17$, $p = 0.38$ for right PFC).

In publication 3, we found that, in the left hemispheres, there are more streamlines between piriform cortex and thalamus than piriform cortex and OFC (χ^2 (df 1) = 8.52, $p = 0.004$). On the contrary, more streamlines were seen in right hemisphere between piriform cortex and OFC (χ^2 (df 1) = 6.5, $p = 0.01$). A partial correlation analysis, controlling for age, revealed that streamlines between both left and right piriform cortex to thalamus exhibited positive correlations with intensity ratings for trigeminal odors ($r = 0.40$, $p = 0.01$, and $r = 0.33$, $p = 0.03$ respectively). A positive correlation between the number of streamlines (piriform cortex to OFC) and threshold score for trigeminal odors ($r = 0.40$, $p = 0.01$) were also seen. However, no such correlation was seen for olfactory odors with any of the streamlines.

Conclusions

CNV which is quite well known and explored in olfaction, in publication 1, we did not find evidence that CNV is clearly and strongly modulated through odorous stimuli – although the majority of the sample showed CNV. Hence, these results suggested that CNV has a very narrow window of applications which is more in the experimental than in the clinical field. In contrast, techniques like tractography, TBSS, are not used in clinical practice. However, they may offer unique insights into olfactory dysfunction. In publication 2, using TBSS anosmic patients showed increased water diffusion in correlation to olfactory function, which suggests myelination in OFC. Previous research has shown increase in gray matter volume in OFC in congenital anosmic subjects. Similarly in publication 3, using probabilistic tractography, white matter streamlines were seen among primary and secondary olfactory areas and correlations to odor threshold and odor identification were found. Correlating streamlines to olfactory function opens new avenues in the clinical interpretation of olfactory loss and, hence, warrants more research. The present studies suggest that connectivity studies based on MRI can help to better understand olfactory dysfunction. It is expected that they will gradually find their way into clinical routine.

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