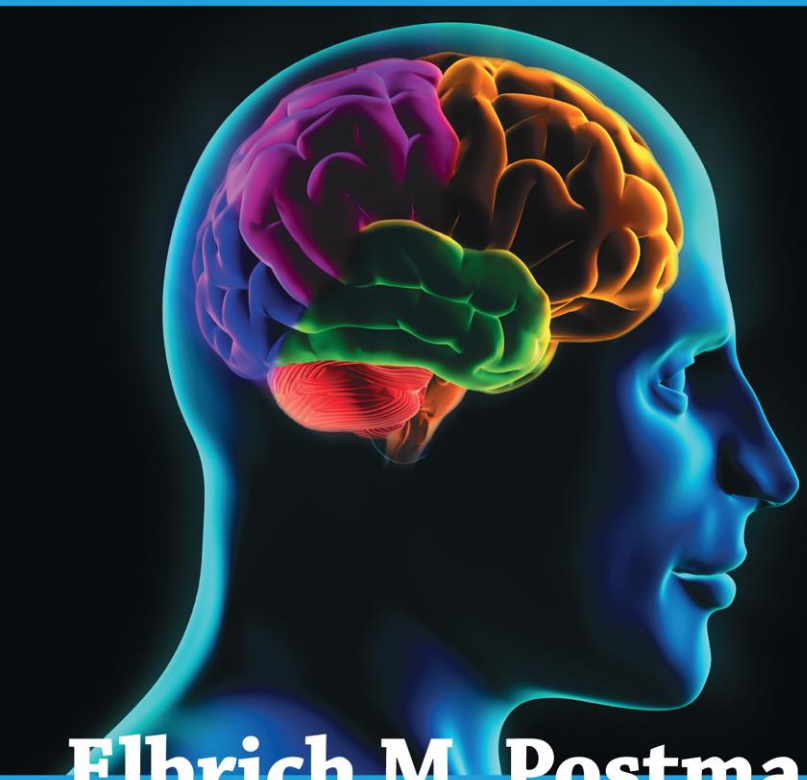
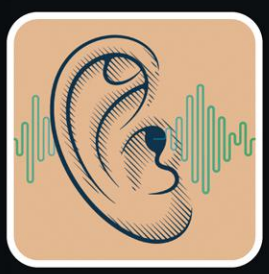
COMING TO YOUR SENSES

Effects of changes in olfactory and gustatory function on eating behavior and the brain





Elbrich M. Postma

Propositions

1. Subjective outcomes are more important than objective outcomes when considering the impact of changes in olfactory and gustatory function on patients.
   (this thesis)

2. Both structure and function must be measured when studying the neurobiology of olfaction.
   (this thesis)

3. Companies should only be allowed to fund research anonymously.

4. The effects of social jetlag get too little attention in nutrition research.

5. Storytelling should be a mandatory part of academic training.

6. Every PhD student needs a hobby that can be enjoyed without the use of any electronic device.

7. Dutch citizens overestimate their health literacy.

Propositions belonging to the Phd thesis entitled:
Coming to your senses: Effects of changes in olfactory and gustatory function on eating behavior and the brain.

Elbrich M. Postma
Wageningen, January 15th 2021

Coming to your senses

Effects of changes in olfactory and gustatory function on eating behavior and the brain

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Coming to your senses

Effects of changes in olfactory and gustatory function on eating behavior and the brain

Elbrich M. Postma

Thesis

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Chapter 1

Introduction

Introduction

For one moment, take the time to think of your favorite food. Can you remember the smell of this food, before you start eating it? And can you recall the taste of this food, once you start eating it? Maybe you notice that your appetite has increased. Your body is already preparing for the consumption of this food, while it might not even be close.

The senses of smell and taste play an important role in daily life. The human sense of smell is better than we often think [1], as it is estimated that humans are able to discriminate more than 1 trillion odors [2]. Information provided by odors affect different behaviors, such as eating behavior, detection of environmental hazards and social communication [3]. Therefore, odors do not only affect how food tastes, but also impact for example personal relationships and are essential in recognizing dangerous situations like a gas leak or fire. On the contrary, the sense of taste is entirely dedicated to eating behavior. The sense of taste is used to perceive the basic taste qualities and to detect nutrients in foods and subsequent consequences for the human body. This can either be positive, like detection of a sweet taste, indicating a food high in carbohydrates and energy, which is rewarding for the body, or negative, like rejecting the intake of a bitter food, as a bitter taste often indicates a toxic component [4].

While smell and taste both have a functional role in eating behavior, the effect of changes in olfactory and gustatory function on eating behavior and the neurobiology of smell and taste is not well enough understood. Hence, within this thesis, we aimed to gain more insight in the effect of changes in olfactory and gustatory function on eating behavior and the neurobiology of smell and taste. From here, we hope to ultimately improve health care and nutritional recommendations for patients.

This introduction starts with an overview of how smell and taste are processed and their role in eating behavior. Next, changes in olfactory and

gustatory function are discussed, followed by their effect on eating behavior and how changes in olfactory function relate to the neurobiology of smell. The introduction ends with the aim and outline of this thesis.

Processing of smell and taste: from the nose and the mouth to the brain

Odor molecules can be inhaled through the nose to the nasal cavity, which is called orthonasal smelling, or they are transferred to the nasal cavity via the mouth, which is called retronasal smelling (Figure 1) [5]. Here, odor molecules are transported to the olfactory epithelium, which is located in the upper part of the nasal cavity. The signals that are excited by the binding of the odor molecules to the olfactory epithelium are transferred to the olfactory bulb through the olfactory nerve (first cranial nerve). This nerve is the connection between the nose and the brain. The olfactory bulb has an important role in transmitting signals to other brain regions involved in olfactory processing. The primary olfactory regions of the brain are the piriform cortex, the entorhinal cortex and the amygdala [6,7]. Subsequently, signals are transferred to the secondary olfactory regions of the brain, which are the orbitofrontal cortex, the anterior cingulate cortex and the insular cortex [6,7]. In these brain regions further processing of odors is conducted, like odor recognition and affective coding [7].

Taste perception starts on the tongue (Figure 1). The taste buds that are located on the tongue distinguish the 5 basic tastes: sweet, salty, sour, bitter, and umami [8]. After binding of taste molecules to the taste receptor cells, located in the papillae in the taste buds on the tongue, the signals are transferred to the brain through the facial nerve, the glossopharyngeal nerve and the vagus nerve, respectively the

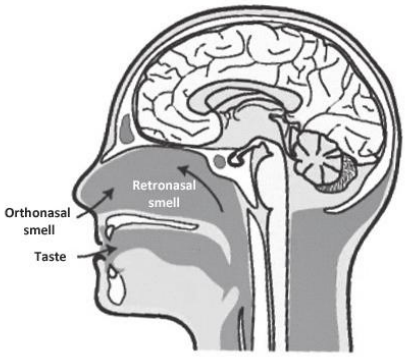

Figure 1. *Location of peripheral odor and taste perception (derived from [14]).*

VII, IX and X cranial nerve [9]. The first place in the brain where the gustatory signals are processed is the nucleus of the solitary tract of the brainstem [10]. Next, they are sent to the ventral posterior medial nucleus of the thalamus; from here, the signals are transferred to the primary taste cortex in the insula and the secondary taste cortex in the orbitofrontal cortex for further processing needed for conscious taste perception [4].

While smell and taste both have a separate pathway from peripheral input to central processing, within the brain, smell and taste can be integrated to flavor perception. Integration of both signals takes place in the anterior ventral insula and subsequently in the orbitofrontal cortex, amygdala, anterior cingulate cortex, frontal operculum and the insula [10–12]. The brain can learn to match flavors to post-ingestive effects of related nutrients by repeated experience, which can result in a preference for these flavors [10]. Therefore, flavor perception is an important determinant of eating behavior [13].

The role of smell and taste in eating behavior

Each day, humans are confronted with numerous food choices, such as what to eat and how much to eat. Eating behavior involves many different processes, like food choice and food intake. Smell and taste are important sensory characteristics of food that determine liking and preferences of foods and play a differential, but complimentary role in eating behavior [15]. Moreover, food preferences can be used to signal needs of the body. For example, following a low-protein diet can increase the preference for savory, high-protein foods [16], in order to restore the bodies' deficiency.

Odors play a role in the preparation of consumption of foods by increasing appetite and affecting food choice. Before the start of consumption, odors can signal the presence of edible foods [17]. The presence of odors can increase appetite [18–20] and exposure to odors may possibly increase the actual amount of food intake [18]. Moreover, odors play a role in food choice [21].

Taste is more focused on nutrient sensing, which plays a role in food

intake and satiation [22]. The basic tastes can be used to detect the nutritional content of foods during consumption [23]. Preferences for the basic taste qualities are innate: already shortly after birth, infants show positive and negative reactions towards different tastes [24]. Food preferences are more complex, as taste is combined with odors to form flavor, and are more sensitive to learning: repeated exposure to a certain flavor can eventually lead to acceptance of this food [25,26].

Concluding, smell and taste are complimentary to each other and both have a functional role in eating behavior. Therefore, smell and taste, and their combined perception in flavor of food, are important determinants for food intake and subsequently nutritional status. However, there are individuals who have to face changes in olfactory and gustatory function. Studying the effect of these changes in olfactory and gustatory function on both neurobiology and eating behavior in patient populations will lead to useful insights on how smell and taste affect eating behavior and the brain.

Causes & characteristics of changes in olfactory and gustatory function

Despite the fact that olfactory and gustatory function play an important role in eating behavior, these senses are not often discussed in daily life or in healthcare. However, 3% up to 20% of the general population suffers from changes in olfactory function [27–30]. Changes in gustatory function are less common, as gustation is an anatomically more robust system than olfaction [4]. Of all patients seeking clinical assistance for changes in olfactory and gustatory function, less than 4% actually suffers from changes in gustatory function [31]. Ageing plays an important role in the prevalence of changes in olfactory and gustatory function: in elderly people the prevalence of both changes in olfactory [32] and taste [33] function increases. In geriatric patients the prevalence of changes in olfactory function might even increase to more than 90% of the patients [32]. Individuals with changes in olfactory and gustatory function often report several complaints, like a decreased quality of life, issues with daily

safety and a depressed mood [34–36]. This makes changes in olfactory and gustatory function a burden on the life of many patients.

In the general population, most cases of primary olfactory dysfunction are caused by upper respiratory tract infection, sinonasal diseases or head trauma [37]. Some individuals are born without the sense of smell: this is called congenital anosmia and is characterized by the absence of the olfactory bulb [38]. Moreover, there is a wide range of diseases to which olfactory dysfunction is related, like neurodegenerative diseases [39–41], schizophrenia [42] and depression [43,44]. Lastly, changes in olfactory function can also be a side effect of treatment, like chemotherapy [45]. In most patients with changes in olfactory function, the sense of smell is decreased or absent, respectively hyposmia or anosmia. These changes are considered as quantitative olfactory loss. The phenomenon of distortion of the sense of smell is called parosmia; this is considered as qualitative olfactory loss.

For primary taste dysfunction, common causes are posttraumatic, upper respiratory tract infection and sinonasal diseases [31,46]. However, in many cases of primary taste dysfunction, the cause of the taste dysfunction is not known. Moreover, taste dysfunction is often reported in relation to the use of medication [47] or in specific patient groups, like cancer patients undergoing chemotherapy or radiotherapy [48]. Among cancer patients, 45% to 85% of patients experience, mostly transient, changes in gustatory function [45]. Whereas changes in olfactory function are mainly quantitative, changes in gustatory function are often qualitative, like an altered perception of existing tastes, parageusia, or the perception of non-existent tastes, phantogeusia [5].

Thus, while changes in olfactory and gustatory function are common among the general population, there is a wide range of causes. These causes should be taken into account while studying the effects of changes in olfactory and gustatory function.

The effect of changes in olfactory & gustatory function on eating behavior

The effect of changes in olfactory and gustatory function on eating behavior has mainly been studied in the context of self-reported changes. Studies including patients with changes in olfactory and gustatory function report several alterations in eating behavior, like reduced appetite and food enjoyment, and an increased use of herbs and spices during cooking [34,35,49–52].

So far, little research has been done on the effect of changes in olfactory function on actual food intake in the general population. In specific populations, like older adults and geriatric patients, some studies found no association between olfactory function and nutritional intake [32,53], while others found that elderly women who suffered from changes in olfactory function had reduced adherence to dietary guidelines and an increased risk for poorer diet quality over time [49,54]. However, as changes in olfactory function can lead to weight gain or weight loss [35,55], it is likely that actual food intake changes in these patients.

Also in regard to the effect of changes in gustatory function on eating behavior, most of the research has been performed in specific populations, like cancer patients undergoing chemotherapy. These patients often suffer from a decreased appetite, have difficulties in maintaining an adequate nutritional intake as a results of changes in gustatory function, and develop several strategies to deal with eating during their treatment [56–58]. Moreover, taste alterations can lead to specific problems, like food aversions [59]. Conflicting results have been found for food preferences and intake in cancer patients undergoing chemotherapy. Some studies found that decreased gustatory function can lead to changes in food preferences and a reduced energy intake [60–62], while others did not find an effect on food preferences and intake [63,64]. The type of chemotherapy regimen received is related to the prevalence of changes in gustatory function [57]. However, as most studies include a heterogeneous patient population, it is difficult to draw conclusions on the effect of changes in gustatory function on eating behavior based on the studies performed so far.

While changes in olfactory and gustatory function are likely to affect eating behavior, most research so far only focused on the effect of olfactory and gustatory function on self-reported alterations in eating behavior. Therefore, we still know little about the effect of changes in olfactory and gustatory function on objectively measurable components of eating behavior, like food preferences and food intake.

Neuroplasticity of changes in olfactory function

The human brain is plastic and olfaction can be trained, as has been shown in experts like perfumers and wine tasters [65,66]. Not only a trained sense of smell can induce changes in the brain; also a decrease in olfactory function can cause reorganization of the brain and is therefore related to several morphological and functional changes in the brain [67].

One of the most studied brain regions in relation to changes in olfactory function is the olfactory bulb (Figure 2). The volume of the olfactory bulb is positively correlated with olfactory function in healthy individuals [68,69]. Some studies replicated this correlation in patients with changes in olfactory function [70,71]. However, others did not find this correlation in patients [72,73]. Moreover, volume of the olfactory bulb is related to olfactory function in patients with neurodegenerative diseases, like Parkinson's disease [74] and Alzheimer's disease [75]. Furthermore, volume of the olfactory bulb is related to

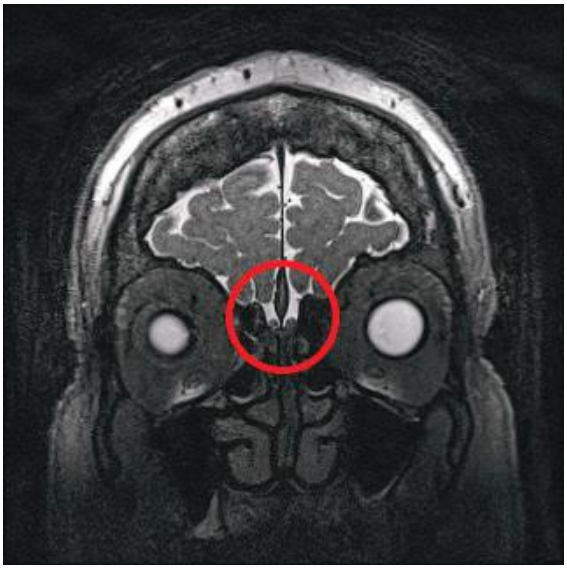

Figure 2. *MRI scan demonstrating the location of the olfactory bulbs, within the red circle.*

duration of changes in olfactory function [76], treatment of changes in olfactory function [77,78], and can be a predictor for recovery in patients with changes in olfactory function [71]. Overall, the olfactory bulb thus seems to be an important indicator of olfactory function. To get to a better understanding of the role of the olfactory bulb in olfactory processing, it is necessary to compare results among studies. However, current studies have mostly been performed in small groups of patients or in heterogeneous populations, which makes it hard to investigate the effect of different causes of changes in olfactory function. Moreover, as measurements of olfactory bulb volume are currently performed manually (see f.e. [79,80]), differences among observers between studies hinder comparisons based on absolute volumes or reference values.

Not only the olfactory bulb, but also other brain regions show changes in volume in relation to olfactory loss. Evidence was found for reduced volume of the piriform cortex in patients with olfactory loss [81–84]. Also areas in the secondary olfactory cortex showed a decreased volume in patients with olfactory loss: the anterior cingulate cortex [81–85]; the orbitofrontal cortex [73,76,81–83,85] and the insular cortex [73,82–85]. However, as research performed so far was either conducted in heterogenous groups or in relatively small patient groups, it is hard to draw conclusions on the relation between changes in olfactory function and morphological changes in the brain.

Also alterations in brain functionality have been found in relation to changes in olfactory function. Hyposmic patients due to primary changes in olfactory function showed a decreased activation in response to odors in olfactory-related brain regions like the right OFC and the left anterior cingulate cortex compared to healthy controls [86]. Moreover, a decreased activation in response to odors in olfactory-related areas was found in elderly compared to younger individuals [87]. In patients with disease-related changes in olfactory function, changes in activation in olfactory-related brain regions were found in patients with Alzheimer's disease [88] and Parkinson's disease [89]. However, in these patients not only changes in olfactory function can lead to neuronal

changes, but also other alterations that are related to the disease. This might distort the way changes in olfactory function and olfactory function are reflected in the brain. Therefore, studying the relation between primary changes in olfactory function and brain activity in relation to odors will give the best insights in how these changes are reflected in the brain. Besides, studies so far mainly investigated brain activity by means of blood-oxygen-level-dependent (BOLD) response, and not functional connectivity in relation to changes in olfactory function. Studying functional connectivity between olfactory-related brain regions will lead to more knowledge on how changes in olfactory function affect neural networks. In addition to studying activation, this will give insight in how olfactory processing as a whole is affected by changes in olfactory function.

Patients with changes in olfactory function can benefit from the plasticity of the human olfactory system. Both medical treatment of olfactory loss [77,78,90] and repeated stimulation of the olfactory system, for example by applying olfactory training [91,92] can lead to increased volumes of brain regions related to the olfactory processing network, like the olfactory bulb. Moreover, olfactory training can improve olfactory function [93] and can cause reorganization of functional networks related to olfactory perception [94]. However, smell training is not effective in all patients. A better understanding of the relation between changes in olfactory function and the morphology and functionality of olfactory-related brain regions is needed. This can lead to more insights in the relation between changes in olfactory function and brain function and health in general. This will provide more possibilities for diagnosis and prognosis and gives guidance for the development of effective treatments to increase chances for recovery in patients with changes in olfactory function.

Aim of the project and outline of this thesis

Changes in olfactory and gustatory function are a widespread problem. However, their effect on eating behavior and the neurobiology of smell and taste is not well enough understood. Hence, within this thesis, we aimed to gain

more insight in effect of changes in olfactory and gustatory function on eating behavior and the neurobiology of smell and taste, in order to ultimately provide patients with sufficient health care and nutritional recommendations (Figure 3). As changes in gustatory function are scarce, we choose to focus on changes in olfactory function when studying the brain in this project. We defined the following aims:

- Investigate the effect of alterations in olfactory and gustatory function on eating behavior in patients with changes in olfactory and gustatory function.
- Investigate possible neurobiological alterations in patients with changes in olfactory function.

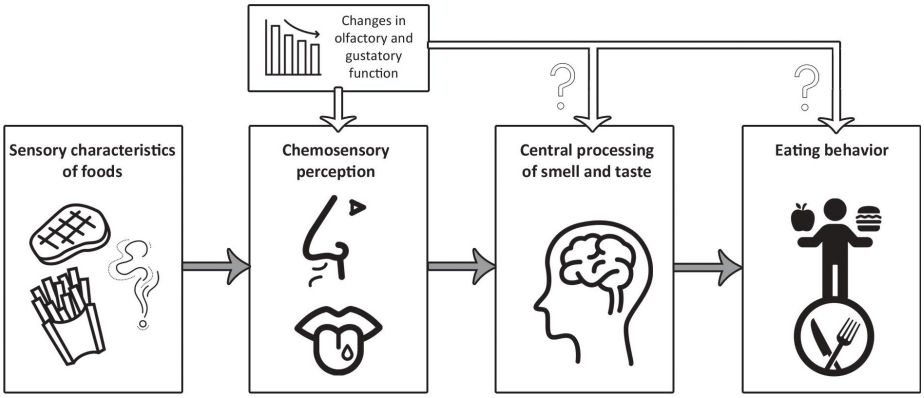

Figure 3. *The relation between olfactory and gustatory function, the neurobiology of smell and taste and eating behavior and the gaps in knowledge on the effect of changes in olfactory and gustatory function.*

First, we investigated the effect of changes in olfactory and gustatory function on food preferences and food intake. In **chapter 2**, we performed a study on food preferences and adherence to dietary guidelines in a population of Dutch patients with primary changes in olfactory function. Subsequently, we studied a specific patient group in which we expected changes in both olfactory and gus-

tatory function, namely cancer patients undergoing chemotherapy. As described in **chapter 3**, a group of patients with colorectal cancer was followed over time during and after chemotherapy treatment to assess the possible changes in olfactory and gustatory function and food preferences.

Secondly, we aimed to get more insight in olfactory processing by investigating possible neurobiological changes in morphology and function of olfactory-related brain areas in patients with changes in olfactory function. The olfactory bulb is the first receptor of olfactory signals in the human brain and therefore of importance to study in the context of changes in olfactory function. To allow comparisons between studies based on objective measurements of olfactory bulb volume, we developed a method to automate measurements of the olfactory bulb volume based on MRI-scans in **chapter 4**. As a next step, we wanted to get a better understanding of the relation between cause and duration of changes in olfactory function and the neurobiology of olfactory processing. Therefore, we studied the relation between changes in olfactory function and morphology of primary and secondary olfactory-related brain regions, as described in **chapter 5**. Finally, in **chapter 6,** we performed an fMRI study to determine how changes in olfactory function affect neural activation patterns and networks in response to odors and how this is related to olfactory function. The results of these studies are summarized and discussed in a general discussion in **chapter 7.**

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Chapter 2

Food preferences and intake in a population of Dutch individuals with self-reported smell loss: An online survey

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Abstract

Olfaction plays a major role in food intake regulation. Losing the sense of smell might therefore affect eating behavior. This study investigated food preferences and intake in individuals suffering from self-reported smell loss with an online survey. Members of the Dutch Anosmia Foundation (DAF) performed the Macronutrient and Taste Preference Ranking Task (n=71) to measure preference for foods high in fat, carbohydrates or protein and low energy foods, and for sweet and savory tastes. To assess dietary intake, adherence to the Dutch Dietary Guidelines for consumption of vegetables, fruit, fiber, fish, saturated fat, trans fatty acids, salt and alcohol was measured (n=105). Results of the DAF participants were compared to local cohort groups. Both the control and DAF participants showed the lowest preference for carbohydrate-rich foods and highest preference for low-energy foods. Participants suffering from congenital smell loss showed an aberrant pattern, with a higher preference for fat. The total adherence score to the Dutch Dietary Guidelines was similar for the control and DAF group, but adherence scores for fiber, trans fatty acids and alcohol were lower in DAF participants. Overall, no major significant differences in food preferences and intake were found for participants who lost their sense of smell during life. Participants suffering from congenital smell loss did show changes in food preferences, suggesting they are potentially more taste-oriented during eating. Together these results show the importance of tailored advice on dietary intake for this patient group.

Introduction

Smell, as one of the sensory properties of food, plays a role in various aspects of human eating behavior. Olfactory cues before eating can increase overall appetite [1,2] and exposure to a food odor can direct appetite towards food products with a similar taste [3,4]. Subsequently, the perception of odors can prepare the body for the intake of associated (macro)nutrients [5,6]. During consumption, odors play a role in determining the flavor of a food [5] and in signaling its nutrient content, through flavor-nutrient learning during previous experiences [7]. Therefore, olfactory signals before and during consumption of foods may affect our eating behavior, i.e. what we eat and how much we eat.

While odors play a vital role in eating behavior, it is estimated that 3% up to 20% of the total population suffers from smell loss [8–10]. Olfactory function can decrease due to ageing, which might lead to for example to higher thresholds for food odors [11,12], but can also be due to other causes such as viral infections or head trauma [13]. Additionally, some individuals are born without the sense of smell: congenital anosmia [13,14]. Smell loss thus affects a broad range of individuals.

Frequently reported complaints among individuals suffering from smell loss are changes in the attitude towards food, such as decreased food enjoyment, and an increased use of spices [15,16]. Aschenbrenner et al. showed changes in food preferences in individuals suffering from smell loss for specific food categories, such as cheeses, fruits or high-fat foods. Importantly, a higher level of smell loss was not related to more changes in dietary intake [15].

In addition to changes in the attitude towards food and altered food preferences in individuals with smell loss, actual food intake might also change, as smell loss can lead to changes in appetite, resulting in weight gain or weight loss [17,18]. Elderly women who suffered from smell loss showed reduced adherence to dietary guidelines, a higher intake of saturated fatty acids, and an increased risk for poorer diet quality over time [19,20]. However, other studies

found no relation between olfactory function and nutritional status [21,22] or eating pleasure, appetite and hedonic ratings of food [23–25] in elderly. As those studies specifically focused on smell loss in elderly, less is known about the effect of smell loss on food preferences and intake in other populations.

This study investigated food enjoyment, food preferences and intake in individuals suffering from self-reported smell loss. All participants were members of the Dutch Anosmia Foundation: a patient association for individuals suffering from smell loss. We hypothesized that food preferences would differ from a healthy control population, and that dietary intake would be of poorer quality in individuals suffering from smell loss.

Materials & Methods

Subjects

Participants were recruited among the members of the Dutch Anosmia Foundation (DAF).All members of the Dutch Anosmia Foundation were invited to join the study by sending an email through the secretary of the DAF. This email contained a randomized log-in code for the online questionnaire to ensure anonymous data collection. Participation was on voluntary basis and all participants agreed upon the use of their results for scientific research. The online questionnaire was sent to 230 members of the DAF. Additionally, a paper version of the questionnaire on adherence to the Dutch Dietary Guidelines was sent to six members as they did not have access to a computer. In total, 108 questionnaires were returned (47%). One participant was pregnant and was therefore excluded from further analysis.

The MTPRT was assessed by 88 DAF members, who all reported to suffer from smell loss. Participants with missing data (n=7) were excluded from the analysis. Additionally, participants who followed a vegetarian or vegan diet (n=10) were excluded, as this might affect their preference for food high in protein and savory food products, since they are mostly meat and fish related [26]. In total, 71 participants were included in the analysis.

The questionnaire on adherence to the DDG was filled in by 107 members of the DAF, who all reported to suffer from smell loss. Within this group, 86 participants (80%) indicated that they were clinically diagnose with a smell disorder. Two participants were excluded due to missing data, resulting in the inclusion of 105 members for further analysis of the data.

For comparison to healthy controls, results from local cohort studies were used. As a control population for the MTPRT, we used data from healthy controls from the 'EetMeetWeet' panel (EMW), a cohort study including participants from the Dutch population [27]. Data from 738 participants were included for analysis. For dietary intake, results from the validation paper of the questi-

onnaire on adherence to the Dutch Dietary Guidelines (n=1.235) were used as reference [28].

Participants were asked for demographic information: sex, age, height and weight, and etiology and duration of smell loss. For etiology, participants could choose one from the following categories: congenital, cold/flu, head trauma, chronic inflammation of the nasal cavity, use of medication, 'I do not know', and other. For duration of disorder, participants could choose from the following categories: 0-6 months, 6 months – 1 year, 1-2 years, 2-5 years, 5-10 years, > 10 years, and congenital. Characteristics of the DAF participants and the healthy controls from the EMW panel are shown in table 1.

Food enjoyment

Participants were asked to respond to three statements regarding their attitude towards food: "Food now tastes different than before"; "I eat less than before"; and "I enjoy eating food less than before". 'Before' referred to the time before the participants lost their sense of smell. Answering options were: completely agree; agree; neutral; disagree; completely disagree; or I was not able to smell my whole life.

Table 1. *Characteristics of the DAF participants and healthy controls (EMW) who filled in the Macronutrient and Taste Preference Ranking Task (MTPR) and the DAF participants who filled in the questionnaire on adherence to the Dutch Dietary Guidelines (DDG).*

	MTPRT	**MTPRT**	**Adherence to DDG**
	Healthy controls (EMW) (n=738)	DAF participants (n=71)	DAF participants (n=105)
Male (%)	37.1	27.8	30.5
Female (%)	61.8	73.2	69.5
Sex unknown (%)	1.1	N/A	N/A
Age (years, mean ± SD, range)	55±15 (19-84)	58±12 (22-82)	58±13 (14-87)
BMI (kg/m2 mean ± SD, range)	25±3.7 (15-40)	26±4.1 (19-38)	25±3.9 (19-38)
Etiology			
Congenital	N/A	14	16
Cold/flu		13	15
Head trauma		19	32
Chronic inflammation		8	15
Other causes		17*	27*
Duration			
< 5 years	N/A	22**	37**
5-10 years		16	20
> 10 years		19	32
Congenital		14	16

* Other includes the options: use of medication (n=1), 'I do not know' (n=9) and other (n=7).

** The category < 5 years consists of participants from the following categories: 6 months – 1 year (n=1), 1-2 years (n=2) and 2-5 years (n=19).

Food preferences

The Macronutrient and Taste Preference Ranking Task (MTPRT) as described by De Bruijn et al. (2017) was used to assess liking and ranking for foods high in carbohydrates, fat or protein, and low-energy foods as well as the taste qualities sweet and savory [26]. The task consists of 32 pictures of food products and includes eight pictures for each macronutrient category. For carbohydrates, fat and low-energy there are pictures of four sweet and four savory food products. The pictures for the protein category only include savory food products. Before performing the task, all participants rated their hunger on a 100 point visual analog scale (VAS) anchored by 'not hungry at all' and 'very hungry'.

Liking for all products in the MTPRT was measured on a 100 point VAS anchored by 'do not like at all' and 'like extremely'. All pictures were shown one by one. Ranking scores were determined by presenting the participants four pictures at the same time, which they had to rank in the order they would prefer to eat the products at that moment. The ranking task consisted of two parts: the first part included sixteen combinations of four pictures, representing all macronutrient categories. The results from the first part were used to calculate preferences for the macronutrient categories. Macronutrient ranking scores were calculated on a scale of 1-4 for each category with the following formula: ranking score = (4*(#rank1) + 3*(#rank2) + 2*(#rank2) + 1*(#rank4))/16. The second part included twelve combinations of four pictures, which represented a sweet and a savory product from two macronutrient categories. The results from this second part were used to calculate preferences for the taste qualities. Ranking scores were calculated on a scale of 1.5-3.5 for both taste qualities with the following formula: ranking score = (4**(#rank1)* + 3**(#rank2)* + 2**(#rank2)* + 1**(#rank4)*)/24 [26].

Figure 1. *Four-choice option from the Macronutrient and Taste Preference Ranking Task. Participants were asked to rank the pictures in the order they would prefer to eat the foods. This picture shows products representing the four macronutrient categories: salty sticks as food high in carbohydrates, chocolate as food high in fat, fish as food high in protein, and strawberries as low-energy food.*

Dietary intake

To measure dietary intake, adherence to the Dutch Dietary Guidelines (DDG) was assessed by means of a questionnaire on the intake of 34 regularly consumed foods in the Netherlands as described by Van Lee et al. [28,29]. Results were used to determine the intake of 8 different dietary components: vegetables, fruit, dietary fiber, fish, saturated fatty acids, trans fatty acids, salt and alcohol. The intake of vegetables, fruit, fish and alcohol was calculated from the reported intake of the food items that represented these specific categories. For intake of dietary fiber, saturated fatty acids, trans fatty acids and salt, all food items in the questionnaire were used. Intake was calculated based on the frequency of consumption and portion sizes as mentioned by the participant in the questionnaire. Adherence to the Dutch Dietary Guidelines was subsequently converted to a 10-point scale, indicating no adherence to the guideline (0) to complete adherence to the guideline (10) based on the calculated intake. For vegetables, fruit, dietary fiber and fish a higher score thus represents a higher intake, whereas a higher score for saturated fatty acids, trans fatty acids, salt or alcohol represents a lower intake. The total adherence score was calculated as the sum of the separate component scores and was measured on a scale of 0-80.

Statistical analysis

The data were analyzed with IBM SPSS Statistics (version 24). Normality of the data was checked with the Shapiro-Wilk test. Data on food enjoyment are reported as percentage of total. All data from the MTPRT are reported as mean with standard error and all data on adherence to the DDG are reported as mean with standard deviation. Results were considered significant at $p \leq 0.05$.

Macronutrient and Taste Preference Ranking Task

Macronutrient and taste liking and ranking scores were calculated as described by above, based on de Bruijn et al. [26]. As ranking scores for sweet and savory are each other's opposite, this paper only reports the ranking scores for sweet.

Liking scores for low-energy foods in the DAF participants were not normally distributed. Therefore, a Wilcoxon signed rank test was used to analyze differences between liking scores for the macronutrient and taste categories within the DAF participants. A Mann-Whitney U Test was used to compare the liking scores between the DAF participants and the healthy controls per category. For both tests the Benjamini-Hochberg procedure was applied to control for multiple comparisons, using a false discovery rate of 0.05 [30].

A one-sample T-test was used to determine whether ranking scores of the DAF participants were significantly different from a 'no-preference' score of 2.5. An independent samples T-test was used to compare ranking scores for macronutrient and taste categories between the DAF participants and the healthy controls. For both tests the Benjamini-Hochberg procedure was applied to control for multiple comparisons, using a false discovery rate of 0.05.

To assess the potential influence of duration and etiology on the ranking scores within DAF participants, a GLM was used. Food preference scores were used as dependent variables, duration or etiology as fixed factor and age, sex and hunger as covariates. In case of significant results, the Bonferroni procedure was used as post hoc test.

Dietary intake

The total adherence score to the Dutch Dietary Guidelines of the DAF participants was compared to the reference value by means of a one-sample T-test. As the separate component scores for dietary intake of the DAF participants were not normally distributed, these were compared to the reference component scores by means of a one-sample Wilcoxon signed rank test. The Benjamini-Hochberg procedure was applied to control for multiple comparisons, using a false discovery rate of 0.05.

The potential influence of duration and etiology of disorder within DAF participants on adherence to the Dutch Dietary Guidelines was analyzed with a GLM for the total score as well for the separate component scores, using the Bonferroni procedure as post-hoc test. Fixed factors were duration or etiology, while age and sex were added as covariates.

Results

Food enjoyment

72% of the participants suffering from smell loss (n = 107, 15% choose the option 'I was not able to smell my whole life') reported to enjoy eating food less than they did before the onset of their smell loss, and 81% of the participants indicated that food tasted different compared to before they lost their sense of smell. However, the majority of participants (58%) reported no decline in their food intake due to their smell loss.

Food preferences

The liking score of the DAF participants for low-energy foods was significantly higher than the liking scores for fat, protein and carbohydrates. Additionally, the liking score for fat was significantly higher than the liking score for carbohydrates. The liking scores of the healthy controls were all significantly higher compared to the liking scores of the DAF participants (see figure 2; see appendix A, table A1 and A2, for p-values and Benjamini-Hochberg procedure).

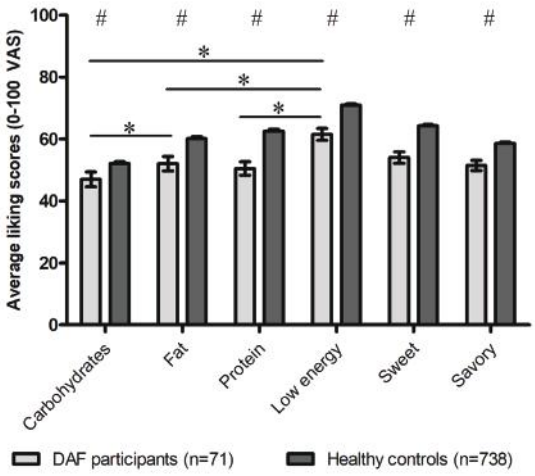

Figure 2. *Mean liking scores ± SEM for the DAF participants and the healthy controls for all macronutrient categories and taste qualities. Significant diffe-*

*rences (p ≤ 0.05) between categories within the DAF participants are indicated with a *; significant differences between the groups are indicated with an #.*

The ranking score for low-energy foods was significantly higher than the no-preference score of 2.5, while the ranking score for foods high in carbohydrates was significantly lower than the no-preference score for the DAF participants. Sweet foods were ranked significantly lower by the healthy controls compared to the DAF participants (see figure 3; see appendix A, table A3 and A4, for p-values and Benjamini-Hochberg procedure).

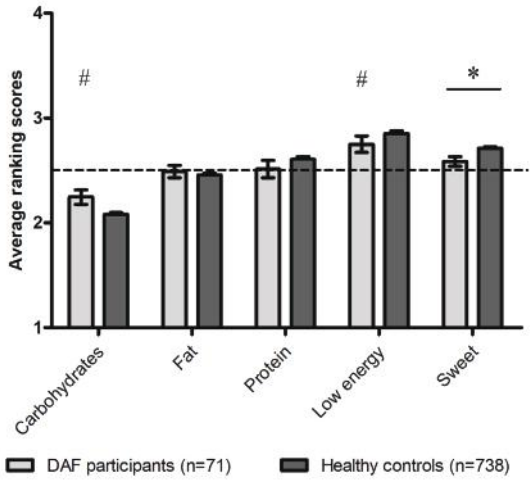

Figure 3. *Mean ranking scores ± SEM for the DAF participants and the healthy controls for all macronutrient categories and the taste quality sweet. The dashed line indicates a 'no-preference score' of 2.5. Significant differences (p ≤ 0.05) from the 'no-preference' score are indicated with an # and significant differences between the groups are indicated with a *.*

There was no significant effect of duration of disorder on the ranking scores for any of the macronutrient categories, nor for the taste qualities (see appendix A, table A5, for p-values and Benjamini-Hochberg procedure).

Etiology significantly affected ranking scores for fat, low energy and

sweet (see appendix A, table A6, for p-values). The post-hoc test showed the most different pattern for the congenital group, with a significant difference between the other causes group and the congenital group in ranking for fat, as is shown in table 2 (see appendix A, table A7, for p-values).

Table 2. *Effect of etiology on ranking scores reported as mean ± SE. Different letters indicate significant differences between groups.*

	Cold/flu (n=13)	Head trauma (n=19)	Chronic inflammation (n=8)	Other causes (n=17)	Congenital (n=14)
Carbohydrates	2.3 ± 0.2	2.1 ± 0.1	2.2 ± 0.2	2.2 ± 0.1	2.4 ± 0.2
Fat	2.5 ± 0.1^{ab}	2.4 ± 0.1^{ab}	2.6 ± 0.2^{ab}	2.3 ± 0.1^{a}	2.8 ± 0.1^{b}
Protein	2.7 ± 0.2	2.5 ± 0.2	2.6 ± 0.3	2.5 ± 0.2	2.4 ± 0.2
Low-energy	2.5 ± 0.2	3.0 ± 0.2	2.6 ± 0.2	3.0 ± 0.2	2.4 ± 0.2
Sweet	2.4 ± 0.1	2.4 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.8 ± 0.1

Dietary intake

There was no significant difference between the total adherence score of DAF participants to the Dutch Dietary Guidelines and the reference value. For the separate components, adherence to the guidelines in the DAF population was significantly lower for dietary fiber, trans fatty acids and alcohol, and higher for salt, as is shown in table 3 (see appendix B, table B1, for p-values and Benjamini-Hochberg procedure). These differences represent a higher intake of trans fatty acids and alcohol, and a lower intake of dietary fibers and salt in the DAF participants.

There was no significant effect of the duration of smell loss on the total adherence score to the dietary guidelines. Within the adherence scores for the separate components, a significant effect of duration of disorder was found for adherence to the guideline for salt. However, post-hoc testing showed no significant differences between the duration groups. No significant effect of duration on adherence to any of the other components was found (see appendix B, table B2 and B3, for p-values and Benjamini-Hochberg procedure).

No significant effect of etiology was found for total adherence score as well as for the scores for the separate components (see appendix B, table B4, for p-values).

Table 3. *Total adherence score and scores for the separate components ± SD for the DAF participants (n=105) and reference values (n=1.235) by van Lee et al. [28]. Components marked with an asterisk are significantly different from the reference value.*

Component	Mean ± SD	Reference value
Vegetables	6.4 ± 3.1	6.7 ± 2.6
Fruit	7.2 ± 3.1	8.0 ± 2.7
Dietary fiber*	6.9 ± 2.2	7.8 ± 1.9
Fish	5.2 ± 3.4	5.5 ± 3.2
Saturated fatty acids	5.6 ± 4.0	5.5 ± 4.0
Trans fatty acids*	8.9 ± 3.2	9.2 ± 2.7
Salt*	7.1 ± 2.6	6.3 ± 2.8
Alcohol*	8.3 ± 3.1	8.6 ± 2.7
Total score	55.6 ± 10.6	57.6 ± 9.6

Discussion

In this study we investigated food preferences and intake in a population of individuals suffering from self-reported smell loss. Food preferences appeared to be similar for DAF participants and a healthy control population, except for the individuals suffering from congenital smell loss. This group showed a higher preference for fat compared to individuals with non-congenital smell loss. Surprisingly, this did not translate into significant differences in adherence to the Dutch Dietary Guidelines for individuals suffering from congenital smell loss. Overall, individuals with smell loss had similar adherence scores to the Dutch Dietary Guidelines as the reference population, regardless of etiology. Nonetheless, lower adherence scores to the guidelines for dietary fiber, trans fatty acids and alcohol and a higher adherence to the guidelines for salt were found in the DAF participants compared to the reference population.

Within the current study, 72% of the DAF participants reported decreased food enjoyment, and overall food liking scores were lower in DAF participants than in the healthy controls. This is not surprising, given that smell is an important component of flavor perception [31], and is in line with literature that reports decreased food enjoyment in individuals suffering from smell loss [16,32,33]. Nonetheless, the overall pattern in food preferences of the DAF participants was similar to the healthy control population. This is in contrast to the results of Aschenbrenner at al., who did find changes in food preferences among patients suffering from smell loss, such as an increased preference for fruits and vegetables [15]. Also Duffy et al. reported changes in food preferences in elderly women suffering from smell loss, for example a decrease in preference for whole-grain breads [19].

By using a quantitative and implicit ranking procedure to measure food preferences, the current study aimed to investigate food preferences in line with the expression of food preferences in daily life. As pointed out by de Bruijn et

al. [26], food preferences are influenced by many factors. Time of day [34] or metabolic state [35] might for example change the preference for savory food products. As we asked participants to perform the task online, this was done in a non-controlled situation. This might lead to inconsistencies, for example participants performing the task in different hunger states. To control for this variable, we added 'hunger' as covariable in our analyses. On the other hand, using online tools to measure food preferences might also better reflect real-life than testing participants in an experimental laboratory setting.

Individuals suffering from congenital smell loss did show altered patterns in food preferences. They reported a significantly higher preference for fat than participants who lost their sense of smell at later age. Additionally, etiology of smell loss affected preference for sweet-tasting foods and low-energy foods. This corroborates findings of Novakova et al., who demonstrated higher liking during prolonged exposure to a sweet food in participants suffering from congenital smell loss, compared to healthy controls [36]. These results imply that individuals suffering from congenital smell loss are more taste (sweet)- or nutrient (fat) oriented while eating. As they lack any olfactory perception during eating, they do not perceive the full flavor of foods. This might explain their increased preference for fat, which is sometimes considered as the sixth basic taste [37], and the effect found for low-energy foods, such as vegetables, which are mostly low in taste and nutrients [38].

This aberrant pattern in food preferences might arise from the differential formation of food preferences in individuals with congenital smell loss compared to healthy individuals. The formation of food preferences starts early in life [39]. Typically, repeated exposure to foods and their associated odors leads to learning about the nutrient content, and/or postingestive effects of these foods [5]. This process of unconscious flavor-nutrient learning plays an important role in the formation of food preferences [7]. Individuals with congenital smell loss simply never had the opportunity to learn from and perceive sensory stimuli in the same way as healthy individuals or those who lost their

sense of smell at later age. In contrast, individuals with non-congenital smell loss can rely on memories from the time they were able to smell when it comes to associations with certain foods. Perhaps they continue to eat according to their previous preferences when they lose their sense of smell. Individuals with congenital smell loss however, are forced to rely solely on taste as a sensory cue to convey nutrient information, and thus show different food preferences, as evidenced by the current results.

Only a few studies have investigated dietary intake in individuals suffering from smell loss (see e.g. [19,20,40]). These studies demonstrated a poorer dietary quality in individuals suffering from smell loss compared to individuals with a normal sense of smell for one or more nutrients. This is in line with the current study, where we found lower adherence to several components of the Dutch Dietary Guidelines in the individuals with smell loss. We found that dietary fiber intake was significantly lower in the DAF participants compared to the reference group. Albeit not significant, fruit and vegetable intake were also somewhat lower in DAF participants. This might have accounted for the lower intake in fibers, as fruit and vegetables are important sources of dietary fibers [41,42]. Additionally, intake of trans fatty acids and alcohol was higher in the DAF participants than in the reference population. These results point towards an unhealthier diet in the DAF participants. However, the questionnaire used in this study only includes a limited number of categories [28]. To get a more detailed overview of the total intake, other methods like food frequency questionnaires or 24 hour recalls could be used.

Although total adherence to the dietary guidelines was not different between the DAF participants and the reference population, the overall score indicated a high-moderate adherence to the dietary guidelines. This result suggests that intake per component could be improved for both populations to achieve a better overall diet quality.

The current study used an online survey to measure dietary intake.

This questionnaire is easy and quick to fill in, but also includes only a limited number of categories. Other methods, such as food frequency questionnaires, give a more complete overview of total dietary intake, but are therefore also more time-consuming, which might lead to unfinished questionnaires [43]. The questionnaire used in this study was previously validated by correlating the results to the outcomes of a 180-item food frequency questionnaire combined with a 24-hour urinary natrium excretion value, demonstrating that the questionnaire was appropriate to measure dietary intake on group level [28]. Similar results were found in a study comparing an online dietary intake tool to a 4-day weighed food record [44], showing that online tools on dietary intake can be used as a reliable screener for dietary intake on group level.

Individuals with congenital smell loss displayed a different pattern of food preferences. However, this did not alter their adherence to dietary guidelines compared to participants with smell loss at later age or the healthy controls. Eating behavior is a complex process which involves both implicit and explicit factors [5,45]. Therefore, individuals can show a discrepancy between their preferred choice and the actual choice made within the context of consumption [46]. Measuring food preferences can give valuable information on eating behavior, but should be treated cautiously in the context of other factors that also play a role in food intake, as individuals suffering from smell loss might use strategies to compensate for their smell loss when eating [40]. The link between food preferences and actual food intake should be further investigated, preferably in a population of patients with clinical, objective assessment of their chemosensory loss.

In our study, smell loss was self-reported. People are not good at distinguishing smell and taste [47,48], and self-report on smell and taste ability is therefore not always reliable. However, all participants were members of a patient association for individuals suffering from smell loss, increasing the likelihood of actual

olfactory dysfunction. One might expect that members of a patient association are more aware of their smell loss than patients who did not join a patient association. However, we have included a wide range of patients in regard to age, etiology and duration of disorder, as is shown in table 1 of the manuscript. Moreover, our results on food enjoyment, as described in the results, are in line with previous studies (see e.g. the review by Hummel and Nordin [32]), which were done in more general patient populations. Therefore we consider the population of the current study as representative for patients suffering from smell loss in general.

Etiology and duration of smell loss were also self-reported in our study. The fact that we did not find clear effects of etiology and duration of disorder on food preferences and food intake might be due to the fact that patients are not well able to remember cause and/or onset of the smell loss they suffer from. While the etiology and duration of congenital smell loss can be well defined, for other people suffering from smell loss it might be harder to indicate the exact cause or duration of their disorder. As 80% of our participants was clinically diagnosed with a smell disorder, we assume that most of the reported causes of smell loss are accurate. In addition, participants were given the option to choose 'I do not know' for etiology of smell loss (selected by 9 participants). Moreover, we used categories for duration of smell loss instead of a continuous measure of duration, as done before by others (see e.g. [13,49]). For future studies in clinically diagnosed populations, we recommend a larger sample size to stratify results according to etiology or duration of disorder.

Conclusion

Our results surprisingly show that there are no major significant differences in food preferences in individuals suffering from smell loss compared to healthy controls, except for those with congenital anosmia. This group demonstrated an aberrant pattern of food preferences, which seems to be more taste- or nutrient oriented. Although there were no significant differences in overall ad-

herence to the Dutch Dietary Guidelines in individuals suffering from smell loss compared to healthy controls, adherence scores for fiber, trans fatty acids and alcohol were lower in the individuals suffering from smell loss. This shows the importance of tailored advice on dietary intake for this patient group. Future research should further explore these findings in a larger population of clinically diagnosed individuals suffering from smell loss, and investigate the effects of duration or etiology on different measures of eating behavior.

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Appendix A: Food preferences

Table A1. *Liking scores for the MTPRT for DAF participants (n=71) were tested with the Wilcoxon signed rank test to analyze differences between liking scores for different categories within the DAF participants. The Benjamini-Hochberg procedure was applied to control for multiple comparisons, using a false discovery rate (Q) of 0.05.*

Comparison	P-value	Rank	Q	M	Critical value	Test
Protein-Fat	0.654927	7	0.05	7	0.050000	0
Protein-Carbohydrates	0.222292	6	0.05	7	0.042857	0
Savory-Sweet	0.051396	5	0.05	7	0.035714	0
Fat-Carbohydrates	0.001993	4	0.05	7	0.028571	1
Low-energy-Fat	0.000665	3	0.05	7	0.021429	1
Low-energy-Protein	0.000015	2	0.05	7	0.014286	1
Low-energy-Carbohydrates	0.000004	1	0.05	7	0.007143	1

Table A2. *Liking scores for DAF participants (n=71) and healthy controls (n=738) were tested with the Mann-Whitney U Test to compare the liking scores between the groups. The Benjamini-Hochberg procedure was applied to control for multiple comparisons, using a false discovery rate (Q) of 0.05.*

Category	P-value	Rank	Q	M	Critical value	Test
Carbohydrates	0.0476850000	6	0.05	6	0.050000	1
Fat	0.0005130000	5	0.05	6	0.041667	1
Savory	0.0000700000	4	0.05	6	0.033333	1
Low-energy	0.0000050000	3	0.05	6	0.025000	1
Protein	0.0000001632	2	0.05	6	0.016667	1
Sweet	0.0000000599	1	0.05	6	0.008333	1

Table A3. *Ranking scores for the MTPRT for DAF participants (n=71) were tested with a one-sample T-test to determine whether ranking scores were significantly different from a 'no-preference's score of 2.5. The Benjamini-Hochberg procedure was applied to control for multiple comparisons, using a false discovery rate (Q) of 0.05.*

Category	P-value	Rank	Q	M	Critical value	Test
Protein	0.872230	5	0.05	5	0.050000	0
Fat	0.859223	4	0.05	5	0.040000	0
Sweet	0.063083	3	0.05	5	0.030000	0
Low-energy	0.002542	2	0.05	5	0.020000	1
Carbohydrates	0.000466	1	0.05	5	0.010000	1

Table A4. *Ranking scores for the MTPRT for DAF participants (n=71) and healthy controls (n=738) were tested with an independent samples T-test to compare ranking scores between both groups. The Benjamini-Hochberg procedure was applied to control for multiple comparisons, using a false discovery rate (Q) of 0.05.*

Category	P-value	Rank	Q	M	Critical value	Test
Fat	0.613926	5	0.05	5	0.050000	0
Protein	0.279721	4	0.05	5	0.040000	0
Low-energy	0.225004	3	0.05	5	0.030000	0
Carbohydrates	0.023725	2	0.05	5	0.020000	0
Sweet	0.008880	1	0.05	5	0.010000	1

Table A5. *Ranking scores for the MTPRT for DAF participants (n=71) were tested with a GLM to assess the potential influence of duration on the ranking scores. Food preference scores were used as dependent variables, duration as fixed factor and age, sex and hunger as covariates.*

Category	Degrees of freedom	F-value	P-value
Carbohydrates	3, 64	0.638	0.594
Fat	3, 64	2.568	0.062
Protein	3, 64	0.380	0.768
Low-energy	3, 64	1.732	0.169
Sweet	3, 64	2.644	0.057

Table A6. *Ranking scores for the MTPRT for DAF participants (n=71) were tested with a GLM to assess the potential influence of etiology on the ranking scores. Food preference scores were used as dependent variables, etiology as fixed factor and age, sex and hunger as covariates.*

Category	Degrees of freedom	F-value	P-value
Carbohydrates	4, 63	0.797	0.532
Fat	4, 63	2.537	0.049
Protein	4, 63	0.365	0.832
Low-energy	4, 63	3.279	0.017
Sweet	4, 63	2.892	0.029

Table A7. *Ranking scores for the MTPRT for DAF participants (n=71) were post-hoc tested with the Bonferroni procedure to assess the influence of etiology on the ranking scores per group for fat, low-energy and sweet.*

		Congenital (n=14)	Cold/flu (n=13)	Head trauma (n=19)	Chronic inflammation (n=8)	Other causes (n=17)
Fat	Congenital	-	0.853	0.304	1.000	0.034
	Cold/flu	0.853	-	1.000	1.000	1.000
	Head trauma	0.304	1.000	-	1.000	1.000
	Inflammation	1.000	1.000	1.000	-	1.000
	Other	0.034	1.000	1.000	1.000	-
Low-energy	Congenital	-	1.000	0.115	1.000	0.084
	Cold/flu	1.000	-	0.281	1.000	0.296
	Head trauma	0.115	0.281	-	1.000	1.000
	Inflammation	1.000	1.000	1.000	-	1.000
	Other	0.084	0.296	1.000	1.000	-
Sweet	Congenital	-	0.174	0.066	1.000	1.000
	Cold/flu	0.174	-	1.000	1.000	0.599
	Head trauma	0.066	1.000	-	0.808	0.271
	Inflammation	1.000	1.000	0.808	-	1.000
	Other	1.000	0.599	0.271	1.000	-

Appendix B: Food intake

Table B1. *Scores for the separate components of the Eetscore for the DAF population (n=105) were compared to the reference component scores by means of a one-sample Wilcoxon signed rank test. The Benjamini-Hochberg procedure was applied to control for multiple comparisons, using a false discovery rate (Q) of 0.05.*

	P-value	Rank	Q	M	Critical value	Test
SFA	0.8400	8	0.05	8	0.050000	0
Vegetables	0.4370	7	0.05	8	0.043750	0
Fruit	0.3260	6	0.05	8	0.037500	0
Fish	0.2350	5	0.05	8	0.031250	0
Alcohol	0.0030	4	0.05	8	0.025000	1
Salt	0.0020	3	0.05	8	0.018750	1
TFA	0.0001	2	0.05	8	0.012500	1
Fiber	0.0001	1	0.05	8	0.006250	1

Table B2. *Scores for the separate components as well as the total adherence score for the Eetscore for the DAF population (n=105) were tested with a GLM to assess the potential influence of duration on the scores. The scores were used as dependent variables, etiology as fixed factor, and age and sex as co-variates.*

Category	Degrees of freedom	F-value	P-value
Vegetables	3, 99	0.294	0.829
Fruit	3, 99	1.373	0.256
Dietary fiber	3, 99	1.984	0.121
Fish	3, 99	0.303	0.823
Saturated fatty acids	3, 99	0.181	0.909
Trans fatty acids	3, 99	0.871	0.459
Salt	3, 99	3.238	0.025
Alcohol	3, 99	0.526	0.666
Total score	3, 99	0.456	0.714

Table B3. *Separate component scores for the Eetscore for the DAF participants (n=105) were post-hoc tested with the Bonferroni procedure to assess the influence of during on the scores per group for salt.*

		< 5 years (n=37)	5-10 years (n=20)	> 10 years (n=32)	Congenital (n=16)
Salt	< 5 years	-	0.050	0.478	0.172
	5-10 years	0.050	-	1.000	1.000
	> 10 years	0.478	1.000	-	1.000
	Congenital	0.172	1.000	1.000	-

Table B4. *Scores for the separate components as well as the total adherence score for the Eetscore for the DAF population (n=105) were tested with a GLM to assess the potential influence of etiology on the scores. The scores were used as dependent variables, etiology as fixed factor, and age and sex as covariates.*

Category	Degrees of freedom	F-value	P-value
Vegetables	4, 98	0.648	0.629
Fruit	4, 98	0.386	0.818
Dietary fiber	4, 98	1.526	0.201
Fish	4, 98	1.201	0.315
Saturated fatty acids	4, 98	0.204	0.935
Trans fatty acids	4, 98	0.738	0.568
Salt	4, 98	0.669	0.615
Alcohol	4, 98	0.428	0.788
Total score	4, 98	0.862	0.490

Chapter 3

Chemosensory perception and food preferences in colorectal cancer patients undergoing adjuvant chemotherapy

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Abstract

Background and aim: Cancer is one of the major public health problems, with colorectal cancer being one of the most occurring types of cancer. During treatment, patients may experience changes in their nutritional intake due to side-effects of treatment, like changes in chemosensory perception, i.e. smell and taste function. This study investigated alterations in chemosensory perception and food preferences in colorectal cancer patients during and after adjuvant chemotherapy.

Methods: To investigate changes during chemotherapy, patients undergoing adjuvant chemotherapy (n=15) were measured before the start, halfway through (approximately 3 months after the start of adjuvant chemotherapy), and within one month after the end of chemotherapy. As a comparison group, colorectal cancer patients not undergoing chemotherapy (n=20), underwent the same measurements at similar time points. To measure changes after treatment, chemosensory perception and food preferences of patients who had undergone chemotherapy treatment were measured once, either at 6, 12 or 24 months after diagnosis (n=20 for all time points). Objective olfactory and gustatory function were measured with the Sniffin' Sticks and the Taste Strips test. Subjective smell and taste perception were determined with a questionnaire, while food preferences were assessed with a computer-based ranking task. Changes during treatment were assessed using linear mixed model analyses, and changes after treatment were assessed with a one-way ANOVA or a Kruskal Wallis test.

Results: Objective olfactory and gustatory function did not differ statistically significantly between any of the groups and at any time point during or after treatment (all $p > 0.05$). In contrast, subjective smell ($F(1,84)=8.17$, $p=0.005$) and taste ($F(1,99)=4.08$, $p=0.046$) perception were rated statistically significantly lower by patients undergoing chemotherapy than the comparison group during treatment. At 6 months after diagnosis, patients who underwent

chemotherapy rated their subjective taste perception significantly lower than patients at 12 and 24 months after treatment ($F(2,57)=12.05$, $p=0.002$). Food preferences did not change during treatment, or thereafter (all $p > 0.05$). However, preference for protein-rich foods was positively correlated with objective gustatory function ($r=0.36$, $p<0.001$).

Conclusions: Similar to other cancer patient populations, mainly subjective smell and taste perception are affected in colorectal cancer patients undergoing adjuvant chemotherapy. Changes in objective olfactory and gustatory function in relation to chemotherapy were not detected by the tests used in our study nor did food preferences change. However, it should be noted that subjective changes in smell and taste perception can affect subsequent flavor perception and food enjoyment, which might negatively impact eating behavior and nutritional intake.

Introduction

Cancer is one of the major public health problems. In 2018, worldwide 18.1 million new cancer cases were diagnosed, while cancer led to 9.6 million deaths [1]. To reduce mortality [2] and increase quality of life [3,4] it is important that patients adhere to specific lifestyle guidelines, including dietary recommendations [5], during as well as after treatment.

During treatment, cancer patients often have difficulties in adhering to a healthy diet and adequate nutritional intake due to physical as well as psychological effects of treatment [6]. Patients treated with chemotherapy can experience acute and long-term side-effects, which include peripheral neuropathy, fatigue, gastrointestinal symptoms and changes in olfactory and gustatory function [7,8]. In patients undergoing chemotherapy, changes in gustatory function occur more frequent (45-84% of patients) than changes in olfactory function (5-60% of patients) [9]. These changes have a high impact on daily life and can add to the disease-related distress experienced by patients [10,11]. Research among cancer patients undergoing chemotherapy [12–17] showed that changes in olfactory and gustatory function occur in various cancer patient populations. Patients reported both quantitative changes, like a decreased gustatory function [18], and qualitative changes, for example a constant metallic taste [19]. Overall, reported changes in chemosensory perception are often temporary and disappear within months after the end of treatment [12,20,21].

Smell and taste are crucial for inducing appetite and sensing nutrients [22], and changes herein may therefore lead to altered food preferences, reduced appetite and food aversions [6,18,23,24]. As a result, changes in olfactory and gustatory function can lead to altered dietary intake [25,26], which may eventually cause an impaired nutritional status in cancer patients.

Colorectal cancer is one of the most occurring types of cancer [27]. A commonly used form of treatment for colorectal cancer is surgical resection, followed by adjuvant chemotherapy for more advanced tumor stages. For patients

with rectal cancer, radiotherapy with or without chemotherapy might also be applied in a neo-adjuvant setting. In studies on self-reported changes in olfactory and gustatory function in colorectal cancer patients undergoing chemotherapy, 50% [28] and 72% [11] of the patients reported changes in gustatory function and reduced food enjoyment. However, more objective and extensive measures of chemosensory perception and food preferences in this patient population are lacking.

Given the high prevalence of colorectal cancer and the detrimental consequences that might result from changes in olfactory and gustatory function, it is highly relevant to examine these changes and their potential dietary consequences more systematically. The aim of this study was therefore to investigate changes in objective and subjective olfactory and gustatory function in colorectal cancer patients during and after chemotherapy and their effect on food preferences.

Methods

The current study was part of the COLON study, a large multi-center prospective cohort study, set up to investigate associations of diet and other lifestyle factors with quality of life, survival and recurrence of disease in colorectal cancer patients [29]. The protocol for the current study was approved by the ethical committee CMO Regio Arnhem/Nijmegen (NL30446.091.09). All patients agreed on participating in the study by signing an informed consent.

Study design and study population

The current study investigated chemosensory perception and food preferences in colorectal cancer patients during as well as after chemotherapy (Figure 1). Patients in the COLON database who fit the criteria with regard to treatment and timing of treatment in relation to the measurements as described below were approached for participation in the study.

During treatment, 20 colorectal cancer patients undergoing adjuvant chemotherapy were included. Due to complications or extended hospital stays, 5 patients dropped out after the first measurement, resulting in 15 patients considered for analyses As a comparison group, 20 colorectal cancer patients who did not undergo adjuvant chemotherapy were included. In both groups, patients were tested at three time points. Patients undergoing chemotherapy were tested after surgery, but before the start of chemotherapy (T1), halfway through chemotherapy (T2; approximately 3 months after the start of adjuvant chemotherapy), and within one month after the end of chemotherapy (T3; depending on the number of cycles of chemotherapy completed). For the comparison group similar moments were chosen, resulting in test sessions approximately within 3 weeks after diagnosis, 3 months after diagnosis and 6 months after diagnosis.

After treatment, 60 colorectal cancer patients who had previously undergone adjuvant chemotherapy were included for one test session after en-

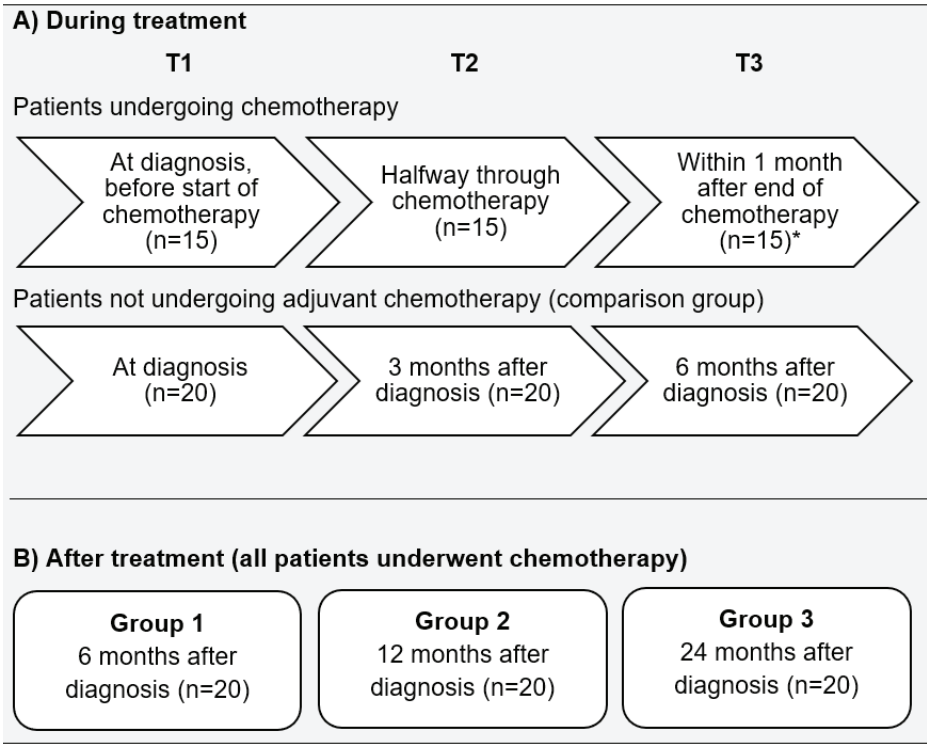

Figure 1. *Schematic study design, showing numbers of patients and time points of test sessions during and after treatment. * Timing of the test session was dependent on the number of cycles of chemotherapy completed. A) In total, 15 (chemotherapy group) and 20 (comparison group) patients were measured at all 3 time points. B) At all time points, 20 patients were measured. These patients differed for the different time points and did not overlap with the 35 patients included in the first part of the study ('during treatment').*

ding chemotherapy. Time points for measurements were 6 months after diagnosis (n=20), 12 months after diagnosis (n=20) or 24 months after diagnosis (n=20).

Stage of disease and treatment characteristics were obtained from the Dutch Colorectal Audit (DCRA) collected as part of the COLON study. Current body weight and height, and smoking habits were self-reported by the patients.

Measurements

Measurements took place at the patients' home. In the case of multiple test sessions per patient, they were performed on approximately the same time of the day. All tests were carried out in the order as described in the following paragraphs. Patients were instructed not to smoke and not to drink or eat anything except water 15 minutes before the measurements. Additionally, patients were asked not to wear perfume of aftershave at the day of measurement.

Objective olfactory function

The Sniffin' Sticks were used to measure objective olfactory function [30]. This test assesses odor threshold, odor discrimination ability and odor identification ability. A forced choice procedure was applied for all separate parts of the test. During the threshold test and the discrimination test patients were blindfolded.

The odor threshold was determined by using 16 triplets of pens with a different concentration of n-butanol, using dilutions in a ratio of 1:2 in a geometric series starting from a 4% n-butanol solution as described in [30]. Per triplet, one pen contained the odor, while the other two pens contained solely solvent. Patients had to pick out the odor-containing pen during a staircase up-down procedure. The average of the last four turning points was calculated as score (T, score: 1-16). During the discrimination task, 16 triplets of pens were offered in a randomized order. All triplets contained two similar pens and one pen with an aberrant odor, which the patients had to pick out. Also within the triplets the order of the pens was randomized. Score was the number of correctly identified pens (D, score: 0-16). The identification test consisted of 16 pens that were a combination of pens from the basic identification test and the extended identification test [31]. This combination was randomized for each test session. Patients were asked to identify each odor by selecting the correct descriptor from a list of four descriptors. Score was the number of correctly identified pens (I, score: 0-16).

The scores of the three tests were summed up to a total TDI-score

(score: 1-48). A higher score represents a better olfactory function. The total score was used to categorize olfactory function: functional anosmia (TDI ≤ 16); hyposmia (16 > TDI < 30.75) or normosmia (TDI ≥ 30.75) [32].

Objective gustatory function

The Taste Strips were used to measure objective gustatory function. The test contained 16 impregnated filter papers, which were impregnated with sweet taste (0.05, 0.1, 0.2, or 0.4 g/ml sucrose), sour taste (0.05, 0.09, 0.165 or 0.3 g/ml citric acid), salty taste (0.016, 0.04, 0.1 or 0.25 g/ml sodium chloride) or bitter taste (0.0004, 0.0009, 0.0024 or 0.006 g/ml quinine hydrochloride) [33]. The tastes qualities were randomized within a concentration and the strips were then presented in order of increasing concentration. Patients had to identify the taste of each strip by placing it in the mouth and choosing between sweet, sour, salty, bitter or tasteless according to a forced choice procedure. After each taste strip, patients took a sip of water to rinse the mouth and neutralize the palate.

A correct identification/response yielded 1 point; the score ranged from 0-4 for the individual taste qualities and from 0-16 points for the total test, in which a higher score represented a better gustatory function. A score of ≥ 10 points was considered as normal gustatory function [33].

Subjective olfactory and gustatory function

Patients filled out the Appetite, Hunger and Sensory Perception (AHSP) questionnaire [34]. The questionnaire included questions on subjective smell perception (6 items, score range: 6-30) and subjective taste perception (8 items, score range: 8-40). All questions were answered on a 5-point Likert scale. A higher score represented a more positive perception of current olfactory and gustatory function.

Food preferences

To assess food preferences, the Macronutrient and Taste Preference Ranking Task (MTPRT) was used [35]. This computer-based task was performed in E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA). Within the task, liking and preference ranking for four macronutrient categories (carbohydrates, fat, protein and low-energy), and two taste qualities (sweet and savory) were assessed.

The task consisted of 32 pictures of food products: eight pictures for each macronutrient category. For high-carbohydrates, high-fat and low-energy, this included pictures of four sweet and four savory food products. For high-protein, only pictures of savory food products were included as sweet foods high in protein are limited in our food supply.

Liking for all products in the MTPRT was measured on a 100 point VAS anchored by 'do not like at all' and 'like extremely'. All pictures were shown one by one.

Preference ranking scores were determined by presenting four pictures at the same time, which patients had to rank in the order they would prefer to eat the products at that moment. The ranking task consisted of two parts: the first part included sixteen combinations of four pictures, representing all macronutrient categories. Preference scores were calculated for the macronutrient categories with the following formula: ranking score = (4*(#rank1) + 3*(#rank2) + 2*(#rank2) + 1*(#rank4))/16, yielding a score of 1-4. The second part included twelve combinations of four pictures, which represented a sweet and a savory product from two macronutrient categories. Preference scores for sweet and savory were calculated with the following formula: ranking score = (4*(#rank1) + 3*(#rank2) + 2*(#rank2) + 1*(#rank4))/24, yielding a score of 1.5-3.5 [35].

Data analysis

Statistical analyses were performed in IBM SPSS Statistics (version 25). Normality of the data was checked with the Shapiro-Wilk test. All p-values < 0.05

were considered statistically significant. All data are presented as mean ± SD or as N (%), unless mentioned otherwise.

During treatment, objective and subjective olfactory and gustatory function and food preferences were analyzed with a linear mixed model to assess differences over time and between patient groups. Patients were added as subjects variable, while time point of measurement was added as repeated variable. A diagonal covariance structure was applied. Fixed factors in the model were group (chemotherapy or comparison group), time point (T1, T2 or T3 as described in the study design), and the interaction between group and time point. Dependent variables were scores for the Sniffin' Sticks, Taste Strips, subjective smell or taste perception as calculated from the AHSP, liking or ranking scores for the macronutrient categories carbohydrates, fat, protein and low-energy products and the taste quality sweet. For the liking part of the MTPRT also the liking score for the taste quality savory was studied; for ranking this score was not studied, as sweet and savory ranking scores are each other's opposites by definition. When significant results were found, the Dunn-Bonferroni procedure was applied to further explore these.

After treatment, a one-way ANOVA was used to compare scores for the Sniffin' Sticks and food preferences between groups (6, 12 or 24 months after diagnosis). A Kruskal Wallis test was used to compare scores for the Taste Strips and subjective smell and taste perception, between groups. When significant results were found, the Dunn-Bonferroni procedure was applied to further explore these.

Correlations between subjective and objective smell and taste function and food preferences, both during and after treatment, were assessed with Pearson correlations. The Benjamini-Hochberg procedure was applied to the correlations for food preferences to account for multiple comparisons, using a false discovery rate of 0.05 [36].

Results

Patient characteristics

Patients measured during treatment were on average similar in age and BMI. There were more former smokers and more men among patients undergoing chemotherapy compared to comparison group. Most patients undergoing chemotherapy had a stage III tumor, while the patients in the comparison group mostly had a stage I or stage II tumor. The majority of the tumors was located in the colon. Patients who were measured after treatment were on average similar in age and BMI. There were less men included at T1 compared to T2 and T3; moreover, at T1 the number of patients with a colon tumor was higher than at T2 and T3 (table 1).

Olfactory function

During treatment, there were no significant differences for overall Sniffin' Sticks score between patients undergoing chemotherapy and the comparison group ($F(1,99)=0.50$, $p=0.48$), between time points ($F(2,66)=0.57$, $p=0.57$) or for the interaction between group and time point ($F(2,66)= 0.15$, $p=0.86$; figure 2a), nor for separate scores for threshold, discrimination and identification (appendix A, table A1). For subjective smell perception, there was a significant effect of group ($F(1,84)=8.17$, $p=0.005$), with patients undergoing chemotherapy rating their subjective smell perception significantly worse than the comparison group. There was no significant effect of time point of measurement ($F(2,56)=1.75$, $p=0.18$) or the interaction between group and time point ($F(2,56)=0.10$, $p=0.90$; figure 2b).

After treatment, overall Sniffin' Sticks score was similar for the patient groups at different time points ($F(2,57)=0.33$, $p=0.72$; figure 2c), as well as the separate scores for threshold, discrimination and identification (appendix A, table A2). Subjective smell perception also did not differ between the groups ($F(2,57)=1.32$, $p=0.52$; figure 2d).

Table 1. *Demographic characteristics at diagnosis for the patients measured during treatment and at time point of measurement for patients measured after reatment; medical characteristics were obtained upon time of diagnosis. BMI: body mass index; CAPOX: chemotherapy regimen of capecitabine + oxaliplatin; N/A: data not available*

	During treatment		**After treatment**		
	Chemotherapy (n=15)	Comparison (n=20)	T1 (n=20)	T2 (n=20)	T3 (n=20)
Age (years, mean ± SD)	66 ± 7.7	67 ± 8.8	63 ± 9.1	65 ± 8.9	66 ± 4.7
Sex (% male)	13 (87%)	14 (70%)	10 (50%)	13 (65%)	13 (65%)
BMI (kg/m2 mean ± SD, range)	28 ± 2.7	26 ± 3.7	27 ± 3.9	27 ± 4.6	26 ± 2.7
Smoking, N (%)					
Never	2 (13%)	12 (60%)	7 (35%)	10 (50%)	9 (45%)
Former	13 (87%)	8 (40%)	13 (65%)	10 (50%)	10 (50%)
Current	0	0	0	0	1 (5%)
Tumor stage*, N (%)					
I	0	8 40%)	1 (5%)	0	0
II	0	6 (30%)	3 (15%)	2 (10%)	1 (5%)
III	11 (73%)	1 (5%)	15 (75%)	15 (75%)	17 (85%)
IV	1 (7%)	0	0	2 (10%)	2 (10%)
N/A	3 (20%)	5 (25%)	1 (5%)	1 (5%)	0
Tumor location					
Colon	10 (67%)	12 (60%)	19 (95%)	15 (75%)	19 (95%)
Rectum	2 (13%)	4 (20%)	1 (5%)	4 (20%)	1 (5%)
N/A	3 (20%)	4 (20%)	0	1 (5%)	0
Chemotherapy regimen, N (%)					
CAPOX	5 (33%)	-	15 (75%)	11 (55%)	17 (85%)
Capecitabine	4 (27%)	-	2 (10%)	4 (20%)	1 (5%)
N/A	6 (40%)	-	3 (15%)	5 (25%)	2 (10%)

* Stages as defined by American Joint Committee on Cancer (AJCC) staging manual (8th edition)

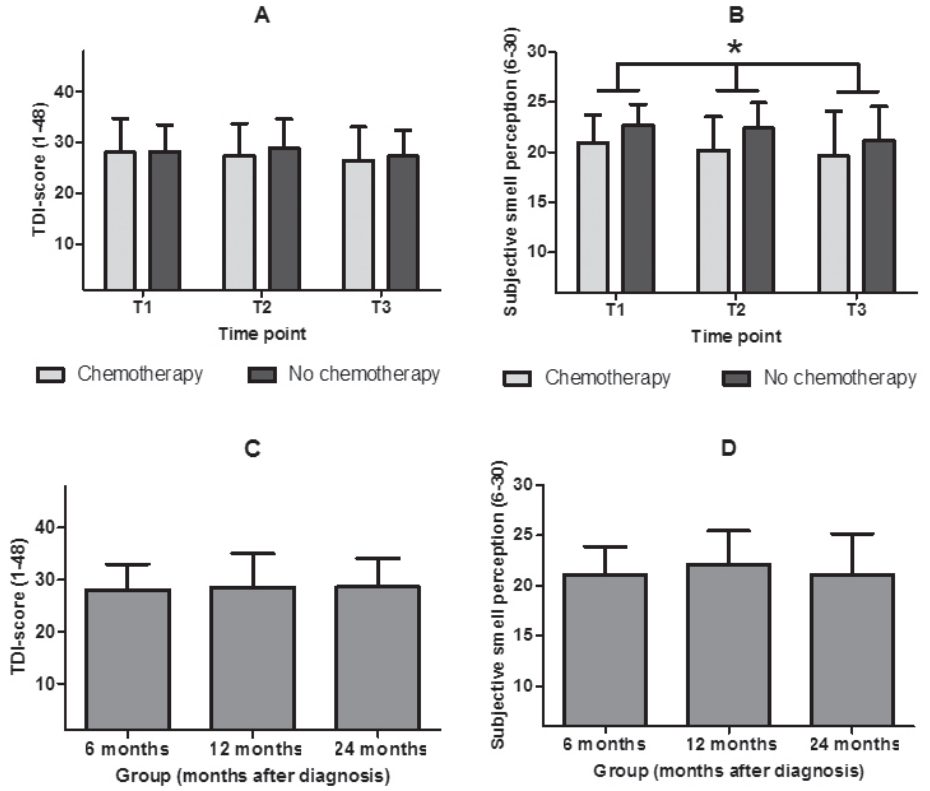

Figure 2. *Mean scores ± SD for a) objective and b) subjective olfactory function during treatment for all three time points and for c) objective and d) subjective olfactory function after treatment. An asterisk indicates a statistically significant difference between groups.*

Gustatory function

During treatment, there were no statistically significant differences for overall Taste Strips score between the patient groups ($F(1,98)=0.32$, $p=0.57$), between time points ($F(2,69)=0.09$, $p=0.92$) or for the interaction between group and time point ($F(2,69)=0.93$, $p=0.40$; figure 3a), nor for separate scores for sweet, sour, salty and bitter taste (appendix A, table A1). For subjective taste perception, a significant effect of group was found ($F(1,99)=4.08$, $p=0.046$). Post hoc testing showed that patients undergoing chemotherapy rated their subjective taste perception significantly worse than the comparison group. There was no

significant effect of time point of measurement (F(2,65)=0.62, p=0.54) or the interaction between group and time point (F(2,65)=2.43, p=0.10; figure 3b).

After treatment, overall Taste Strips scores were similar for the groups at different time points (F(2,57)=0.50, p=0.78; figure 3c), as well as the separate scores for sweet, sour, salty and bitter (see appendix A, table A2). For subjective taste function, there was a significant difference among groups at different time points (F(2,57)=12.05, p=0.002; figure 3d). Post-hoc testing showed that 6 months after diagnosis, patients rated their subjective taste perception significantly worse than patients at 12 and 24 months after diagnosis.

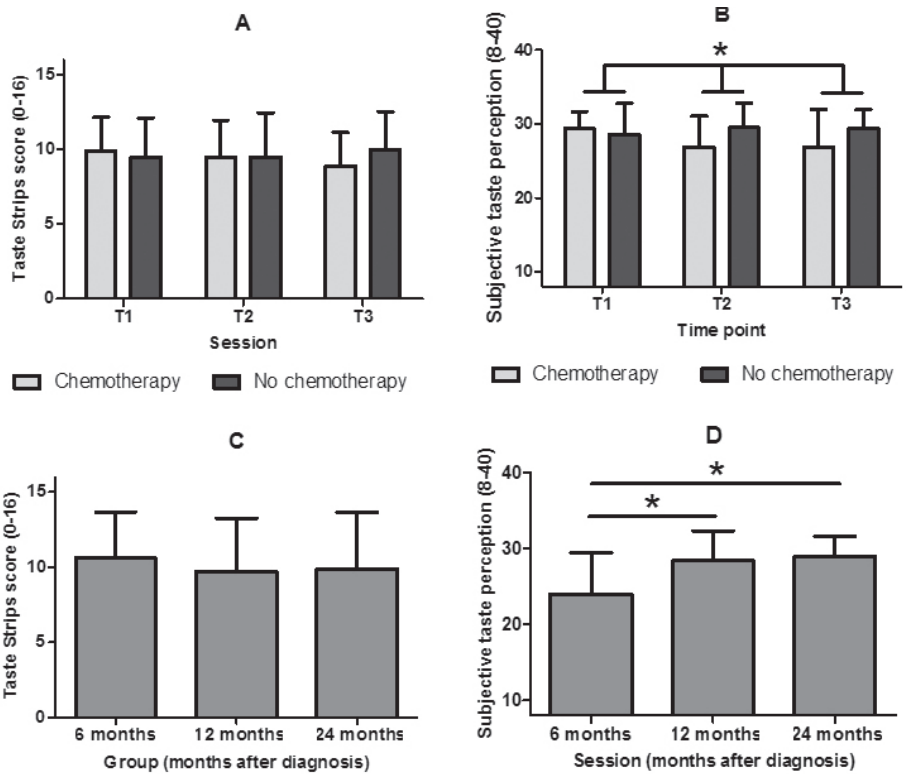

Figure 3. *Mean scores ± SD for a) objective and b) subjective gustatory function during treatment for all three time points and for c) objective and d) subjective gustatory function after treatment. An asterisk indicates a statistically significant difference between groups.*

Correlations between objective and subjective olfactory and gustatory function

Both during and after treatment, subjective, but not objective, smell and taste function, were positively correlated (Table 2). Additionally, objective and subjective smell function showed a positive correlation, while objective and subjective taste function were not correlated.

Table 2. *Correlations between objective and subjective olfactory and gustatory function during and after treatment; significant correlations are highlighted in bold.*

Timing of measurement	Objective smell – objective taste	Objective smell – subjective smell	Objective taste – subjective taste	Subjective smell – subjective taste
During (n=35)	r=0.05, p=0.63	**r=0.42, p<0.001**	r=0.04, p=0.69	**r=0.50, p<0.001**
After (n=60)	r=0.18, p=0.18	**r=0.38, p=0.003**	r=0.20, p=0.87	**r=0.32, p=0.013**

Food preferences

During treatment, only liking for sweet tasting foods was statistically significantly different among groups ($F(1.97)=5.60$, $p=0.02$): patients undergoing chemotherapy liked sweet foods less compared to the comparison group. No other effects of group or session were found on liking of any of the macronutrients or the taste quality savory (all $p>0.05$). For ranking, both patients undergoing chemotherapy and the comparison group showed the lowest preference scores for high-carbohydrate foods and the highest preference scores for high-protein foods at all time points. There were no effects of group or session on ranked preferences for any of the macronutrients nor for sweet tasting foods (all $p>0.05$) (appendix B, table B1).

After treatment, there were no significant differences in any of the liking scores between time points for any of the macronutrients or taste qualities (all

p>0.05). For ranking, high-carbohydrate foods were least preferred at all time points. At 6 months after diagnosis, high-protein foods and low-energy products were ranked highest, while at 12 and 24 months after diagnosis only high-protein foods were ranked highest. There were no significant differences in any of the preference scores between time points for any of the macronutrients or for sweet tasting foods (all p>0.05; appendix B, table B2).

No significant correlations between any of the liking or preference scores and objective olfactory function, subjective smell perception or subjective taste perception were found during treatment. However, objective gustatory function was significantly positively correlated to preference for high-protein foods and negatively correlated to preference for low-energy products (table 3). After treatment, there were no significant correlations between any of the liking or preference scores and objective olfactory and gustatory function or subjective smell and taste perception.

Table 3. *Correlations between objective gustatory function and food preference scores during and after treatment; significant correlations, after adjustment with the Benjamini-Hochberg procedure, are highlighted in bold.*

Timing of measurement	Carbo-hydrates	Fat	Protein	Low-energy	Sweet
During (n=35)	r=-0.18, p=0.06	r=0.09, p=0.35	**r=0.36, p<0.001**	**r=-0.28, p=0.004**	r=-0.22, p=0.03
After (n=60)	r=0.31, p=0.02	r=0.06, p=0.65	r=-0.20, p=0.13	r=-0.02, p=0.89	r=0.04, p=0.76

Discussion

This study aimed to investigate chemosensory perception and food preferences in colorectal cancer patients undergoing chemotherapy as compared to colorectal cancer patients who did not receive adjuvant chemotherapy, during and after treatment. There were no statistically significant differences in objective olfactory and gustatory function between patients undergoing chemotherapy and the comparison group nor after treatment. However, subjective smell and taste perception were rated worse by patients during chemotherapy than by those who did not undergo adjuvant chemotherapy. Patients undergoing chemotherapy rated their subjective taste perception significantly lower 6 months after diagnosis, than patients at 12 and 24 months after diagnosis. Food preferences did not differ between the groups nor at any time point.

Patients undergoing chemotherapy rated their subjective smell and taste perception significantly worse than the comparison group, while we did not find any differences in objective olfactory and gustatory function. These results are in line with previous studies, that also mainly reported changes in subjective smell and taste perception in other populations of cancer patients during and shortly after chemotherapy treatment, despite heterogeneity in patient groups and measuring methods [37]. We used the ASHP to assess subjective smell and taste perception [34], as was also done in previous studies by De Vries et al. [26] and IJpma et al. [16], while other studies [38,39] used the Chemotherapy-induced Taste Alteration Scale [40]. The latter questionnaire also includes questions on experiencing aberrant tastes, which might not be detected by objective clinical tests, such as the Taste Strips. This concurs with the fact that we did find a correlation between objective and subjective olfactory perception, but not between objective and subjective gustatory perception. Experiencing a constant metallic taste [19,41] is one of the most self-reported taste-related complaints in cancer patients undergoing chemotherapy [42]. However, metal-

lic taste is not considered one of the basic tastes [43]. It is therefore questionable whether experiencing a metallic taste can actually be seen as a change in taste perception, or should rather be regarded as altered flavor perception [44]. Patients might perceive changes in flavor perception as alterations in chemosensory perception regardless of objectively detectable changes in olfactory and gustatory function. Our results highlight the importance of subjective testing in addition to objective testing, as subjective smell and taste perception play an important role in eating behavior in daily life.

We found no changes in food preferences over the course of chemotherapy treatment, which confirms results from previous studies on food preferences in other specific cancer patient populations based on the same [12] or a comparable task [15,16]. However, we did find that a lower objective gustatory function was correlated with lower preference for high-protein foods. It is known that cancer patients often have difficulties maintaining an adequate protein intake [45] and that high-protein foods like meat are often reported to be aversive by patients undergoing chemotherapy [18,46]. A qualitative study among patients undergoing a chemotherapy regimen containing oxaliplatin, which was also used in the current patient population, showed that changes in taste perception in these patients were mostly related to broader changes in flavor perception and food enjoyment [44]. Moreover, in patients with metastatic or irresectable esophagogastric cancer, lower self-reported taste function similarly correlated with a lower preference for protein. These patients underwent the same chemotherapy regimen as patients in the current study, although in palliative setting [47]. Therefore, changes in taste perception can alter flavor perception, which might subsequently impact food preferences and nutritional intake in cancer patients undergoing chemotherapy.

A particular strength of the study was the inclusion of a baseline measurement (i.e. before start of chemotherapy) for all patients that were measured during treatment. Moreover, we included a group of colorectal cancer patients who

did not undergo adjuvant chemotherapy as a comparison group, to investigate changes in olfactory and gustatory function that may occur specifically as result of chemotherapy treatment, and their duration, rather than these effects occurring as results of the cancer or other treatment procedures.

However, for the interpretation of our results, it should be taken into account that our study population included patients who were diagnosed with different stages of colorectal cancer. As the stage of disease affects the treatment that is applied [48], research in a homogeneous population of patients in the same stage of disease is warranted to further investigate the effect of the tumor as well as other (preceding) treatment procedures. Furthermore, it should be noted that chemotherapy strategies might have deviated for individual patients, for example due to experienced treatment-related toxicity.

In the current study, actual dietary intake was not measured. Our study in breast cancer patients showed that side-effects of treatment, such as changes in subjective taste perception, a dry mouth and difficulties in chewing, were associated with a lower energy intake and specifically lower intake of protein and fat during chemotherapy [26]. This suggests that subjective complaints, which might not be detected by objective clinical tests, and subsequent changes in flavor perception, are relevant in the context of eating behavior. To gain further insights in the relation between changes in flavor perception and eating behavior, a next step would be to investigate olfactory and gustatory function as well as actual food intake in the same patient group.

Changes in olfactory and gustatory function should be detected early during treatment to allow early interventions and to prevent the detrimental effects they may can result in. A recent trial showed that smell and taste training during chemotherapy can improve gustatory function in patients with changes in gustatory function. However, this did not directly improve quality of life or decrease the risk of malnutrition [49]. As changes in gustatory function can lead to a reduced energy intake [50], it would be highly relevant to investigate the

relation between this training and dietary intake, which was not included in the current study.

Conclusion

This study showed that in colorectal cancer patients undergoing chemotherapy mainly subjective olfactory and gustatory function are affected during and shortly after treatment. Objective changes in olfactory and gustatory function in relation to chemotherapy were not detected by the clinical tests used in our study. However, the combination of alterations in subjective smell and taste perception might lead to a changed flavor perception, which can impact food preferences and eating behavior. Our results provide leads to specifically consider protein intake in patients who are affected by (subjective) changes in gustatory function. As an adequate nutritional status is important during treatment and recovery and improves wellbeing of patients, colorectal cancer patients would benefit from recommendations on dietary intake that are tailored to changes in olfactory and gustatory function.

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Appendix A: Objective and subjective smell and taste scores

Table A1. *Separate and total objective smell and taste scores and subjective smell score during treatment; scores are shown as mean ± SD. Statistically significant differences are indicated in bold.*

	Patients undergoing chemotherapy			Comparison group			Group		Time point		Interaction	
	T1	T2	T3	T1	T2	T3	F	P	F	P	F	P
Threshold	5.9 ± 2.87	6.6 ± 3.22	5.2 ± 3.08	6.6 ± 2.84	7.3 ± 2.60	6.6 ± 2.74	2.53	0.12	1.17	0.32	0.19	0.83
Discrimination	10.7 ± 2.40	10.8 ± 2.18	10.0 ± 2.62	10.7 ± 2.05	10.6 ± 2.01	9.9 ± 1.94	0.07	0.78	1.33	0.27	0.01	0.99
Identification	11.5 ± 2.92	10.1 ± 2.74	11.3 ± 2.46	10.9 ± 2.69	11.2 ± 2.80	10.9 ± 2.21	0.004	0.95	0.42	0.66	0.97	0.39
Total TDI-score	28.1 ± 6.63	27.4 ± 6.36	26.4 ± 6.65	28.2 ± 5.29	29.0 ± 5.68	27.3 ± 5.13	0.50	0.48	0.57	0.57	0.15	0.86
Subjective smell	20.9 ± 2.79	20.2 ± 3.32	19.6 ± 4.52	22.7 ± 2.06	22.4 ± 2.54	21.1 ± 3.46	**8.17**	**0.005**	1.75	0.18	0.10	0.90
Sweet	2.8 ± 0.78	3.1 ± 0.88	2.5 ± 0.74	3.0 ± 0.83	2.8 ± 0.95	3.1 ± 1.00	0.93	0.34	0.27	0.76	1.64	0.20
Sour	1.8 ± 0.78	1.8 ± 0.94	2.0 ± 0.76	1.9 ± 0.85	2.0 ± 0.92	2.4 ± 0.93	1.59	0.21	1.43	0.25	0.19	0.83
Salty	2.6 ± 1.06	2.2 ± 0.86	1.8 ± 0.68	2.3 ± 1.14	2.3 ± 1.13	2.3 ± 0.92	0.36	0.55	1.65	0.20	1.32	0.27
Bitter	2.7 ± 1.11	2.4 ± 1.40	2.5 ± 1.41	2.2 ± 1.24	2.4 ± 1.23	2.4 ± 1.18	0.76	0.39	0.01	0.99	0.31	0.74
Total taste score	9.9 ± 2.26	9.5 ± 2.45	8.9 ± 2.23	9.5 ± 2.61	9.5 ± 2.96	10.1 ± 2.52	0.32	0.57	0.09	0.92	0.93	0.40
Subjective taste	29.4 ± 2.29	26.9 ± 4.15	26.9 ± 5.04	28.6 ± 4.22	29.5 ± 3.33	29.4 ± 2.54	**4.08**	**0.046**	0.62	0.54	2.43	0.10

Table A2. *Separate and total objective and subjective smell and taste scores after treatment; scores are shown as mean ± SD. Different letters indicate a significant difference between time points.*

	6 months after diagnosis (n=20)	12 months after diagnosis (n=20)	24 months after diagnosis (n=20)	F	P
Threshold	6.4 ± 2.07	6.8 ± 3.25	6.0 ± 2.73	0.44	0.64
Discrimination	11.3 ± 2.43	10.4 ± 2.37	11.9 ± 2.28	1.79	0.18
Identification	10.3 ± 2.69	11.2 ± 2.69	11.5 ± 2.21	1.32	0.28
Total TDI-score	28.0 ± 4.97	28.5 ± 6.54	28.6 ± 5.50	0.33	0.72
Subjective smell	21.1 ± 2.77	22.1 ± 3.36	21.1 ± 4.09	1.32	0.52
Sweet	2.9 ± 1.02	2.7 ± 1.03	3.0 ± 1.12	1.20	0.55
Sour	2.2 ± 0.93	2.1 ± 1.17	2.1 ± 0.76	0.31	0.86
Salty	3.0 ± 0.92	2.4 ± 1.46	2.6 ± 1.35	1.62	0.44
Bitter	2.5 ± 1.54	2.5 ± 1.43	2.3 ± 1.33	0.56	0.76
Total taste score	10.6 ± 3.07	9.7 ± 3.56	9.9 ± 3.74	0.50	0.78
Subjective taste	24.0 ± 5.47[a]	28.4 ± 3.91[b]	28.9 ± 2.70[b]	12.05	0.002

Appendix B: Macronutrient and taste liking and preference scores

Table B1. *Liking and preference scores for all macronutrients and the taste categories sweet and savory during treatment; scores are shown as mean ± SD. For ranking only the score for sweet is shown, as this is the opposite of the score for savory. Statistically significant differences are indicated in bold.*

	Patients undergoing chemotherapy			Comparison group			Group		Time point		Interaction	
	T1	T2	T3	T1	T2	T3	F	P	F	P	F	P
Liking												
Carbohydrates	50.6 ± 18.96	53.9 ± 19.59	49.2 ± 17.49	56.6 ± 13.45	57.7 ± 13.16	57.6 ± 15.65	3.58	0.06	0.23	0.79	0.17	0.84
Fat	63.3 ± 14.30	65.3 ± 17.16	65.0 ± 11.64	65.9 ± 12.45	67.5 ± 15.09	66.6 ± 14.98	0.58	0.45	0.14	0.87	0.009	0.99
Protein	67.1 ± 10.84	67.7 ± 13.37	67.7 ± 14.25	65.8 ± 16.27	68.6 ± 13.80	70.2 ± 11.47	0.06	0.81	0.31	0.74	0.17	0.85
Low-energy	63.2 ± 15.87	63.9 ± 14.82	61.6 ± 14.97	65.3 ± 12.62	67.1 ± 12.83	68.4 ± 13.57	2.11	0.15	0.07	0.93	0.26	0.77
Sweet	61.9 ± 10.45	64.3 ± 13.10	63.1 ± 9.79	69.1 ± 14.89	69.5 ± 14.74	69.1 ± 12.93	5.60	0.02	0.09	0.92	0.05	0.96
Savory	61.8 ± 8.85	63.0 ± 10.37	60.3 ± 9.39	63.0 ± 9.35	65.1 ± 9.66	65.9 ± 10.87	2.42	0.12	0.26	0.77	0.47	0.63
Preference												
Carbohydrates	2.1 ± 0.48	2.0 ± 0.51	2.0 ± 0.56	2.1 ± 0.53	2.1 ± 0.54	2.1 ±. 051	0.80	0.37	0.001	1.00	0.06	0.94
Fat	2.6 ± 0.55	2.5 ± 0.48	2.5 ± 0.49	2.6 ± 0.50	2.6 ± 0.50	2.6 ± 0.50	0.21	0.65	0.45	0.64	0.07	0.93
Protein	2.9 ± 0.38	2.9 ± 0.55	2.9 ± 0.56	2.7 ± 0.81	2.9 ± 0.76	2.7 ± 0.70	1.21	0.28	0.08	0.93	0.23	0.80
Low-energy	2.4 ± 0.78	2.6 ± 0.79	2.6 ± 0.75	2.5 ± 0.55	2.4 ± 0.60	2.6 ± 0.64	0.001	0.97	0.18	0.83	0.48	0.62
Sweet	2.7 ± 0.25	2.6 ± 0.29	2.7 ± 0.23	2.7 ± 0.56	2.7 ± 0.52	2.8 ± 0.47	0.72	0.40	0.09	0.91	0.03	0.97

Table B2. *Liking and preference scores for all macronutrient and taste categories after treatment; scores are shown as mean ± SD.*

	6 months after diagnosis (n=20)	12 months after diagnosis (n=20)	24 months after diagnosis (n=20)	F	P
Liking					
Carbohydrates	49.7 ± 16.34	50.5 ± 14.34	54.7 ± 15.56	0.604	0.55
Fat	60.9 ± 17.45	67.1 ± 12.31	69.0 ± 13.34	1.711	0.19
Protein	63.9 ± 19.60	67.0 ± 17.94	68.3 ± 19.76	0.277	0.76
Low-energy	74.2 ± 12.29	64.5 ± 12.98	68.9 ± 13.09	2.902	0.06
Sweet	65.8 ± 9.82	65.0 ± 12.59	71.3 ± 10.41	1.955	0.15
Savory	64.5 ± 10.52	62.1 ± 9.86	64.6 ± 12.60	0.338	0.72
Preference					
Carbohydrates	2.0 ± 0.51	2.1 ± 0.36	2.0 ± 0.34	0.059	0.94
Fat	2.6 ± 0.63	2.8 ± 0.45	2.7 ± 0.52	0.746	0.48
Protein	2.7 ± 0.77	3.0 ± 0.76	2.9 ± 0.66	0.626	0.54
Low-energy	2.7 ± 0.74	2.2 ± 0.54	2.5 ± 0.55	3.023	0.06
Sweet	2.7 ± 0.35	2.7 ± 0.24	2.7 ± 0.27	0.097	0.91

Chapter 4

Automatic quantification of the olfactory bulb volume in MRI scans using convolutional neural networks

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Abstract

The olfactory bulb (OB) plays a key role in the central olfactory pathway. The volume of the OB and its plasticity are important for diagnosis, prognosis and treatment of patients with olfactory loss, like patients with primary olfactory loss or patients with olfactory loss related to neurodegenerative diseases. Until now, measurements of OB volume have been limited to time consuming manual segmentations, hampering large scale studies. Hence, the aim of this study was to exploit convolutional neural networks to achieve automatic and fast measurements of OB volume in MRI scans of patients with primary olfactory loss. As reference, we used OB volumes based on manual measurements.

Manual measurements consisted of applying planimetric manual contouring of which subsequently segmentations were obtained that were used as reference for the automatic segmentation task. To automatically quantify OB volumes, each OB was automatically localized and segmented. Evaluation was performed on MRI scans from two sets of patients (N=66 and N=42), that differed from each other with respect to the field strength used to obtain the MRI scans (3T or 1.5T) and acquisition parameters, such as repetition time, echo time, slice thickness, and the number of coronal slices. Automatic segmentation was evaluated using the Dice coefficient and the average symmetrical surface distance between the manual segmentation and automatic OB segmentations. Moreover, volumes determined from the manual and automatic segmentation were compared.

For segmentation of the olfactory bulbs, a Dice coefficient above 0.8 and average symmetrical surface distance below 0.24 mm were achieved. Volumes determined from the manual and automatic segmentations were significantly correlated (total OB volume: $p<0.001$).

These results demonstrate that automatic segmentation and quantification of OB in MRI scans can be performed accurately. These automated measurements can be utilized in both research and health care and may lead to more

insight in the role of the OB in diagnosis, prognosis and treatment of olfactory loss. Moreover, they may enable early detection and follow-up of disease-related olfactory loss.

Introduction

On a daily basis, humans are exposed to thousands of different odors. These can be pleasant odors, like the smell of a tasty food. In contrast, odors can also function as a warning signal, for example to detect leaking gas. Moreover, the sense of smell plays an important role in several professions: wine tasters and perfumers are known to have a better sense of smell than the general individual [1]. In spite of the importance of the sense of smell, 3% up to 20% of the general population exhibits olfactory loss [2–4]. These individuals often report several complaints related to their olfactory loss, such as a decreased quality of life, issues with daily safety or a diminished appetite [2,5,6]. To be able to treat these patients, it is important to gain a better understanding of the human olfactory pathway.

As the olfactory bulb is the first recipient of odor signals in the human brain, it is an important part of the olfactory pathway. Typically, odor perception starts when odor molecules from the air enter the nose and bind to receptors in the olfactory epithelium on the roof of the nasal cavity [7]. The activated olfactory receptor neurons transmit signals to the olfactory bulb in the brain, from where they are transferred to the primary olfactory regions of the brain [7]. Volume of the olfactory bulb has been associated with several outcome measures, like olfactory function (e.g. [8–10]) and volumes of other olfactory regions of the brain (e.g. [11,12]). Moreover, olfactory bulb volume is affected in patients with primary olfactory loss, for example patients with olfactory loss after traumatic brain injury [11], after upper respiratory tract infection [13] or due to chronic rhinosinusitis [14,15]. All these patient populations have in common that they show a reduced olfactory bulb volume in comparison to healthy individuals. It is known that the volume of the olfactory bulb can play a role in prognosis for recovery in patients with primary olfactory loss: a larger olfactory bulb volume is related to a better recovery of smell ability [10]. Moreover, olfactory training [16] and medical treatment, like a functional endoscopic sinus surgery [15,17,18]

were both found to be related to an increase in olfactory bulb volume in patients with olfactory loss. The volume of the olfactory bulb and its plasticity therefore seem to be important for diagnosis, prognosis and treatment of patients with primary olfactory loss.

Several patient populations are affected by disease-related changes in their sense of smell. The relation between olfactory loss and olfactory bulb volume has been studied in patient populations in which olfactory loss is a prominent feature, for example patients with depression [19,20], schizophrenia [21,22] and patients with neurodegenerative diseases [23,24]. These studies found reduced volumes of the olfactory bulb in patients compared to volumes in health controls. Therefore, olfactory bulb volume can be an early indicator of the onset of disease or be an indicator for being at risk of disease. The importance of the volume of the olfactory bulb in disease and related outcome measures shows that studying the volume of this brain area is clinically relevant. In addition, more research might lead to a better understanding of the role of the olfactory bulb in relation to functioning of the brain during disease in general.

The olfactory bulb is a small structure. Therefore, a specialized scanning sequence is needed to visualize the olfactory bulb with a structural MRI scan. Manual segmentation is the commonly used method to measure olfactory bulb volume in these scans [25,26]. This method includes manual tracing of the outlines of the olfactory bulb in all slices that display the olfactory bulb to calculate total volume. Manual segmentation allows comparisons of groups within a study or comparison of relative differences in volume between studies. However, while most previous work shows a high intra- and interrater reliability of repeated measurements within studies (see e.g. [17,26]), differences among observers may still be present between studies. This makes it problematic to compare absolute volumes between different studies, or to establish cut-offs for olfactory bulb volume abnormalities that can be applied to different patient populations. Additionally, the current manual segmentation method is time con-

suming, as it takes on average 10 minutes per patient for a trained observer. In recent years, deep learning techniques, especially convolutional neural networks (CNNs), have become increasingly popular for the automatic analysis of medical images [27]. These automated algorithms allow processing of large datasets in a short timeframe with increased reproducibility compared to manual analysis. CNNs were previously used to measure small volumes in the brain in MRI scans, such as white matter hyperintensities [28].

The aim of this study was to exploit CNNs for automatic and fast segmentation of olfactory bulb and subsequent measurements of their volume from anatomical MRI scans. The automatic segmentation and volume measurements were evaluated against manual reference in clinical patients exhibiting primary olfactory loss.

Methods

Study population

This study used two different datasets including clinical patients with olfactory loss. Dataset 1 was obtained from the Smell and Taste Center in Ede, The Netherlands. This dataset contained patients who visited the Smell and Taste Center between August 2015 and July 2017 and who signed an informed consent on the use of their patient files for research. In total, 100 patients were included as a random sample. Dataset 2 was obtained from the Smell and Taste Center at the University Hospital Carl Gustav Carus in Dresden, Germany. This dataset contained patients who were clinically diagnosed with olfactory loss, and who signed an informed consent on the use of their patient files for research. In total, 70 patients were included from this dataset.

In both datasets, patients' objective smell ability was measured using the Sniffin' Sticks test [29]. The scores of the test (score: 1-48) were used to categorize patients in one of the olfactory functioning groups: functional anosmia (TDI ≤ 16); hyposmia (16 > TDI < 30.75) or normosmia (TDI ≥ 30.75) [30].

This study was approved by the review committee for scientific research of Hospital Gelderse Vallei, Ede, the Netherlands (BC/1703-143).

MRI image acquisition

MRI scans in dataset 1 were acquired on a 3T Siemens Magnetom Verio scanner (Siemens, Erlangen, Germany). For each scan, a 32-channel head coil was used. To image the olfactory bulb, a coronal T2-weighted 2D turbo spin-echo scan of 28 slices was made, using GRAPPA factor 2 (repetition time: 4630 ms; echo time: 153 ms; field of view: 205 x 256 mm; in-plane voxel size: 0.47 mm; slice thickness: 1.0 mm (no gap); 28 slices; flip angle= 145◦; total scan time: 4.30 minutes).

For dataset 2, MRI scans were acquired on a 1.5T Siemens Prisma scanner (Siemens, Erlangen, Germany). To image the olfactory bulb, a 32-chan-

nel head coil was used and a coronal T2-weighted sequence was made (repetition time: 2300 ms; echo time: 2.98 ms; field of view: 256 x 240 mm; in-plane voxel size: 0.47 mm; slice thickness: 1.2 mm; 32 slices; flip angle= 9◦; total scan time: 9.20 minutes).

Manual segmentations of olfactory bulb volume

The volume of the left and right olfactory bulb was determined by applying planimetric manual contouring (PMC) as manual segmentation method. For dataset 1, MIPAV software (version 7.4.0, Centre for Information Technology, National Institutes of Health, Bethesda, Maryland, USA) was used. For dataset 2, AMIRA software (version 6.0, Department for Scientific Visualization, Zuse Institute Berlin (ZIB)) and AVIZO software (version 9.4, ThermoFisher Scientific, Waltham, Massachusetts, USA) were used.

PMC was done on all slices of a scan according to a standardized protocol as described previously [31]. The measurement started with the selection of slices on which the olfactory bulbs were visible between the posterior parts of the eyeballs in the coronal plane. The first slice with a visually detectable olfactory bulb was used at the starting point of the measurement. The olfactory bulbs were delineated manually in each successive slice of the brain. The change in diameter at the beginning of the olfactory tract was used to define the end of the olfactory bulb [25,31]. The surface area of both bulbs in each slice was calculated and the total surface area was multiplied with the slice thickness to obtain the volume of the left and right olfactory bulb in mm3. Additionally, for each olfactory bulb the center of the corresponding surface area was computed and served as reference landmark location for the center of the bulb.

To minimize variability in the dataset for training of the algorithm, only two measurements of the same bulb that differed < 10% in volume were included. In dataset 1, manual measurements were conducted in duplicate by one observer on different days. When the repeated measurements differed more than 10% in volume from each other, a third measurement was conducted to

obtain two measurements that differed less than 10%. In dataset 2, manual segmentations were performed by two independent observers on different days. The observers were blind to any patient characteristics.

For the final dataset, 61 patients from dataset 1 and 36 patients from dataset 2 were included. Additionally, respectively 5 and 6 patients with no (visible) olfactory bulb were added to dataset 1 and 2, to train the CNN to recognize the absence of an olfactory bulb. Patient characteristics are described in Table 1.

Training of the neural network

Automatic segmentation of the olfactory bulbs in MRI scans was performed in three consecutive steps (Figure 1). First, two localization CNNs were used to automatically localize the center of the left and right olfactory bulb, respectively [32]. These landmarks were used to define region of interest containing the bulbs that were used as input for the segmentation CNN [33–35] that automatically segmented olfactory bulbs in each slice. Finally, bulbs volumes were determined from the obtained segmentations.

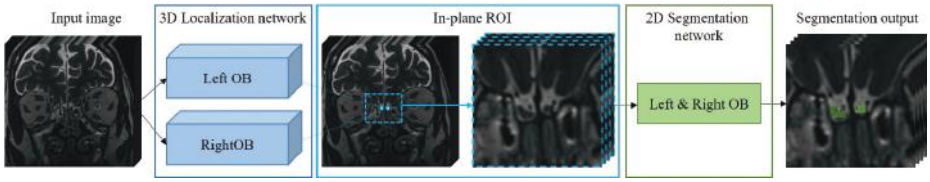

Figure 1. *Automatic segmentation of the olfactory bulbs in MRI scans using convolutional neural networks (CNNs). First, two CNNs (3D Localization network) are used to localize the center of the left (Left OB) and right olfactory bulb (Right OB), respectively. Subsequently, an ROI containing both olfactory bulbs is extracted and used as input for the segmentation CNN (2D Segmentation network) which automatically segments both olfactory bulbs to determine their volume.*

Table 1. *Patient characteristics for both datasets; characteristics are displayed as mean ± SD or N (%).*

	Dataset 1 (N=66)	**Dataset 2 (N=42)**
Age (years)	59 ± 16.3	54 ± 15.4
Male/female ratio	28/38 (42%/58%)	17/25 (40%/60%)
Duration of olfactory loss		
0-2 years	15 (23%)	24 (57%)
2-5 years	16 (24%)	8 (19%)
5-10 years	16 (24%)	3 (7%)
> 10 years	15 (23%)	2 (5%)
Whole life	4 (6%)	5 (12%)
Cause of olfactory loss		
Idiopathic	22 (34%)	8 (19%)
Chronic rhinosinusitis	20 (30%)	0
Post-infectious	12 (18%)	29 (69%)
Trauma	4 (6%)	0
Congenital	4 (6%)	5 (12%)
*Other**	4 (6%)	0
Sniffin' Sticks score	16.1 ± 7.6**	17.0 ± 7.0
Smell disorder		
Functional anosmia	34 (57%)	23 (38%)
Hyposmia	3 (5%)	21 (50%)
Normosmia	17 (40%)	4 (10%)

* = toxic/drugs (N=3) and iatrogenic (N=1)

** = could only be determined for 60 patients due to missing data

Automatic localization of the olfactory bulbs

Because olfactory bulbs are very small, there is a large class imbalance between pixels labelled as foreground, i.e. olfactory bulb, and pixels labelled as background. Therefore, to deal with the class imbalance and to simplify the segmentation task, first the center of each olfactory bulb was automatically localized using our previously proposed approach for detection of anatomical landmarks [32]. In our previous work, we employed a network that performed regression of 3D displacement vectors that point from the center of an analyzed patch towards the landmark location. In addition, the network simultaneously performed classification of image patches based on the presence of the landmark in the patch. To obtain the final landmark location, the output of both tasks were combined by computing a weighted average landmark location. For this, predicted displacement vectors were used to obtain an estimated landmark location for each analyzed patch while posterior classification probabilities were used as weights to indicate the importance of each patch during averaging of the estimated landmark locations [32]. In the current work, to localize the center of the olfactory bulbs, two networks were used - one for the left and one for the right olfactory bulb (Figure 2).

The architecture of both CNNs was identical (Figure 2). In our previous work, localization CNNs were used to localize landmarks in coronary CT angiography (CCTA) scans [32], which are large scans (512x512 voxels in-plane) compared to the MRI scans used in our current study (256x256 voxels in-plane). Therefore, to adjust to the smaller scan size, the last convolutional layer of the network was removed. Therefore, in our current work a localization CNN did not contain six but five convolutional layers (Figure 2), which reduced the receptive field of the network from 70x70x70 voxels to 55x55x55 voxels. Each convolutional layer contained 32 (3x3x3) kernels, of which the first were each followed by a (2x2x2) max-pooling layer. Subsequently, the network was split into two output streams: one for the regression task and one for the classification task. Both output streams were similar in design and contained two dense

layers, with 64 or 96 kernels, respectively, and one output layer. The output layer for prediction of the displacement vectors (regression task) predicted the displacement in the coronal, sagittal and axial direction. The output layer for the classification of image patches (classification task) predicted whether the landmark of interest was present in an image patch or not and outputted posterior classification probabilities between 0 and 1.

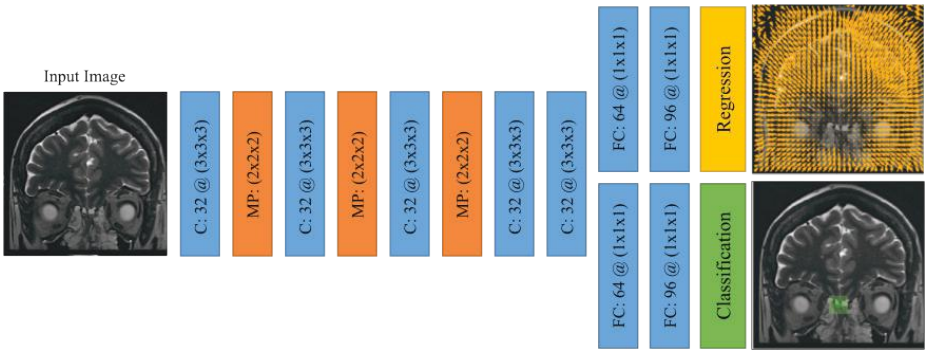

Figure 2. *Architecture of the convolutional neural network (CNN) used to automatically localize the center of the left or right olfactory bulb. The CNN contained 5 convolutional layers (C), each with 32 (3x3x3) kernels, 3 (2x2x2) max-pooling layers (MP), and 2 output streams: one for regression of the displacement vectors (regression) and one for classification of the image patches (classification). Both output streams contain 2 dense layers (D) with 64 and 96 (1x1x1) kernels, respectively. The output layer for the regression task contains three output nodes, one for the displacement in coronal, sagittal, and axial direction, while the output layer for the classification task contains one output node to determine the presence of the landmark in the patch.*

Before analysis, images were resampled to an isotropic voxel size of 0.47 mm to compensate for the large voxel size in the coronal direction. Contrary to our previous work [32], networks were trained during 100,000 training iterations, instead of 60,000. Furthermore, to adjust for the small number of slices in the coronal direction, during every iteration a mini-batches containing 10 randomly

sampled sub-images of size 56x56x56 voxels, instead of 72x72x72 voxels, was shown to the network.

Automatic segmentation

After automatic localization of the centers of both olfactory bulbs, the obtained landmark locations indicating the centers of the olfactory bulbs were resampled back to the original image resolution and images were cropped to in-plane ROIs of 61x61 pixels containing both olfactory bulbs. To deal with the large voxel size in the coronal direction compared to the pixel size in-plane, for automatic segmentation of the olfactory bulbs, a 2D CNN was employed that analyzed 2D coronal slices of the extracted ROIs.

The CNN architecture consisted of six convolutional layers, each with 32 (3x3) kernels. Additionally, the third and fourth convolutional layer contained dilated convolutions [33] (Figure 3). Contrary to normal convolutions, dilated convolutions have larger spacings between kernel elements [33]. By stacking convolutional layers with increasing dilation rates, the receptive field of the network can rapidly increase while the number of network parameters does not grow exponentially. The receptive field of the network was set to 21x21 pixels, while the network contained 51,234 trainable parameters. Convolutional layers were followed by two dense layers and one output layer. The output layer contained three output nodes, one for each class: background, left olfactory bulb, and right olfactory bulb, and predicted posterior classification probabilities between 0 and 1 for all classes, using the softmax activation function. To prevent overfitting, batch normalization [36] and dropout (p=0.5) [37] were applied to the dense layers.

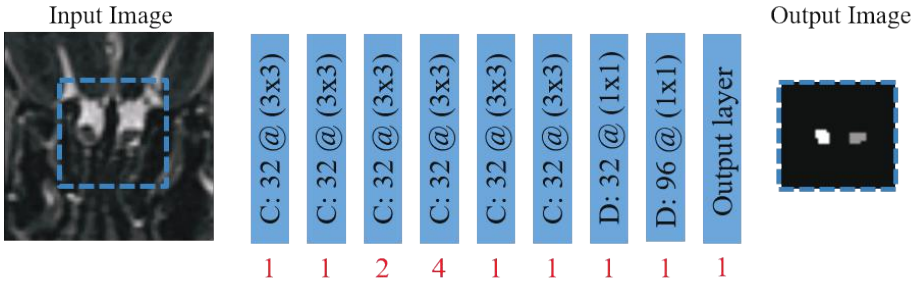

Figure 3. *Architecture of the convolutional neural network (CNN) used to automatically segment the olfactory bulbs. The CNN contained six convolutional layers (C) with each 32 (3x3) kernels, two dense layers (D), with 32 and 96 (1x1) kernels, respectively, and one output layer. The output layer contained three output nodes, one for every class: background, left olfactory bulb, and right olfactory bulb. Increasing dilatation rates in convolutional layers are indicated with red numbers. During training, sub-images with a size of 35x35 pixels are shown to the CNN of which the center 21x21 pixels are classified (dashed blue square).*

Similar to the localization networks described in section 2.5.1, the segmentation network was trained during 100.000 training iterations. In every iteration, mini-batches containing 40 randomly sampled sub-images of size 35x35 pixels were shown to the network, of which the center 21x21 pixels were classified. To alleviate class imbalance, 20% of the sub-images in a mini-batch contained foreground pixels. Furthermore, to decrease overfitting, during training, data augmentation by randomly rotating sub-images between -10 and +10 degrees was applied. Network weights were optimized with Adam [38] using the Dice coefficient as loss function [39].

Training and testing

Before experiments, dataset 1 was randomly divided into a training dataset (40 MRI scans), a validation dataset (2 MRI scans), and a test dataset (19 MRI scans). Before training, three scans without clearly visible olfactory bulbs were

added to the training data. Dataset 2 was randomly divided into a training set (20 MRI scans), a validation set (2 MRI scans) and a test set (14 MRI scans). The training and validation data was used to develop the method with the results from the manual segmentation method as reference label. During training, networks were evaluated on the validation set every 10,000 iterations. The best performing settings were defined as final parameter settings and used during testing. The test data was used for the evaluation to assess performance of the method. Note that the test data was not used during method development.

Performance of the localization networks was assessed by computing the Euclidean distance error between automatic and reference location of the center of each olfactory bulb. Evaluation of the segmentation network was performed by computing the Dice coefficient as an overlap measurement between automatic segmentations and manual segmentations. The Dice coefficient ranges from 0 (no overlap) to 1 (complete overlap). Additionally, to evaluate automatic segmentation along the bulbs surface the average symmetrical surface distance (ASSD) in millimeters was computed between automatic and manual segmentations. An ASSD of 0 mm represents a perfect match between both segmentations. The correlation between olfactory bulb volumes as measured with the manual segmentations and calculated from the segmentations from the CNN, was calculated by performing Pearson's correlation in IBM SPSS Statistics (version 25).

Results

Results for dataset 1

The first step involved the localization of the centers of the olfactory bulbs. For the left olfactory bulb, the average Euclidian distance error was 1.36 ± 0.80 mm, and for the right olfactory bulb it was 1.47 ± 0.99 mm. Secondly, the olfactory bulbs were segmented. For the left olfactory bulb, the average Dice coefficient was 0.80±0.14 and the average ASSD was 0.23±0.31 mm. For the right olfactory bulb, the average Dice coefficient was 0.82±0.10 and the average ASSD was 0.17±0.18 mm. Total time needed for the localization and segmentation of an MRI scan was 3.01 seconds. Subsequently olfactory bulb volumes were calculated. Results from the automatically obtained measurements were compared with the volumes obtained with the PMC method (Table 2).

Table 2. *Volume of the left and right OB in mm3 measured with the PMC method and the volumes based on the segmentations from the CNN in the test data of dataset 1 (n=19), including only scans with a visually detectable olfactory bulb; results are shown as mean ± SD.*

	PMC method	Automated measurements	Correlation
Volume left olfactory bulb	43.62 ± 12.90	40.04 ± 15.04	r=0.82, n=19, p<0.001
Volume right olfactory bulb	47.28 ± 12.37	42.18 ± 10.32	r=0.50, n=19, p=0.03
Total volume olfactory bulbs	90.90 ± 24.79	80.21 ± 20.83	r=0.79, n=19, p<0.001

For the left olfactory bulb, the average absolute volumetric difference was 7.49±5.42 mm3 and for the right olfactory bulb, the average absolute volumetric difference was 7.88±9.74 mm3. All volumes showed a significant moderate to strong positive correlation between results from the PMC method and the volumes calculated based on the segmentations from the CNN.

Results for dataset 2

The previously trained localization CNNs were also evaluated on dataset 2. On average, automatic localization of the center of the left and right olfactory bulb in the test set resulted in a Euclidean distance error of 3.38±2.66 and 3.54±3.44 mm. Even though distance errors were higher compared to results obtained on dataset 1, ROIs could still be successfully extracted.

Table 3 lists the evaluation measures of the results obtained on the test set with the automatic segmentation method. For the left olfactory bulb, the average Dice coefficient was 0.57±0.17 and the average ASSD was 0.45±0.56 mm. For the right olfactory bulb, the average Dice coefficient was 0.57±0.17 and the average ASSD was 0.56±0.57 mm. Therefore, to improve results, the segmentation CNN was additionally trained using the training data of dataset 2 during 40,000 iterations. To compare results, a network trained with only training data of dataset 2 and a network trained with the training data of both datasets combined were tested as well. These results show that training the network with the training data of dataset 1 combined with additional training of the network with the training data of dataset 2 yielded the best performance for dataset 2 in terms of the evaluation measures. For the left olfactory bulb, the average Dice coefficient was 0.72±0.13 and the average ASSD was 0.26±0.29 mm. For the right olfactory bulb, the average Dice coefficient was 0.74±0.10 and the average ASSD was 0.23±0.19 mm. These results are close to the average Dice coefficients and ASSDs obtained with the segmentation network trained and tested on dataset 1.

Subsequently, for this training, olfactory bulb volumes were calculated. The average absolute volumetric difference between automatically obtained measurements and volumes obtained with the PMC method was 6.57±4.63 mm3 for the left olfactory bulb and 6.59±4.43 mm3 for the right olfactory bulb.

Table 3. *Mean ± SD accuracy measures for left and right olfactory bulb volume measurements after training the segmentation network with dataset 1, dataset 2 or both datasets.*

		Left			Right		
Training set	**Test set**	**Dice**	**ASSD**	**Abs. vol. difference**	**Dice**	**ASSD**	**Abs. vol. difference**
DS1	DS2	0.57 ± 0.17	0.45 ± 0.56	11.06 ± 5.18	0.57 ± 0.17	0.56 ± 0.57	9.04 ± 5.44
DS1 - DS2	DS2	0.72 ± 0.13	0.26 ± 0.29	6.57 ± 4.63	0.74 ± 0.10	0.23 ± 0.19	6.59 ± 4.43
DS2	DS2	0.62 ± 0.25	1.52 ± 3.87	5.03 ± 4.05	0.70 ± 0.12	0.29 ± 0.24	6.52 ± 3.60
DS1 + DS2	DS2	0.66 ± 0.16	0.36 ± 0.36	8.57 ± 5.55	0.66 ± 0.10	0.28 ± 0.16	16.29 ± 5.07
DS1 + DS2	DS1	0.79 ± 0.11	0.23 ± 0.29	7.38 ± 6.73	0.80 ± 0.09	0.17 ± 0.12	9.45 ± 6.71

DS = dataset; DS 1 – DS 2 = trained on dataset 1 and additionally on dataset 2; DS 1 + DS 2 = trained on a mix of both datasets; ASSD = average symmetrical surface distance; abs. vol. difference = absolute volumetric difference in mm3

Results on MRI scans with no detectable olfactory bulbs

Clinical populations of patients with olfactory loss also include patients who have no detectable olfactory bulbs, e.g. patients with congenital anosmia [40]. Therefore, the ability of the network to recognize the absence of an olfactory bulb was evaluated by using available scans of patients with no visually detectable olfactory bulbs (Table 4). Here, the Dice coefficient and ASSD could not be calculated as manual segmentations were not possible in these scans.

Table 4. *Mean ± SD accuracy measures for left and right olfactory bulb volume measurements by the segmentation network for dataset 1 (n=2) and 2 (n=6); both datasets included only patients with no visually detectable olfactory bulb.*

Training method	Test set	Abs. vol. difference left	Abs. vol. difference right
DS1	No bulb DS1	1.53 ± 1.53	3.74 ± 3.74
DS1	No bulb DS2	2.24 ± 1.47	2.55 ± 3.72
DS1 - DS2	No bulb DS2	2.86 ± 1.72	1.67 ± 1.94
DS2	No bulb DS2	1.01 ± 0.64	0.75 ± 0.72
DS1 + DS2	No bulb DS1	0.99 ± 0.99	2.31 ± 0.77
DS1 + DS2	No bulb DS2	1.67 ± 1.60	3.34 ± 2.68

DS = dataset; DS 1 – DS 2 = trained on dataset 1 and additionally on dataset 2; DS 1 + DS 2 = trained on a mix of both datasets; abs. vol. difference = absolute volumetric difference in mm^3

Discussion and conclusion

In this study, we presented a method utilizing CNNs to automatically perform measurements of olfactory bulb volume in MRI scans. The method accurately localized the centers of the olfactory bulbs in both datasets. Moreover, segmentation of the olfactory bulbs and calculation of olfactory bulb volumes were performed with a high Dice coefficients (> 0.8) and low average symmetrical surface distance (< 0.24 mm).

Volumes based on the segmentations from the CNN had a high accuracy, and were well in line with the manual reference method. In dataset 1, the average location of the olfactory bulb was deviating less than half a voxel between the CNN and manual segmentation. Moreover, volumes of manual segmentation and the segmentation CNN were moderate to strong positively correlated. We found comparable Dice and ASSD results for segmentation of dataset 2 by using the segmentation CNN that was first trained with dataset 1 and additionally trained with dataset 2. In previous studies using manual segmentation to measure olfactory bulb volume, inter- and intra-rater reliability were used to evaluate the volumes of manual measurements conducted by different observers (see e.g. [17,26]). Inter-observer agreement based on absolute volumes is often high. However, the outcome of volumetric measurements can be highly correlated, while the actual location of the olfactory bulb may not necessarily overlap, thus making reproducibility of manual segmentation dependent on the observers. Moreover, quality of manual segmentation depends on the experience of the observers [41]. Our study showed that it is possible to determine the location of the olfactory bulb and measure its volume automated and fast by using CNNs.

Besides localization and segmentation of the olfactory bulbs, the CNNs were also able to successfully detect the absence of an olfactory bulb. In these scans, on average segmentation results yielded volumes smaller than 4 mm3. This volume is unlikely in detectable bulbs. The current datasets included pa-

tients who did not have an olfactory bulb due to congenital anosmia and patients who suffered from olfactory loss after head trauma and did not have a visible olfactory bulb. In the latter, the olfactory bulb might be damaged due to lesions caused by the head trauma [42]. However, the CNNs were also able to segment olfactory bulbs in brains that showed such a deviating anatomy. This indicates that the CNNs developed in our study could be applicable for a wide range of patients with olfactory loss.

For the current study we had access to two datasets of patients with from olfactory loss, to train and evaluate our method. These datasets were acquired on different scanners. Evaluation of the results on dataset 2 showed that the accuracy of the measurements in this dataset was lower compared to the accuracy in dataset 1. The number of scans in the training dataset of dataset 2 (20 scans) was less than half compared to the number of scans in the training dataset of dataset 1 (43 scans). Hence, the network trained on dataset 1 had more examples it could learn from compared to the network trained with only the training data of dataset 2. Therefore, enlarging the training dataset of dataset 2 could improve results. Additionally, scans from dataset 2 were made on a 1.5-T MRI scanner rather than a 3-T scanner and had a longer acquisition time, which probably led to lower quality of the data. Using a 3-T scanner and a shorter acquisition time might result in better image quality, and could therefore also yield better results. However, in clinical practice, it might not always be possible to use a high field scanner to perform scans of the olfactory bulb. Our results show that the results based on the scans obtained on a 1.5-T scanner were still accurate once additional training was performed, suggesting that the segmentation CNN can also be applied at 1.5-T scanners, although 3-T would be preferable.

Applying the segmentation CNN as developed in our study to measure olfactory bulb volume will make it easy and reliable to apply these measurements in clinical care, as these measurements are fast and not subject to observer

bias. It will enable the follow-up of olfactory bulb volume over time, as the CNN makes it possible to compare absolute volumetric differences based on scans at different time points. This can be applied to follow progression of disease over time, or for instance to monitor the effect of treatment for olfactory loss, like olfactory training, on olfactory bulb volume. This will not only improve possibilities for diagnosis and treatment, but will also lead to more insight in the relation between olfactory bulb volume and brain health in general.

Our current results are based on training the CNNs on manual segmentations. While previous studies showed that this method can lead to reliable results based on inter- and intra-rater comparisons [26,41], surprisingly little is known about how these segmentations relate to the actual volume of the olfactory bulb. In this study, manual segmentation was considered as the approximate ground truth. To determine how measurements of olfactory bulb volume in MRI scans relate to actual volume, ground truth should be measured. This would, for example, be possible by using post-mortem data. Implementing results from such ground truth measurements could be applied to improve manual measurements. Training the localization and segmentation CNNs on these improved measurements will even further increase accuracy of the CNNs.

In this study, we employed CNNs for automatic localization and segmentation of the olfactory bulbs. These segmentations produced by these networks can be used to calculate olfactory bulb volume in different patient populations, like patients exhibiting primary olfactory loss, affecting 3% to 20% of the general population, as well as in patients exhibiting disease-related olfactory loss. As there are many individual differences in volume of the olfactory bulb, for example between men and women [8,31] or due to ageing [43], it is important to train the CNNs on a broad sample of patients, including different patients populations, as well as on a sample of healthy individuals in order to obtain absolute normative data. Moreover, this allows the investigation of total brain volume as determinant of olfactory bulb volume, as total brain volume affects grey matter volume [44]. Therefore, in the future we would like to expand our dataset

to make these CNNs applicable for volumetric measurements of the olfactory bulb in both health individuals and multiple patient populations. This will allow comparisons between outcomes of different studies, not only based on relative but also on absolute volumetric differences.

To further validate the CNNs, we aim to expand our dataset with additional MRI data of various patient groups and healthy individuals on which manual segmentation of olfactory bulb volume have been performed. By this, we will increase the variability of the dataset on which the CNNs are trained, which will enable the use of our CNNs in different settings for research and in daily practice. The ultimate goal is to develop a stand-alone web-based interface that other researchers can use to obtain automatically measured OB volumes. These results could then subsequently directly be used for analysis and also be applied on the level of the individual patient. More information on the progress of this project will be available online at: https://osf.io/2vuac/.

As the results from the current datasets were promising, we consider the use of the CNNs as described in the current study as an opportunity to enable the use of automated assessment of olfactory bulb volume in clinical and research settings. This would allow further research on olfactory bulb volume in relation to disease. In neurodegenerative diseases like Parkinson's disease of Alzheimer's disease, changes in olfactory ability can be an early signal for development of disease [24,45]. Further investigations on changes in olfactory bulb volume may give more insight in the role of changes in olfactory bulb volume in detection of neurodegenerative diseases in an early stage. In the future, this might increase the possibilities for an early start of treatment [46,47].

Conclusion

In this study we used CNNs to segment olfactory bulbs in MRI scans and subsequently perform measurements of olfactory bulb volumes. The method enables automatic measurements of olfactory bulb volume in patients and saves time compared to manual segmentation. When findings are confirmed in larger studies, this approach may facilitate the use of this method for olfactory bulb volume measurements in other patient populations, for longitudinal studies on olfactory bulb volume, comparisons of bulb volume between studies, as well as meta-analyses. Automatic analysis may allow getting further insight in the role of the olfactory bulb in diagnosis, prognosis and treatment of olfactory loss.

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Chapter 5

Morphological changes in the brain due to olfactory loss: a voxel-based morphometry study on etiology and duration of olfactory dysfunction

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Abstract

Losing the sense of smell can have a major impact on daily life and is quite common: 3% up to 20% of the general population suffers from olfactory loss. Olfactory loss can change morphology of olfactory-related regions in the brain. Aim of this study was to provide insight in the role of etiology and duration of olfactory loss and olfactory function on the morphology of olfactory-related regions in the brain. The study population included 257 patients who were clinically diagnosed with olfactory loss. Patients' olfactory function was measured using the Sniffin' Sticks, and a 3T MRI scanner was used to acquire a T1-weighted scan of the brain. Voxel-based morphometry analysis was applied to segment the scans. Hereafter, an ROI analysis was conducted, including two full factorial models to investigate the effect of etiology and duration of olfactory loss on gray matter density, and a multiple regression model to investigate the relation between olfactory function and gray matter density. Both etiology and duration of olfactory loss had a significant effect on gray matter density in four clusters, representing the gyrus rectus and orbitofrontal cortex (OFC), bilaterally. Patients with congenital anosmia had reduced density in the gyrus rectus compared to patients who lost their sense of smell at later age, while the density of the OFC of patients with congenital anosmia was increased compared to the other patients for both etiology and duration of olfactory loss. Moreover, there was a significant association between density of the left OFC and olfactory function. These results imply that morphological changes in patients with acquired olfactory loss are mainly reflected in the OFC. These changes did not depend on etiology or duration of olfactory loss, but on olfactory function. Patients with congenital anosmia showed a distinct morphology of the gyrus rectus and the OFC, most likely due to the fact that they were never able to smell. Morphology of olfactory-related areas in the brain therefore seems sensitive to olfactory function and the subsequent degree of sensory input.

Introduction

Olfaction is important in daily life: odors do not only affect how food tastes, but also play a role in the detection of danger, for example smelling a fire or leaking gas. Although olfactory loss is not often discussed, 3% up to 20% of the general population suffers from olfactory loss [1–3]. The most common causes of primary olfactory loss are post-viral loss, chronic rhinosinusitis and head trauma [4]. Patients with olfactory loss report decreased quality of life, issues with daily safety and diminished appetite [5,6]. Olfactory loss can therefore have a major impact on daily life.

Olfactory loss can impact both peripheral and central processing of odors. After entering the nose, odors stimulate the olfactory epithelium located at the roof of the nasal cavity. Through the olfactory nerve, the olfactory receptor cells in the epithelium first project to the olfactory bulb, which is the key odor processing structure, located at the base of the prefrontal cortex. A larger volume of the olfactory bulb is correlated with better olfactory function, in both healthy people [7,8] and patients with olfactory loss [9,10]. Beyond the olfactory bulb, the piriform cortex, the entorhinal cortex and the amygdala are considered as primary olfactory-related areas [11]. Other regions that are known to play a role in odor processing, so-called secondary olfactory-related regions, are the orbitofrontal cortex, the anterior cingulate cortex and the insular cortex [11,12].

The central olfactory system is plastic and is affected by peripheral olfactory input. Training of the olfactory system can lead to changes in morphology of olfactory-related regions, as has been shown in for example wine tasters or perfumers [13]. These experts train their sense of smell in a professional way, which can lead to increased volume of the right insula and entorhinal cortex [14]. While these experts have an improved sense of smell due to their training, patients with olfactory loss are subject to decreased olfactory input. This might, similarly, induce morphological changes in both primary and secondary olfactory-related regions of the brain. Previous studies demonstrated a decre-

ased volume of the piriform cortex in patients with olfactory loss [15–18]. Additionally, evidence for a decrease in volume of the anterior cingulate cortex was found [15–19], as well as the orbitofrontal cortex [15–17,19–21] and the insular cortex [16–20]. Moreover, reduced olfactory input in patients with olfactory loss can lead to reorganization of neural processes [22,23]. Although it is known that olfactory loss may lead to changes in olfactory-related brain regions, so far there are no studies that compared the morphology of olfactory-related brain regions in patients with olfactory loss due to different etiologies or durations.

While most patients lose their sense of smell at a later age, some people are born without the sense of smell [24]. Previous research showed an increase of gray matter volume in the piriform cortex in patients with congenital anosmia compared to healthy controls [25,26]. Findings on morphology of the orbitofrontal cortex are conflicting. Frasnelli et al. reported an increase of cortical thickness of the medial orbitofrontal cortex bilaterally, and Peter et al. found increased gray matter volume in the medial orbital gyrus bilaterally [25,27]. In contrast, Karstensen et al. found reduced gray matter volume in the left medial orbitofrontal cortex [26]. Moreover, research on the differences in brain morphology between patients with congenital anosmia and acquired olfactory loss is non-existent.

Understanding the consequences of olfactory loss and reduced peripheral input on the olfactory system in the brain can deepen our insight in the processes that underlie morphological changes (over time). This will open up further possibilities for treatment of olfactory loss. Therefore, this study aimed to obtain more insight in the effect of olfactory loss on olfactory-related brain areas by investigating morphometry of these regions. We included a clinical population of patients with olfactory loss, containing different etiologies and durations of olfactory loss. Moreover, we investigated the relation between olfactory function and morphology of olfactory-related brain regions.

Materials and Methods

Selection of patients

The patient population consisted of 369 patients who were clinically diagnosed with olfactory loss and visited the Smell and Taste Center in Hospital Gelderse Vallei (Ede, the Netherlands) between August 2015 and December 2018. Patients were included for analysis in the current study when they: suffered from functional anosmia or hyposmia; suffering from a quantitative smell disorder; olfactory loss due to one of the following etiologies: post-viral; chronic rhinosinusitis; idiopathic; or congenital anosmia. In total, 257 patients were included for analysis. Duration of olfactory loss was divided into five categories: 0-2 years; 2-5 years; 5-10 years; > 10 years; and congenital ('never been able to smell').

All patients signed informed consent on the use of their patient data for research. This study was approved by the review committee for scientific research of Hospital Gelderse Vallei, Ede, the Netherlands (BC/1703-143).

Measurement of olfactory function

Patients' olfactory function was measured using the Sniffin' Sticks [28]. This test measures odor threshold with n-butanol (T, score: 1-16), discrimination ability (D, score: 0-16) and identification ability (I, score: 0-16). The scores of the three separate parts of the test are summed up to a composite TDI-score (score: 1-48) and were used to categorized patients into functional anosmic (TDI ≤ 16); hyposmic (16 > TDI < 30.75) or normosmic (TDI ≥ 30.75) [29]. For 10 out of 257 patients, TDI-score could not be obtained.

Image acquisition and voxel-based morphometry preprocessing

MRI data was acquired on a 3T Siemens Magnetom Verio scanner (Siemens, Erlangen, Germany). Using a 32-channel head coil, a 1-mm isotropic sagittal T1-weighted 2D MP-RAGE scan was made (TE/TR: 2.26/1900 ms; flip angle: 9o; 192 slices FoV: 256 x 256 mm).

Voxel-based morphometry (VBM) analysis was done using the Computational Anatomy Toolbox (CAT12, r1363) implemented in SPM12 (v7219, Wellcome Centre for Human Neuroimaging, UCL, London, UK) and executed in Matlab R2018 (The Mathworks, Natick, MA, USA) by following the CAT12 manual (http://dbm.neuro.uni-jena.de/cat/index.html). All images were manually reoriented to the same point of origin by using the Montreal Neurological Institute (MNI) template as a reference. Spatial normalization and tissue classification were performed by using the default settings in CAT12, including the standard tissue probability maps in SMP12 [30]. The T1-weighted images were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). Voxels were resliced to 1.5 x 1.5 x 1.5 mm and total intracranial volume (TIV), GM volume and WM volume were calculated. The gray matter volumes were used to check sample homogeneity in CAT12. There was a large average homogeneity ($r > 0.85$). Outliers were manually inspected, but no volumes were excluded due to poor quality. As a next step, the GM and WM segmentations were used to create a group-specific probabilistic template in the Diffeomorphic Anatomical Registration through Exponentiated Lie algebra (DARTEL) toolbox to refine inter-subject registration [31]. Finally, the GM and WM segmentations were smoothed with the default settings, using an isotropic 3D Gaussian kernel with a full width at half maximum of 8 mm.

The effect of etiology and duration on gray matter density was investigated using a region of interest (ROI) analysis including a priori ROIs that are known to play a role in olfactory processing from meta-analyses [11,32]. The Automated Anatomic Labeling (AAL) atlas [33] in the WFU pickatlas toolbox [34] was used to create an ROI mask containing the following regions (bilaterally): anterior cingulate cortex (ACC), amygdala, caudate, hippocampus, insula, inferior OFC, medial OFC, superior OFC, piriform cortex, entorhinal cortex, parahippocampal gyrus, putamen, gyrus rectus and superior temporal pole.

Analysis of patient characteristics

Analyses were performed with IBM SPSS Statistics (version 25). Results were considered significant at $p < 0.05$. Normality of the data was checked with the Shapiro-Wilk test. All data are reported as mean ± SD or N (%) unless mentioned otherwise.

Patients were divided into 4 groups based on etiology and 5 groups based on duration of olfactory loss, as described in section 'Selection of patients'. Groups were compared for age and Sniffin' Sticks score by means of a Kruskal Wallis test. When significant results were found, the Dunn-Bonferroni procedure was applied to further explore these.

To determine differences in total gray matter volume between the etiology or duration groups, a one-way ANCOVA was used, with total gray matter volume as dependent variable, and etiology or duration as fixed factor. Age, sex and TIV were added as covariates. When significant results were found, the Dunn-Bonferroni procedure was applied to further explore these.

MRI data analysis

Two full factorial models were used to test the effect of etiology and duration of olfactory loss on gray matter density. An additional full factorial model with etiology as factor and duration as covariate was designed to determine whether duration of olfactory loss would be a confounder in the results for etiology of olfactory loss. In all models, sex, age and TIV were added as covariates. Covariates were mean centered, and an absolute threshold of 0.2 was applied to ensure analysis was restricted to gray matter voxels only. A threshold of $p < 0.05$ family-wise error-corrected at the cluster level was employed. The MarsBaR toolbox [35] was used to extract mean GM density in significant clusters for each patient for post-hoc testing. Post-hoc testing was done in SPSS by comparing mean GM density in significant clusters between the different groups for etiology and duration using Mann-Whitney U tests.

Additionally, a multiple regression model including TDI-score as a co-

variate was used to test the association between olfactory function and regional gray matter density. As TDI-scores were not normally distributed, the scores were log-transformed. Analysis at $p < 0.05$ (FWE) at cluster level yielded no significant results. Therefore, we performed an exploratory analysis using $p < 0.001$ (uncorrected) with a cluster extent threshold of $k > 159$ voxels, based on the expected number of voxels per cluster [36].

Results

Patient characteristics

In the patient population, 148 patients (57.6%) were functional anosmic (average TDI-score: 11.1 ± 2.72) and 99 patients (38.5%) were hyposmic (average TDI-score: 22.0 ± 4.38).

Among the groups for etiology, age ($\chi2(3)=49.49$, $p < 0.001$) and olfactory function ($\chi2(3)=43.76$, $p < 0.001$) were significantly different (for differences between groups, see Table 1). There was no significant effect of etiology on total gray matter volume ($F(3,250)=1.124$, $p = 0.340$). For the groups for duration, age ($\chi2(4)=47.977$, $p < 0.001$) and olfactory function ($\chi2(4)=49.889$, $p < 0.001$) also were significantly different (for differences between groups, see Table 1). Moreover, there also was no significant effect of duration on total gray matter volume ($F(4,249)=1.993$, $p = 0.096$).

Effect of etiology on gray matter density

There was a main effect of etiology group on gray matter density within the a priori defined ROIs, showing four significant clusters: two in the gyrus rectus and two in the orbitofrontal cortex (OFC), bilaterally (Table 2 and Figure 1A). Post-hoc testing displayed that patients with congenital anosmia had reduced density in the gyrus rectus compared to the other patient groups, while OFC density in patients with congenital anosmia was increased compared to the other patient groups (Figure 1B).

Table 1. *Demographics of patients (n=257) who were included in the analysis, categorized on etiology and duration of olfactory loss.*[1]

	Age (years)	Males/ females (n)	Years of olfactory loss					Sniffin' Sticks score	Total gray matter volume (mm^3)
			0-2	2-5	5-10	> 10	Congenital		
Etiology									
Post-viral (n=87)	61 ± 11.6a	26 / 61	41	27	8	11	0	18.6 ± 6.23a	570 ± 64
Chronic inflammation (n=63)	59 ± 11.9a	39 / 24	9	8	20	26	0	14.8 ± 6.46b	584 ± 58
Idiopathic (n=80)	62 ± 13.5a	38 / 42	10	31	19	20	0	14.3 ± 5.48b	573 ± 69
Congenital (n=27)	33 ± 16.4b	12 / 15	0	0	0	0	27	10.1 ± 2.94c	634 ± 63
Duration									
0-2 years (n=60)	61 ± 10.6a	15 / 45	N/A					19.8 ± 6.43a	570 ± 60
2-5 years (n=66)	62 ± 12.4a	31 / 35	N/A					15.6 ± 5.16b	566 ± 68
5-10 years (n=47)	59 ± 14.1a	26 / 21	N/A					14.8 ± 6.26b	589 ± 67
> 10 years (n=57)	61 ± 12.9a	31 / 26	N/A					13.8 ± 5.98b,c	578 ± 62
Congenital (n=27)	33 ± 16.4b	12 / 15	N/A					10.1 ± 2.94c	634 ± 63

[1] Age, Sniffin' Sticks scores and gray matter volumes are reported as mean ± SD. Rows for etiology or duration that do not share similar superscript letters are significantly different. In case no letters are shown, there were no significant differences for that row.

Table 2. *Significant clusters from the F-test for a main effect of etiology of olfactory loss.*

	Hemi-sphere	MNI coordinates (x y z)	Z-value (peak)	Cluster size (voxels)
Gyrus rectus	L	-11 26 -20	> 8.00	941
Gyrus rectus	R	12 24 -17	7.43	694
Superior orbitofrontal cortex	R	20 36 -20	5.45	121
Medial orbitofrontal cortex	L	-20 35 -15	5.27	146

All regions are reported at $p < 0.05$ (FWE) at cluster level.

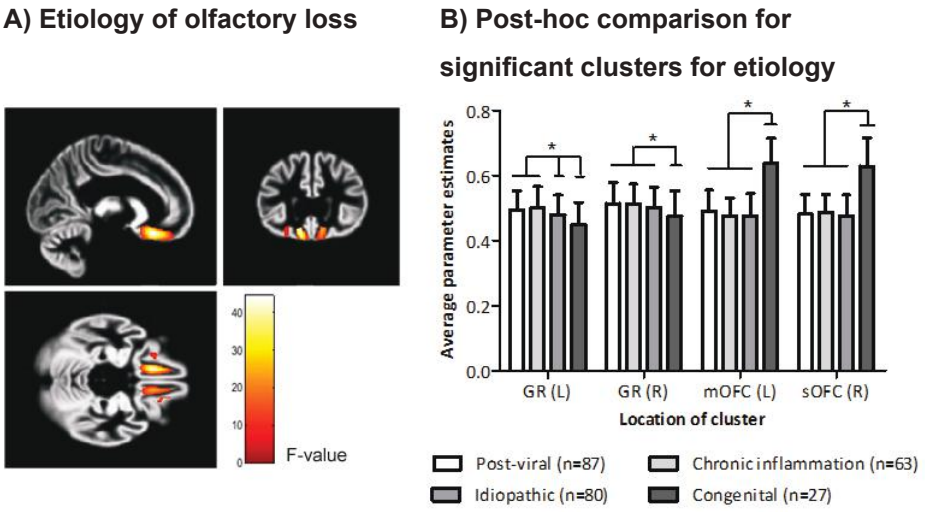

Figure 1. *A) Main effect of etiology group on ROI gray matter density in the gyrus rectus (GR) and medial and superior orbitofrontal cortex (mOFC; sOFC), reported at p < 0.05 (FWE); results are shown as F-map overlaid on the gray matter group template; B) Average gray matter density per cluster per group, reported as mean ± SD; an asterisk indicates significant differences between groups.*

Effect of duration on gray matter density

There was a main effect of duration group on gray matter density within the a priori defined ROIs, similarly revealing four significant clusters in the bilateral

gyrus rectus and OFC (Table 3 and Figure 2A). Likewise, post-hoc testing showed that patients with congenital anosmia had reduced density in the gyrus rectus but increased density in the OFC compared to the other groups (Figure 2B).

Table 3. *Significant clusters from the F-test for a main effect of duration of olfactory loss.*

	Hemisphere	MNI coordinates (x y z)	Z-value (peak)	Cluster size (voxels)
Gyrus rectus	L	-11 26 -20	> 8.00	800
Gyrus rectus	R	12 24 -17	7.34	659
Superior orbitofrontal cortex	R	20 36 -20	5.43	125
Medial orbitofrontal cortex	L	-20 35 -15	5.08	125

All regions are reported at $p < 0.05$ (FWE) at cluster level.

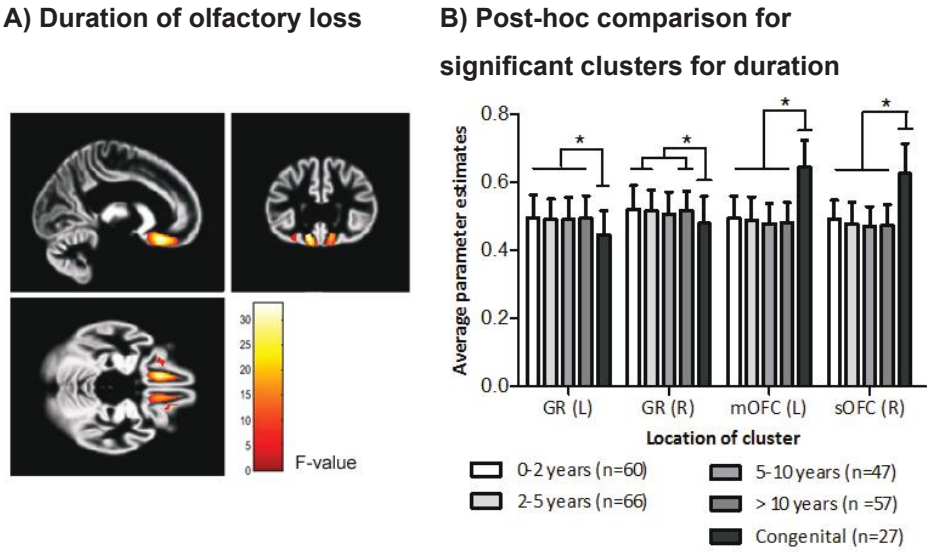

Figure 2. *A) Main effect of duration group on ROI gray matter density of the gyrus rectus (GR) and medial and superior orbitrofrontal cortex (mOFC; sOFC) for duration of olfactory loss, reported at $p < 0.05$ (FWE); results are shown as F-map overlaid on the gray matter group template; B) Average gray matter density per cluster per group per group, reported as mean ± SD; an asterisk indicates significant differences between groups.*

When adding duration of olfactory loss as covariate to the model for the effect of etiology on gray matter density, the same four significant clusters were displayed: the left (MNI (-11,26,-20), Z > 8.00) and right (MNI(-12,24,-17), Z = 6.19) gyrus rectus, and the left (MNI (-20,35,-15), Z = 5.00) and right (MNI (20,36,-20), Z = 5.54) OFC.

Relation between gray matter density and olfactory function

There was a s positive significant association between olfactory function and gray matter density in the left superior OFC (MNI(-18, 20, -15), Z = 3.73, k=193, r=0.21) (Figure 3).

A) Relation with olfactory loss **B) Relation between olfactory function and GM density**

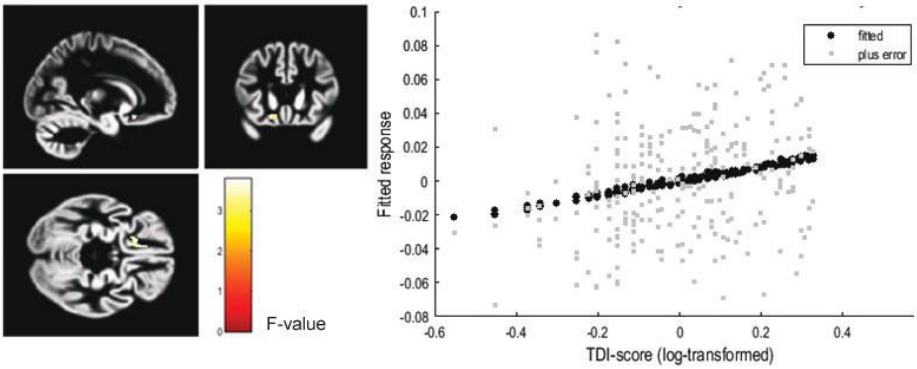

Figure 3. *Association between olfactory function and mean gray matter density in the left superior OFC (MNI coordinates: -18,20,-15), reported at p < 0.001; A) shown on the gray matter group template; B) regression showing F-values as fitted response.*

Discussion and conclusion

This study investigated morphological changes in olfactory-related brain regions in patients with olfactory loss by different etiologies and varying in duration. Etiology and duration of olfactory loss both affected the GM density of the gyrus rectus and the orbitofrontal cortex. Patients with congenital anosmia had increased density in the medial and superior OFC, while density of the gyrus rectus was decreased compared to the other patient groups. Olfactory function was positively associated with GM density of the left superior OFC.

While the gyrus rectus surrounds the olfactory sulcus, it is not commonly acknowledged as olfactory region. It is mostly related to cognitive processes, such as memory recall [37,38]. However, it has been shown to be involved in both anatomical and functional olfactory networks [32] and was therefore included as a region of interest in the current study. The gray matter density of the gyrus rectus was reduced in our patients with congenital anosmia compared to the other patient groups. Previous studies showed increased GM density in the gyrus rectus in perfumers, who have trained their olfactory function, compared to healthy controls. Moreover, increased GM density in the left gyrus rectus was associated with a longer duration of training [39]. This concurs with our finding that patients who were never able to smell have decreased GM density in this region. This finding also dovetails with the reduced GM density of the gyrus rectus seen in patients with anosmia after traumatic brain injury [19] and due to chronic rhinosinusitis [20] compared to healthy controls; both studies did not find this reduction in GM density in hyposmic patients, who had a better olfactory function. Altogether, this reduction in gray matter density in the gyrus rectus points towards an important role of the gyrus rectus in olfactory function which may depend on the degree of sensory input.

The OFC is known to be part of the secondary olfactory cortex [40] and is involved in for example odor identification [41] and hedonic processing

of odors [42]. In the current patient population, a significant positive relation between TDI-score and GM density was shown in the left superior OFC, while others found a positive correlation between TDI-scores and gray matter volume of the right OFC in healthy individuals [8,43]. Additionally, Bitter el al. demonstrated that volume of the right OFC was significantly decreased in anosmic [16] and hyposmic [15] patients compared to healthy controls, just as bilateral reduction in gray matter volume in the medial OFC in patients with olfactory loss after traumatic brain injury [19] and in the right medial OFC due to chronic rhinosinusitis [20]. We conclude that gray matter density in the OFC is associated with olfactory function in patients with olfactory loss. However, results on the specific location within the OFC from literature are conflicting and warrant further research on the exact mechanism behind this association.

Patients with congenital anosmia, who had poorest olfactory function, showed an increased density of the medial and superior OFC compared to the other patients. These results are in line with studies that compared patients with congenital anosmia to healthy controls, and found an increase in cortical thickness of the medial OFC [25] and increased gray matter volume in the medial orbital gyrus bilaterally [27]. However, they contrast with Karstensen et al., who showed a reduced gray matter volume of the medial OFC in patients with congenital anosmia [26]. While results are conflicting, they all suggest that congenital anosmia triggers changes in the OFC, due to life-long lack of sensory input. Overall, further research is needed to better understand the effect of congenital anosmia on gray matter density of the medial OFC. The mechanism behind this effect might be fundamentally different than found in patients with acquired olfactory loss, as patients with congenital anosmia were never able to smell throughout their whole life.

Neuroanatomical changes in patients with olfactory loss have so far mainly been studied with regard to etiology of olfactory loss. Our results suggest that the effect of etiology of olfactory loss on density of olfactory-related brain areas is not mediated by duration of olfactory loss, as effects of etiology

and duration were consistently apparent in the same brain regions, namely the gyrus rectus and orbitofrontal cortex. However, we did see that olfactory function decreased over time: patients with a longer duration of olfactory loss had the lowest scores on the Sniffin' Sticks, in line with previous findings [4]. We postulate that both etiology and duration of olfactory loss affect olfactory function and thereby both may result in morphological changes in the OFC and gyrus rectus following olfactory loss due to changes in the degree of sensory input.

So far, there are few possibilities for treatment of olfactory loss. In some patients, neuroanatomical changes related to olfactory loss can be reversed by olfactory training [44]. Studies on regaining olfactory ability show that olfactory training can be useful for different etiologies of olfactory loss [45] and that a shorter duration of olfactory loss is related to a higher success rate of olfactory training [46]. This indicates that the olfactory system in the brain is plastic and sensitive to peripheral input, corresponding to studies on functional connectivity networks related to olfactory processing (see e.g. [23,47]). This confirms that remaining olfactory function provides potential for treatment and recovery, as shorter duration of olfactory loss is on average is related to better olfactory function [4].

Our unique collaboration with the Smell and Taste Center in Ede allowed us to include a large population of clinically diagnosed patients (n=257), whereas other studies only included smaller and inhomogeneous samples of patients (e.g [16], n=17; [18], n=19) or only a specific group of patients (e.g. [20], n=21; [17], n=16). This allowed us to investigate etiology and duration of olfactory loss within a robust patient population. As there was no healthy control group in the current study, we chose to focus on regions in the brain that are known to play a role in olfactory processing [32], which allowed us to compare results with literature. While previous studies also reported changes in other olfactory brain regions like the piriform cortex, the anterior cingulate cortex and the insu-

lar cortex (see e.g. [15–20]), no significant changes were found in these brain regions in the current study, despite our large sample size. Possibly, changes in these brain regions occur regardless of etiology or duration of olfactory loss or olfactory function. In a follow-up, inclusion of a group of healthy control participants would be warranted to further investigate morphological changes in patients with olfactory loss.

In the current study, patients with olfactory loss after head trauma were excluded, as this can lead to morphological changes unrelated to the olfactory loss per se. Han et al. found decreased volume in the gyrus rectus and the OFC of patients with olfactory loss compared to healthy controls as well as a higher degree of gray matter reduction in patients with anosmia compared to patients with hyposmia [19], both consistent with our results. This shows that our current findings might be generalized to a more diverse patient population. However, analysis of brain lesions in patients with olfactory loss after traumatic brain injury showed that 22 out of 41 patients had lesions in the OFC [19]. This indicates that morphological changes in olfactory-related brain regions in patients with olfactory loss after traumatic brain injury should be interpreted with care.

Conclusion

All together our results show that morphological changes in olfactory-related brain regions due to olfactory loss are reflected in density of the gyrus rectus and the OFC. We suggest that morphological changes do not depend on etiology or duration of olfactory loss, but rather on olfactory function and the subsequent degree of sensory input, in patients who lost their sense of smell at a later age. Patients with congenital anosmia showed an increased density of the OFC compared to patients with acquired olfactory loss, suggestive of a compensatory mechanism due to the lack of sensory input throughout life. Our results confirm the vital role of the OFC in olfaction.

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Chapter 6

Severity of olfactory deficits is reflected in functional brain networks - an fMRI study

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Abstract

Even though deficits in olfactory function affect a considerable part of the population, the neuronal basis of olfactory deficits remains scarcely investigated. To achieve a better understanding of how smell loss affects neural activation patterns and functional networks, we set out to investigate patients with olfactory dysfunction using functional magnetic resonance imaging (fMRI) and olfactory stimulation. We used patients' scores on a standardized olfactory test as continuous measure of olfactory function. 48 patients (mean olfactory threshold discrimination identification (TDI) score = 16.33, SD = 6.4, range 6 - 28.5) were investigated. Overall, patients showed piriform cortex activation during odor stimulation compared to pure sniffing. Group independent component analysis indicated that the recruitment of three networks during odor stimulation was correlated with olfactory function: a sensory processing network (including regions such as insula, thalamus and piriform cortex), a cerebellar network and an occipital network. Interestingly, recruitment of these networks during pure sniffing was related to olfactory function as well. Our results support previous findings that sniffing alone can activate olfactory regions. Extending this, we found that the severity of olfactory deficits is related to the extent to which neural networks are recruited both during olfactory stimulation and pure sniffing. This indicates that olfactory deficits are not only reflected in changes in specific olfactory areas but also in the recruitment of occipital and cerebellar networks. These findings pave the way for future investigations on whether characteristics of these networks might be of use for the prediction of disease prognosis or of treatment success.

Introduction

Anosmia, or the loss of the sense of smell, occurs in approximately 5% up to 20% of the population, with an increase of this percentage with ageing [1]–[4]. Whereas smell ability is mostly assessed by using objective tests such as the Sniffin' Sticks test [5], structural and functional changes in the brain can also be used to explain and understand olfactory loss (for a review see [6]). Using functional magnetic resonance imaging (fMRI) to measure activity and functional connectivity during odor administration in patients with smell disorders will contribute to a fundamental understanding of how the olfactory system works and might lead to better predictions on prognosis and the effect of treatment options like olfactory training for patients suffering from smell loss.

Studies that investigate the effects of olfactory disorders on functional activity in the olfactory system during the administration of odors in general show decreased activation in olfactory areas of the brain [7]. Changes in brain activity after smell loss have been investigated extensively in patients suffering from neurodegenerative disorders, like Alzheimer's disease [8], [9] and Parkinson's disease [10], and in ageing patients [11]. A drawback of these populations is that neuronal changes related to the disease but unrelated to smell loss might distort how smell loss and remaining function are reflected in the brain.

The olfactory system of the brain can be activated by the sensorimotor act of sniffing alone, even without the presence of an odor [12]–[14]. Kollndorfer et al. found more connections between brain regions responsible for processing olfactory stimuli in healthy controls than in anosmic patients during sniffing odorless air, although there was no difference in the spatial extent of the olfactory network between the groups [15]. This indicates that sniffing and smelling are intertwined in healthy persons, but this connection seems affected in anosmic patients. Studies on repeated stimulation of the olfactory system, for example by consciously smelling odors during olfactory training, show that this stimulation can lead to activation of the neuroplasticity capacities of the brain

[12], [16], [17]. Stimulation of the olfactory system can lead to improvement of olfactory function and concurrent changes in functional networks in patients who suffer from smell loss, indicating that smell loss is not always irreversible. For example, a study by Kollndorfer et al showed an increase in functional connectivity in response to chemosensory stimulation with a trigeminal compound after olfactory training in anosmic patients [18]. This suggests that, even when patients are diagnosed with a smell disorder, functionality of the olfactory system in the brain might be maintained. However, it is not known how maintenance of the olfactory system is influenced by severity of olfactory loss. More knowledge on the neural networks within the olfactory system might lead to a better understanding of how and why olfactory training can lead to improvement of olfactory function [19] and to a better prediction of the effectiveness of olfactory training in diverse patient groups.

In this study we set out to determine how decline in olfactory functioning affects neural activation patterns and networks in the olfactory system of the brain and how this is related to the severity of smell loss. While previous studies on brain activation in olfactory disorders have focused on comparing patients to healthy controls, in our study we used patients' scores on a standardized olfactory test as continuous measure of olfactory function, enabling us to assess the impact of the severity of the smell disorder on neural activation and networks. Moreover, as described above heterogeneous patient populations might be a confounding factor in previous investigations of the neuronal alterations after olfactory loss. Therefore, in this study we only included patients who lost their sense of smell by causes that are not known to cause direct changes in the brain, like infection of the upper respiratory tract and sinonasal diseases [20]. Furthermore, previous studies indicated that group independent component analysis (ICA) can provide supplementary information in chemosensory stimulation studies in addition to model-dependent analyses [21], [22]. Therefore, in addition to traditional general linear model analyses, we applied this approach to extract functionally connected networks. Thus, in the present

study we investigated neural responses and functional networks during odor administration in a sample of anosmic and hyposmic patients.

Methods

Patient sample

A total of 124 patients suffering from olfactory dysfunction took part in the clinical care assessment offered by the Smell and Taste Centre at Hospital Gelderse Vallei (Ede, the Netherlands), in collaboration with the division of Human Nutrition of Wageningen University (Wageningen, the Netherlands). All patients visited the centre between July 2015 and October 2016. Of this initial sample, 76 patients were not included in the present study for various reasons (MRI abnormalities: 14, head trauma: 24, chronic diseases including mental health problems and cardiovascular diseases: 8, incomplete MRI or behavioral data: 18, excessive movement artifacts: 3, congenital anosmics: 8, no olfactory deficit according to the olfactory testing: 1). Assessment of MRI abnormalities was based on patients' clinical T2 scans and carried out by a radiologist. Patients exhibiting major neural alterations (such as tumors, severe white matter deviations, atrophies or early signs of neurodegenerative diseases) were excluded. Patients suffering from posttraumatic smell loss were excluded as they might show neuronal changes unrelated to their olfactory deficits [23], [24]. Congenital anosmics were not included as the low sample size did not allow treating them as a subgroup, and their neuronal processing might differ fundamentally from acquired anosmics. Thus, in the present study we analyzed data of 48 patients (29 anosmics, 19 hyposmics) suffering from olfactory loss. Patient sample characteristics are listed in Table 1. All patients gave permission for the use of their medical records for this study.

Table 1. *Detailed description of patient sample.*

	Hyposmic	**Anosmic**
N	19	29
Age [mean (SD)]	57.9 (11.34)	60.3 (14.53)
Female/male	11/8	19/10
Disease duration		
< 2 years	9	5
2-10 years	4	10
> 10 years	6	14
Cause of olfactory dysfunction		
Post-infectious	10	5
Sinonasal	7	13
*Idiopathic/other**	2	11

*Other includes ageing and medicine use.

Procedure

As part of the standard clinical care assessment, all patients participated in clinical established testing of olfactory function ([25], [26], see next section for details). The clinical assessment further comprised tests that were not included in the present analysis (such as assessment of gustatory function using "Taste Strips" [27]) and assessment of retronasal olfactory function as in [28]). Moreover, an ENT physician performed a nasal endoscopy to examine nose and mouth of the patients and conducted a medical history review to determine possible cause and duration of the disorder. All included patients took part in structural and functional MRI measurements (see section 2.2.2). The use of clinically collected data for research purposes was approved by the local ethical committee (Review committee for scientific research of Hospital Gelderse Vallei, Ede, the Netherlands; BC/1703-143). All patients provided written informed consent.

Assessment of olfactory function

Olfactory function was assessed according to the procedure described by Hummel et al [26]. Patients were presented with pen-like odor sticks ("Sniffin' Sticks", Burghart Instruments, Wedel, Germany) in three tasks, assessing odor detection threshold, odor discrimination and odor identification ability. In the odor threshold task, patients had to determine repeatedly in a forced-choice procedure which of three sticks contained a target odorant (n-Butanol). The odorant and two distractor sticks without an odor were presented in a staircase up and down procedure. Out of 7 reversals, the last 4 turning points were averaged to obtain the threshold score. In the discrimination task, 16 triplets of odorants were presented (two containing the same odorant, while one stick contained an aberrant odorant). Patients were instructed to point out the odd odorant in a forced-choice procedure. During the threshold and discrimination task, patients were blindfolded. The odor identification task consisted of 16

odors. Patients had to select the right label for each odor from a list of four descriptors provided. Odor identification was assessed for each nostril separately. For the present analysis, the average score of both nostrils was used. Threshold scores range from 1-16, while the scores for the discrimination and identification tasks range from 0-16. The three subscores were summed up to obtain the total Threshold-Discrimination-Identification score (TDI score). Based on clinical definitions [5], we distinguished anosmia (TDI score <= 16) and hyposmia (16 < TDI score < 30.3).

MRI data acquisition

All scans were acquired on a 3T Siemens Magnetom Verio MRI scanner (Siemens, Erlangen, Germany; software version VB19), using a 32-channel head coil, at Hospital Gelderse Vallei, Ede, the Netherlands. For all scans, GRAPPA factor 2 was used. A 2D echo-planar imaging (EPI) sequence was used for collecting the functional data with 847 scans and 45 axial slices (slice thickness = 3mm, matrix size of 64 × 64, TE/TR of 25/2240 ms, FoV 192 × 192 mm2, 900 flip angle). The stack was tilted at an angle of 30° to the anterior-posterior commissure line for all patients. A sagittal T1-weighted 2D isotropic MP-RAGE scan (192 slices, TE/TR of 2.26/1900 ms, slice thickness = 1 mm, FoV = 256 × 256 mm2, 90 flip angle) was acquired for anatomical reference.

Olfactory stimulation during the functional scan was performed using an 8-channel computer-controlled olfactometer (Burghart, Wedel, Germany). Odors were administered birhinally to the patient through 2 nose pieces that were placed in the nostrils of the patient. Two high caloric, pleasant food odors, equivalent in intensity and used in previous behavioral and fMRI studies [29], [30], were used: a sweet odor, chocolate (IFF, 10810180; 8.5% dissolved in propylene glycol), and a savory odor, beef (IFF, 10878095), 0.04% dissolved in demineralized water). Odor stimuli were embedded in a stream of odorless, humidified air (80%, air flow 8 L/min, 36°C). Stimulus duration of the odor pulse was 200 ms. As control, blank trials were incorporated, during which visual

cues were presented in equal length as the odor trials, while no odor was presented.

The fMRI paradigm consisted of two blocks separated by one minute rest. In total, 20 chocolate odor trials, 20 beef trials and 20 blank trials were presented. Additionally, 10 combined chocolate & picture and 10 beef & picture trials were presented. During these trials, patients were shown a picture of a chocolate muffin (chocolate & picture trials) or a steak (beef & picture trials) in addition to the odor. All trials were equally divided between the two blocks and were randomized within the blocks. All trials were preceded by a white fixation cross turning red. Patients were instructed to sniff through the nose when they saw the red fixation cross (duration 3200 ms). To sustain patients‘ attention, 30 of the odor/blank trials were followed by the question „How intense did you perceive the odor?“, with the anchors “not strong at all” and “very strong”. 11 of the combined odor & picture trials were followed by the question „How well did the picture and the odor match?“, with the anchors “not matching at all” and “very matching”. Patients responded to these questions by moving a cursor along a visual analogue scale (VAS, range 0-100) by button presses on a button box with the thumb of the right hand. The cursor always started at the center of the VAS. Trials were presented with varying inter-stimulus interval (ISI, between 11 and 20 s). Presentation of the visual cues and pictures and triggering of the olfactometer was done with the use of E-Prime 2.0 (Psychology Software Tools Inc). See Figure 1 for details on stimulus timing.

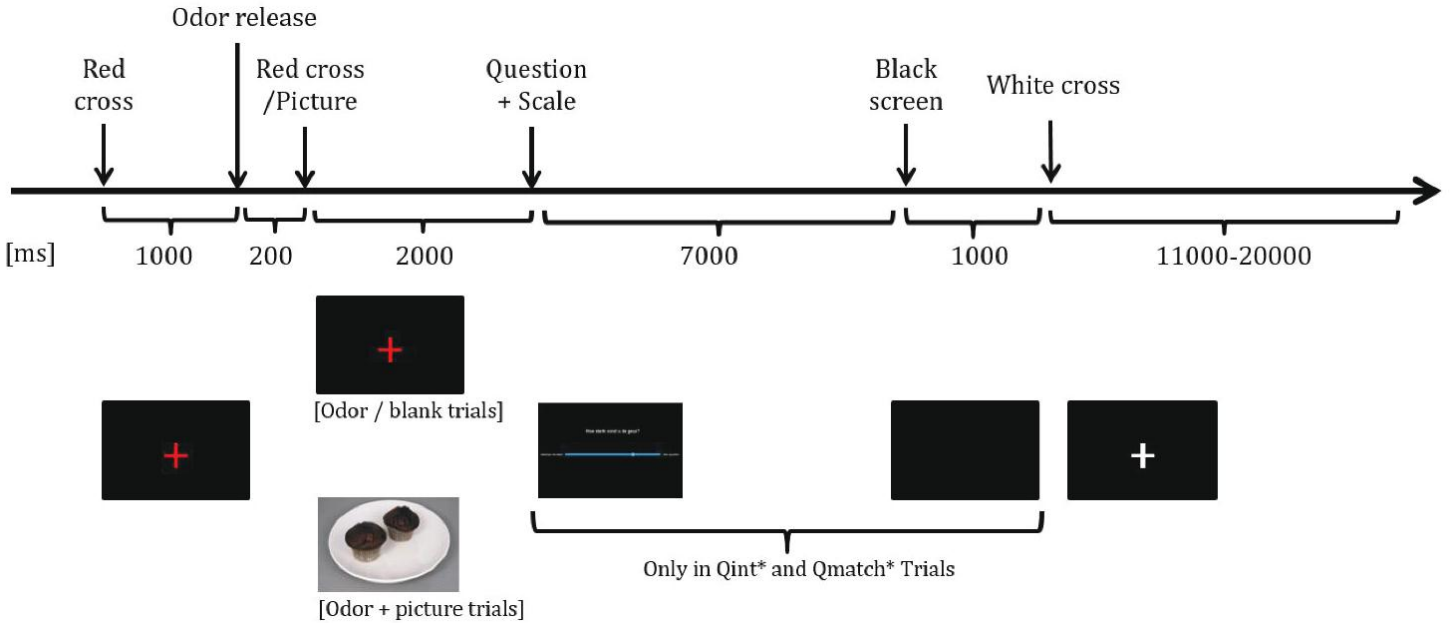

Figure 1. *Details on stimulus timing during the olfactory paradigm. *QInt = intensity question ("How intense did you perceive the odor?"), QMatch = matching question ("How well did the picture and the odor match?").*

MRI data analysis

Processing of the fMRI data

Functional MRI data was preprocessed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab R2011a, including slice-time correction, motion correction, realignment, and spatial smoothing with a 6-mm Gaussian kernel (full-width at half-maximum). Before second level analysis, the ArtRepair toolbox was used [31] to reduce the residual errors of more than 0.5 mm movement between scans which remained after realignment.

Our setup to investigate the preprocessed imaging data included two steps: We first investigated which activations were evoked by the odor stimuli compared to pure sniffing (during blank trials) by using a classical general linear model (GLM) analysis. In a second step, to investigate the functionally connected networks responsible for processing the sensory stimuli, we assessed group independent component analysis (ICA). Both analyses provide complimentary information when it comes to the investigation of functional task-related imaging data. GLM reveals which activation patterns are evoked by the stimulus paradigm under the assumption of a regression model and the haemodynamic response function, whereas ICA is based on a blind sort separation algorithm without the use of an a priori paradigm, that separates functionally connected networks solely based on their temporal patterns. Please see other chemosensory research for reference [18], [21], [32], [33].

GLM analysis

For subject-level analysis, the following conditions were modeled: chocolate odor, beef odor, blanks, chocolate & picture, beef & picture trials and questions (intensity/matching). Six motion regressors were included as regressors of no interest. Subsequently, for each patient parameters were estimated for the comparison (subsequently referred to as ‘contrast’) of odor (chocolate and beef) to the blank condition. The odor (chocolate and beef) > blank contrast

images of the patients were entered into a group-level one-sample t-test to assess activation in response to olfactory stimulation across the whole sample. For this contrast, only the pure odor trials (without pictures) were used. Significance was assessed at a whole-brain FWE corrected threshold (pfwe < .05). In a subsequent multiple regression analysis, we assessed whether overall olfactory function (regressor: TDI scores) was related to the odor-related activity (odor > blank contrast images). For this analysis, a small volume correction (SVC, sphere of 20 voxels around peak of activation) statistical thresholding approach was applied. Moreover, a region of interest analysis of piriform cortex activation was carried out for both subgroups (hyposmics and anosmics) on the odor > blank contrast images to assess whether residual activation of piriform cortex was present for both subgroups. A functional mask for the piriform cortex from a meta-analysis [34] was used for these subgroup analyses and significance was assessed at a FWE-corrected threshold (pfwe < .05).

Group ICA

We conducted group independent component analysis (ICA) on the preprocessed fMRI data using the GIFT toolbox [35]. The number of components was estimated as 44 (mean of estimated components across all patients) using the minimum description length (MDL) algorithm included in the group ICA of fMRI (GIFT) toolbox. Statistical reliability of independent components was assessed using the ICASSO method, that validates the independent component time-series via clustering and visualization [36]. Using ICASSO, the component estimation was performed 20 times with varying initial conditions of the algorithm. In a 2-step principal component analysis (PCA) reduction procedure, components were reduced from 91 (maximum estimated by the implemented MDL algorithm) to 44. For group ICA, the Infomax algorithm was used. Subsequently, the extracted components were inspected visually and 14 artifactual components (overlapping substantially with known motion, susceptibility, vascular or ventricular artifacts) were excluded.

In a next step, to assess which network was most related to odor processing, the network time courses were submitted to a multiple regression with a regressor specifying all odor presentation onsets (including pure odor trials and odor & picture trials). This step was carried out for each patient and resulted in individual beta weights for the odor regressor. The component C37 (subsequently referred to as “sensory processing network”) showed the highest task-relatedness and contained a number of regions associated with olfactory processing previously, and was thus examined further. In order to examine this network in more detail and to compare odor stimulation to pure sniffing, the time course of the sensory processing network C37 was subjected to a further multiple regression including the odor onsets (beef and chocolate combined in one regressor) and blank onsets as regressors of interest. Six motion regressors and the other events of the paradigm (odor & picture onsets, onsets of questions on matching/intensity) were additionally included in the regression model as regressors of no interest. The resulting beta weights of the odor and blank regressors (reflecting the extent to which the network's time course was related to these two regressors) were subsequently correlated with TDI scores to assess whether network recruitment during these trials was related to patients' olfactory function.

In a final step, in an explorative analysis we examined whether any additional network besides C37 was associated with sensory processing and olfactory function. Thus, the time courses of each of the remaining 29 (non-artifactual) components were subjected to the multiple regression model described above (model with odor onsets, blank onsets, odor+picture onsets, onsets of questions on matching/intensity). Task-relatedness was defined as a significant beta-weight in a one-sample t-test ($p < .05$ after FDR-correction to correct for the number of components tested). 19 task-related components emerged from this analysis. For these components, beta weights of odor and blank trial regressors were correlated with TDI scores and evaluated for significance ($p < .05$ after FDR-correction to correct for the number of components tested).

The averaged spatial component maps of the components of interest were entered into a one-sample t-test, thresholded at $p < .05$, FWE-corrected to determine the main brain regions comprised in the component maps. For visualization of fMRI analyses results in Figures 3 -5, whole-brain component maps were exported to the "Multi-image analysis GUI" (MANGO, http://ric.uthscsa.edu/mango) and overlaid on a standard anatomical template in MNI space.

Intensity and matching ratings

Behavioral ratings was analyzed with IBM SPSS Statistics (version 24). Ratings were first averaged per patient. Average intensity ratings for odors versus blanks were compared using a paired T-test (combining hyposmic and anosmic patients). To compare odor intensity ratings from anosmic patients to hyposmic patients, only questions for beef and chocolate odor were included (blanks were excluded), using an independent-samples T-test. A Pearson correlation was used to assess the relationship between Sniffin' Sticks score (TDI) and averaged odor intensity ratings per patient. Ratings of the matching questions were compared between hyposmic and anosmic patients using an independent-samples T test.

Results

Odor intensity and matching of odors and pictures

The group as a whole (n=48) rated odors (mean 24.9 ± 25.6) as more intense than blanks (11.2 ± 14.8; $p < .001$). Hyposmic patients rated the odors with higher intensity than anosmic patients (41.3 ± 23.8 vs 14.2 ± 21.0; $p < .001$). Olfactory function scores (TDI) were significantly positively correlated to odor intensity ratings ($r = 0.45$, $p = .001$). Hyposmic patients rated the match between the odor and (congruent) picture as higher (55.3 ± 20.4) than the anosmic patients (33.7 ± 20.1; $p < .001$).

Olfactory activation in hyposmics and anosmics (GLM)

The results of the whole-brain one-sample t-test showed an increased activation in the piriform cortex (see Figure 2 and Table 2) for odor trials compared to the pure sniffing trials (blank trials), approaching significance at a whole-brain FWE-corrected height threshold ($pfwe = .055$). No significant relation emerged from the multiple regression analysis with TDI scores ($pfwe > .1$). ROI-based one-sample t-tests indicated that there was piriform cortex activation for odors compared to blank trials in both subgroups (hyposmics and anosmics, see Table 2).

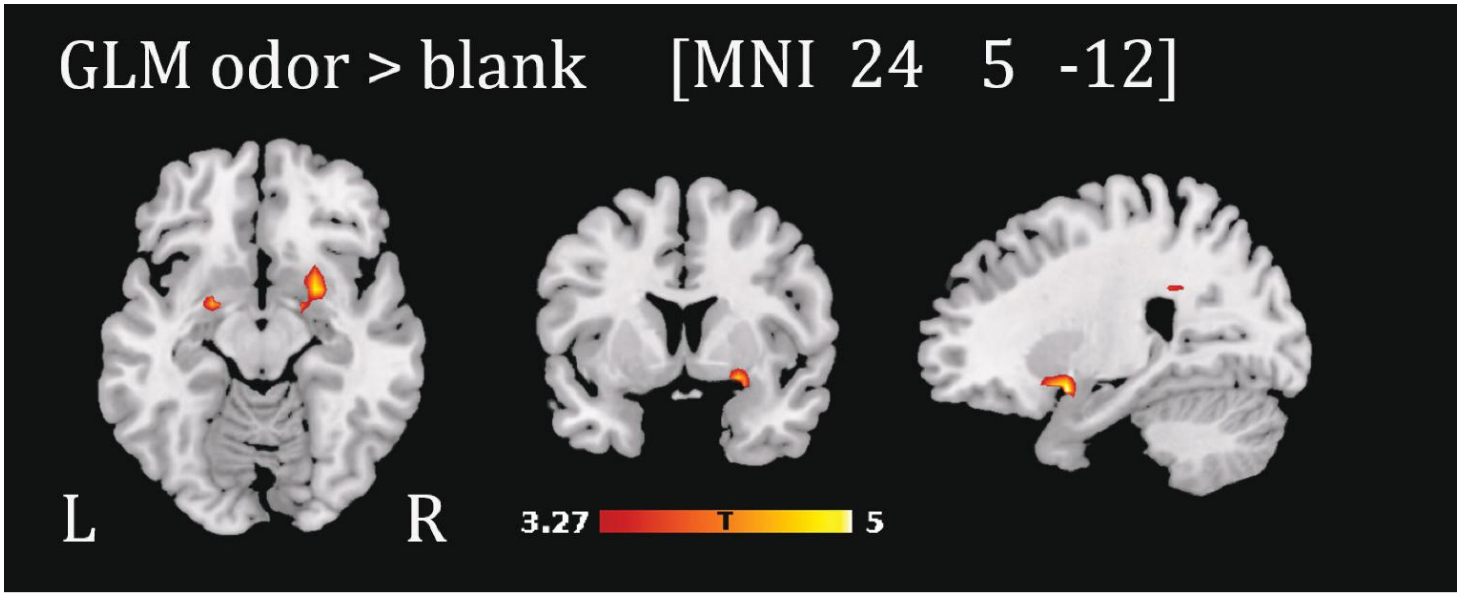

Figure 2. *Activation pattern in the piriform cortex during olfactory stimulation compared to blank trials (results of one-sample t-Test on contrast images odor > blank trials for whole sample, n=48). For illustration purposes, activations are shown at punc. < .001.*

Table 2. *Significant clusters of activation during the olfactory paradigm when odor > blank trials.*

Brain region (peak/nearest grey matter)	Side	Peak MNI coordinates			Peak T-value	pFWE
		x	y	z		
Whole-brain, whole sample odor > blank						
Piriform cortex	R	24	2	-16	5.35	0.055
ROI-analysis*, hyposmics odor > blank						
Piriform cortex	R	27	2	-16	6.03	<.001
	L	-21	-4	-10	4.80	.006
	R	18	-1	-10	4.25	.019
ROI-analysis*, anosmics odor > blank						
Piriform cortex	L	-21	2	-16	4.48	.004
	R	21	8	-16	3.99	.013

Results of one-sample t-tests. MNI = Montreal Neurological Institute. L = right, L = left. *ROI-analysis = region of interest analysis using a mask of the piriform cortex from [34]. Only activations significant at a height-level threshold of $p < 0.05$ with family-wise error (FWE) correction are displayed.

Recruitment of networks during odor administration (group ICA)

Figure 3 shows the spatial extent of the sensory processing network C37, comprising amongst others insula, thalamus, cingulate gyrus and putamen (See Table 3 for the top brain regions of the component map). Recruitment of this network during odor trials was positively correlated with total TDI scores ($r(46) = .30$, $p = .039$), as well as recruitment during blank trials ($r(46) = .37$, $p = .011$).

The beta weights for odor trials of two further components showed a significant correlation with TDI scores at $pFDR < .05$): C3 (subsequently termed "occipital network"): $r(46) = .42$, $p = .003$ and C5 (subsequently termed "cerebellar network"): $r(46) = .41$, $p = .004$. For both components, beta weights for blank trials were positively correlated with TDI scores as well (C3: $r(46) = .41$, $p = .004$ and C5: $r(46) = .35$, $p = .014$). The spatial component maps of these two networks are shown in Figure 4 and Figure 5 (see Table 3 for the top brain regions comprised in the component maps).

Table 3. *Main brain regions included in the three component maps (*)*

Component	Standardized region name	AAL region	No of voxels
C37 ("sensory processing network")	Median cingulate and paracingulate gyri (R)	Cingulum_Mid_R	452
	Median cingulate and paracingulate gyri (L)	Cingulum_Mid_L	421
	Superior frontal gyrus, medial (L)	Frontal_Sup_Medial_L	307
	Superior temporal gyrus (L)	Temporal_Sup_L	283
	Insula (L)	Insula_L	270
	Insula (R)	Insula_R	241
	Anterior cingulate and paracingulate gyri (L)	Cingulum_Ant_L	237
	Thalamus (L)	Thalamus_L	237
	Middle temporal gyrus (L)	Temporal_Mid_L	225
	Thalamus (R)	Thalamus_R	220
	Lenticular nucleus, putamen (L)	Putamen_L	220
	Supplementary motor area (L)	Supp_Motor_Area_L	217
	Superior temporal gyrus (R)	Temporal_Sup_R	209
	Anterior cingulate and paracingulate gyri (R)	Cingulum_Ant_R	194
	Lenticular nucleus, putamen (R)	Putamen_R	190
	Rolandic operculum (L)	Rolandic_Oper_L	176
	Lingual gyrus (L)	Lingual_L	166
	Supplementary motor area (R)	Supp_Motor_Area_R	156
	Lingual gyrus (R)	Lingual_R	150
	Rolandic operculum (R)	Rolandic_Oper_R	149
	Caudate nucleus (L)	Caudate_L	139
	Caudate nucleus (R)	Caudate_R	137
	Middle temporal gyrus (L)	Temporal_Mid_R	130
	Superior frontal gyrus, medial (R)	Frontal_Sup_Medial_R	113
C3 ("occipital")	Middle occipital gyrus (L)	Occipital_Mid_L	526
	Middle temporal gyrus (L)	Temporal_Mid_L	319
	Lingual gyrus (L)	Lingual_L	261
	Lingual gyrus (R)	Lingual_R	258
	Middle occipital gyrus (R)	Occipital_Mid_R	252
	Inferior occipital gyrus (L)	Occipital_Inf_L	207
	Calcarine fissure and surrounding cortex (L)	Calcarine_L	202
	Calcarine fissure and surrounding cortex (R)	Calcarine_R	198
	Fusiform gyrus (L)	Fusiform_L	187
	Fusiform gyrus (R)	Fusiform_R	156
	Precentral gyrus (L)	Precentral_L	146
	Superior occipital gyrus (R)	Occipital_Sup_R	114
	Superior occipital gyrus (L)	Occipital_Sup_L	112
C5 ("cerebellar")	Hemispheric lobule VI (L)	Cerebellum_6_L	378
	Hemispheric lobule VI (R)	Cerebellum_6_R	352
	Crus I (L)	Cerebellum_Crus1_L	313
	Fusiform gyrus (L)	Fusiform_L	311
	Crus I (R)	Cerebellum_Crus1_R	280
	Fusiform gyrus (R)	Fusiform_R	241
	Hemispheric lobule IV/V (L)	Cerebellum_4_5_L	222
	Lingual gyrus (L)	Lingual_L	222
	Lingual gyrus (R)	Lingual_R	165
	Vermic lobule IV/V	Vermis_4_5	142
	Hemispheric lobule IV/V (R)	Cerebellum_4_5_R	133

* Brain regions classified by AAL (automatic anatomic labeling) atlas (http://www.gin.cnrs.fr/AAL?lang=en, [37]). Labeling was conducted on binary masks of the thresholded component maps (at pFWE < .05, k = 100). Please note that for this reason, no voxel intensity information is provided. L = left, R = right.

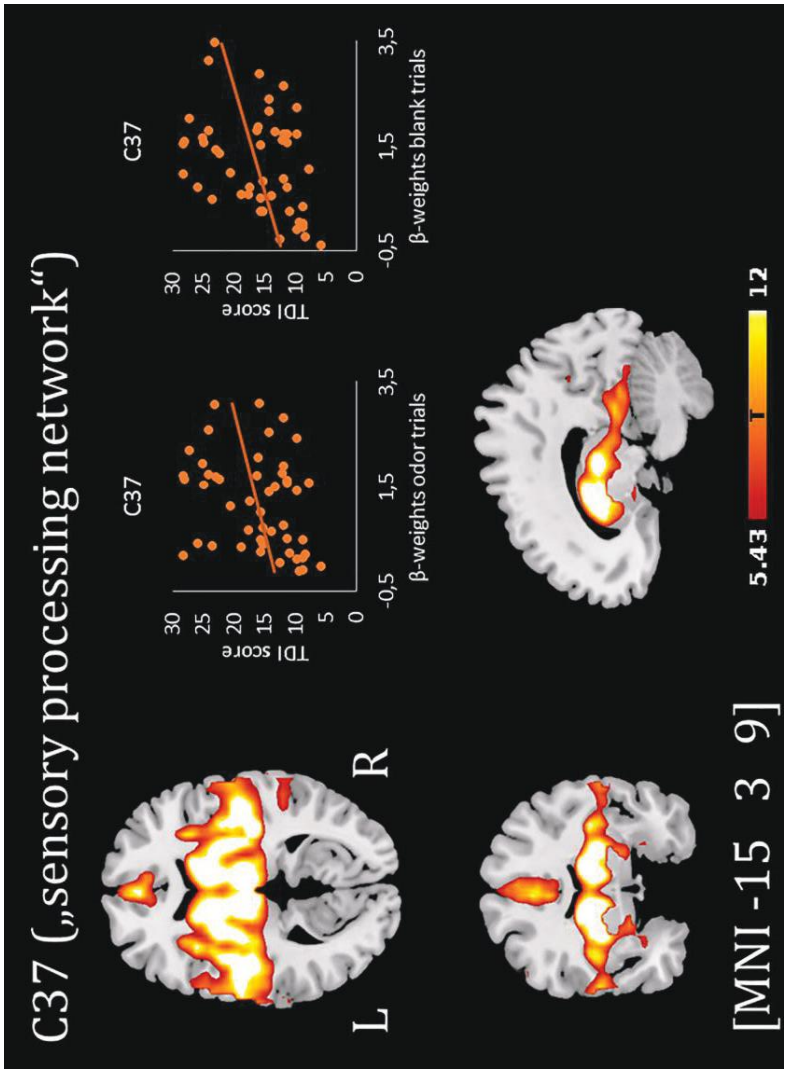

Figure 3. *Component map of the olfactory network (C37, thresholded at p < .05 FWE-corrected) and scatterplots showing the positive correlation between TDI scores and β-weights of the regressor odor / blank trials.*

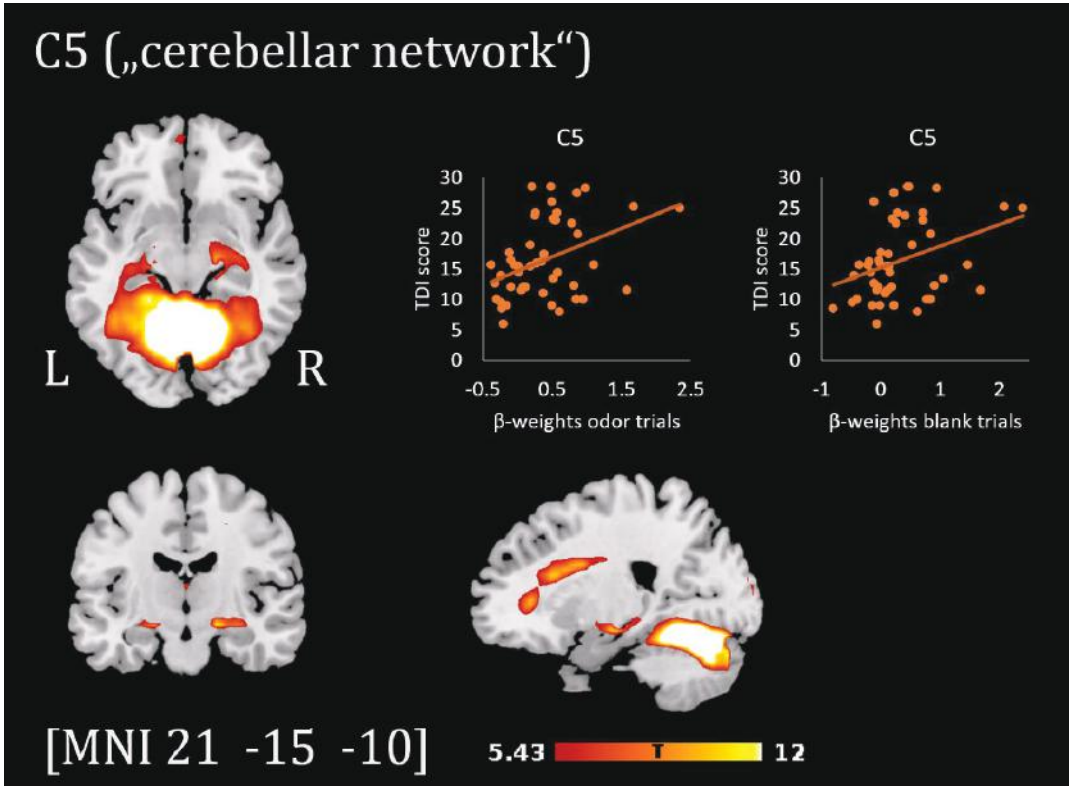

Figure 4. *Component map of the cerebellar network (C5, thresholded at p < .05 FWE-corrected) and scatterplots showing the relation between TDI scores and β-weights of the regressor odor / blank trials.*

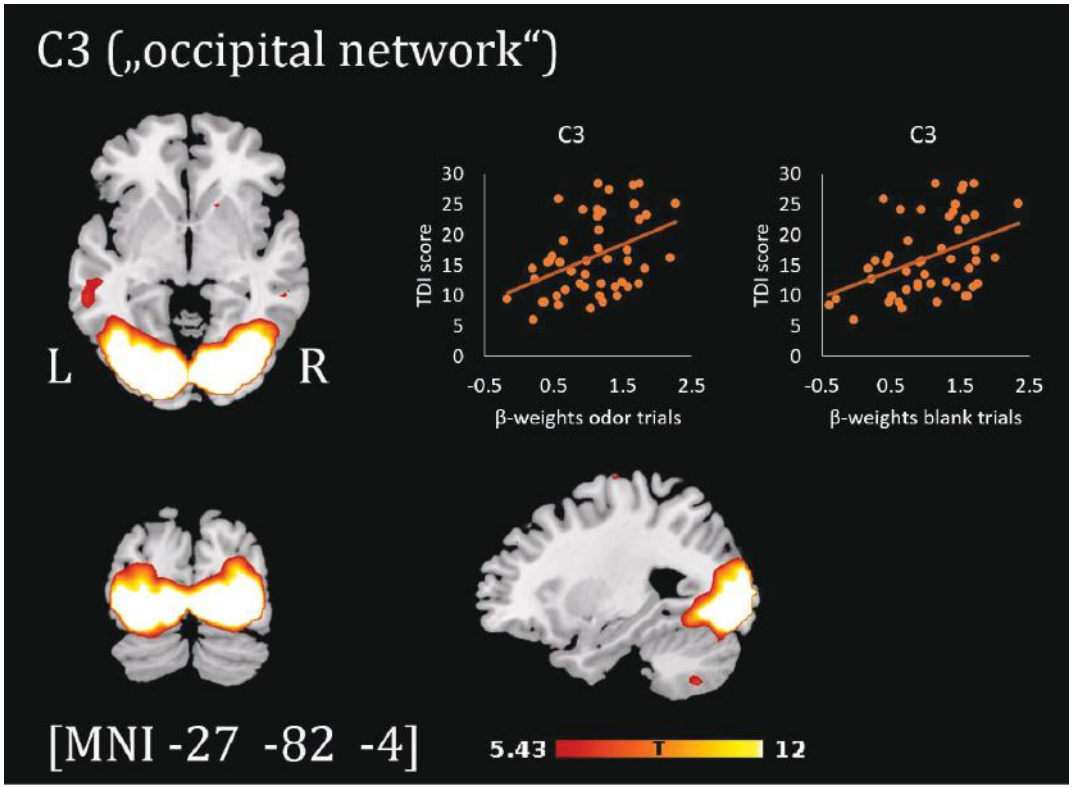

Figure 5. *Component map of the occipital network (C3, thresholded at p < .05 FWE-corrected) and scatterplots showing the relation between TDI scores and β-weights of the regressor odor / blank trials.*

Discussion

In the present study, we set out to determine for the first time how the severity of olfactory loss is reflected in functional brain activity and brain networks. To this end, we investigated neuronal activation in response to olfactory stimulation and pure sniffing in patients suffering from varying degrees of olfactory deficits. We applied two conceptually distinct approaches: general linear model (GLM) and functional connectivity analysis (independent component analysis, ICA). While the GLM analysis showed odor-evoked activity in piriform cortex during olfactory stimulation as compared to pure sniffing, group ICA identified large-scaled sensory processing networks recruited not only for odor processing but also for sniffing without odor stimulation. Task-modulation of three networks was significantly correlated with scores of olfactory function [26].

In both hyposmic and anosmic patients we observed increased piriform cortex activation in response to olfactory stimulation as compared to pure sniffing. In general, piriform cortex activation during olfactory stimulation is well in line with a large number of previous studies showing the essential role of this region for olfactory processing (e.g. [34], [38]–[40]). It is striking that the odor-specific piriform cortex activation was not only present in hyposmic, but also in anosmic patients. Since the anosmic patients had no functional olfactory perception based on their Sniffin' Sticks score, the observed piriform cortex activation in anosmics might be in line with evidence that odors can alter behavior and brain activity even if they are not consciously perceived [41]–[45]. This finding suggests that the pathway from the olfactory epithelium to the piriform cortex might still be intact in these patients and that the olfactory dysfunction occurs after piriform processing. A recent study indicates that functional connections from piriform cortex to olfactory areas can be re-established using olfactory training [12]. Thus, one might speculate whether odor-related activity in piriform cortex in anosmics is a prerequisite of susceptibility for such reorganization processes and whether the extent of this activity could play a role in

disease prognosis and prediction of treatment success. It should be noted, that the observed piriform related activity in the odor > blank contrast could be driven by larger sniffs made in the odor condition. However, as patients received similar sniff instructions in both conditions, this seems unlikely.

In the current study, we used two pleasant food-related odors for stimulation. Food odors are biologically salient stimuli, and are processed differently in the brain compared to other types of odors [46], [47]. This might contribute to the perception of these odors remaining preserved, even in patients with severe olfactory dysfunction. This issue deserves to be further investigated in future studies comparing the processing of food-related to non food-related odors in olfactory dysfunction directly. Additionally, it is possible that some of the piriform cortex activation might be caused by trigeminal stimulation, although the odors were not selected to contain trigeminal properties, in contrast to for example Kollndorfer et al [18]. It is therefore recommended in future studies to thoroughly assess selected odors on trigeminal properties.

ICA revealed three networks which were recruited during the olfactory task and correlated with olfactory function scores: an olfactory, an occipital and a cerebellar network. These results support and extend a previous investigation on olfactory networks in participants with normal olfactory function [48]. In this study, five functionally connected networks were involved in an olfactory task containing odor trials and no-odor control trials. The two olfactory networks found in [48] overlap to a large extent with the sensory processing network identified in our study, as they comprise traditional olfactory regions such as caudate, thalamus, putamen, and hippocampus. Interestingly, [48] also identified a visual/occipital network modulated by their olfactory task, comprising parts of middle occipital gyrus. Notably, a network comprising the cerebellum was also identified but not described further due to the component selection criteria employed [48]. Extending the findings of [48], in the current study we were able to show that the task-modulation of the networks during our olfactory paradigm was related to an external parameter, namely the scores participants

achieved in an olfactory test. Thus, our results confirm the relevance of the olfactory, occipital and cerebellar networks by showing that the extent to which these networks are modulated by the olfactory task reflects olfactory function. The sensory processing network identified in our study contained primarily regions previously associated with olfactory function (insula, thalamus, piriform cortex, cingulate cortex), but also further regions (superior temporal gyrus, superior frontal gyrus). Despite being not regarded as typical olfactory regions, an association of these regions with olfactory functions is in line with results of a recent a voxel-based morphometry study investigating grey matter (GM) volume in anosmics [49]. In this study, GM volume of superior temporal gyrus and superior frontal gyrus was decreased in anosmics compared to controls, possibly indicating an association of these regions with olfactory function.

The second network related to olfactory function in our study comprised occipital regions. Though visual input changed slightly when odor stimuli were released (the fixation cross changed color to signal the presentation of olfactory stimuli), this does not explain why recruitment of the network was related to scores achieved in the olfactory test. The occipital network comprised mainly the inferior and middle occipital gyrus, the middle temporal gyrus and the fusiform gyrus, areas not traditionally assumed to be main olfactory processing regions. However, a growing number of neuroimaging studies has reported activation of the visual cortex even during odor stimulation, particularly in olfactory identification and matching tasks [50]–[52]. Moreover, a decrease in grey matter volume of the fusiform gyrus, the middle temporal gyrus, and the middle occipital gyrus was demonstrated in previous voxel-based morphometry studies in anosmics compared to healthy controls [49], [53], pointing to a possible role of these areas in olfactory processing. It has been suggested that during attempted identification of a smell, people might visualize the potential source of the odor [54]. Thus, one might speculate whether patients scoring lower on the olfactory test in our study recruited the occipital network for visualization of the odor source less than patients achieving higher scores. This is in line with

previous evidence that patients with olfactory deficits show a reduced olfactory imagery capacity [55], [56]. Furthermore, in a repetitive transcranial magnetic stimulation investigation, stimulation of the visual cortex led to improved odor discrimination performance as compared to sham stimulation, thus even pointing to a potential direct contribution of visual cortex to olfactory processing [54]. The interconnection of the visual and olfactory system was also underlined by a recent study on olfactory-visual conditioning [57].

The recruitment of a cerebellar network also showed a correlation with olfactory function scores. Due to the requested button press to rate the stimuli during some (but not all) trials, a preparatory function of this network can be suspected. Moreover, previous neuroimaging studies demonstrating cerebellar activation in response to olfactory stimulation (e.g. [58]–[60]) and with a reported impairment of olfactory function in patients with cerebellar lesions [61], [62]. In particular, the cerebellum was suggested to be part of the "olfactomotor system" involved in the control of sniffing [13], [14], [58]. The particular importance of the cerebellar network for sniffing is further underlined by the correlation between network recruitment during pure sniffing trials and smelling function, with patients scoring higher in the olfactory test showing a higher task-modulation of this network during sniffing. This result is well in line with an observed decreased functional connectivity of cerebellar regions in anosmic patients compared to normosmics during sniffing of odorless air [15].

Interestingly, similarly to the findings observed for the cerebellar network, the recruitment of the olfactory and occipital networks during pure sniffing trials was significantly correlated with olfactory function scores as well. Thus, our results support previous findings that sniffing alone can lead to activation of olfactory regions and extend them by showing that the extent of network recruitment is related to smelling function. Interestingly, in a previous olfactory study on persons with a normal olfactory function [48] olfactory network time courses were also task-modulated in no-odor control trials, as was the case in the present study. As discussed in [48], this might reflect anticipation or expec-

tation of odor stimulation by participants or reflect carry-over effects from odor to non-odor trials.

In the present study, we analyzed a relatively homogeneous sample of patients suffering from olfactory deficits, as we excluded those patients that might show neuronal changes unrelated to olfactory loss (e.g. hyposmia after head trauma or in the course of neurodegenerative diseases). Thus, although the correlative nature of our study impedes strong causal interpretations, we are confident that the recruitment of the functional networks can reflect the severity of olfactory deficits and not the effects of other potentially confounding factors. Still, an important question that could not be investigated in the present study is the relation between duration of olfactory disorder, brain activity patterns and recruitment of functional networks, as duration of olfactory disorder was confounded with severity of the olfactory disorder within our population (see Table 1). Duration of olfactory dysfunction therefore deserves to be investigated in further studies to gain more knowledge on the direction of the observed effects. Additionally, it was not possible to include patients with congenital anosmia in this study due to a low number of patients with this disorder in our population (n=8). As Frasnelli et al. found that patients with congenital anosmia display fundamental changes in brain structure compared to healthy controls [63], it is recommended for studies further investigating the effects of duration of smell loss on functional networks to include patients with congenital disorders as well.

Our results indicate that even patients classified as anosmics based on olfactory testing scores can show activation in olfactory brain areas when stimulated with odors as compared to pure sniffing. Moreover, the recruitment of an olfactory, a cerebellar and an occipital network was related to olfactory function. Future studies might shed more light on the intriguing question whether such activation patterns might be predictive of disease progression or potential regain of olfactory function and of the success of treatment programs such as olfactory training.

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Chapter 7

General discussion

General discussion

Changes in olfactory and gustatory function are a widespread problem. However, so far their effect on eating behavior and the neurobiology of changes in olfactory and gustatory function are not well enough understood. To gain more insight in the effect of changes in olfactory and gustatory function on eating behavior and the neurobiology of smell and taste, the overall aims of this thesis were:

- To investigate the effect of alterations in olfactory and gustatory function on eating behavior in patients with changes in olfactory and gustatory function.
- To investigate possible neurobiological alterations in patients with changes in olfactory function.

This general discussion will first give an overview of the main findings from this thesis. Subsequently, the aims as stated above are discussed, followed by subsequent insights on the relation between eating behavior and the neurobiology of olfactory-related brain regions in patients with changes in olfactory function. Next, methodological considerations will be discussed, as well as the relevance and implications of the findings from this thesis for clinical practice and suggestions for future research. Finally, the discussion will end with an overall conclusion.

Table 1. *Overview of the main findings of this thesis per chapter*

Chapter	Patient population	Methods	Main findings
Changes in olfactory and gustatory function and eating behavior			
2	Patients with self-reported changes in olfactory function (N=105)	• Macronutrient and Taste Preference Ranking Task • Eetscore (adherence to Dutch Dietary Guidelines)	• Reduced food enjoyment in patients • Aberrant pattern in food preferences in patients with congenital anosmia compared to patients with acquired smell loss and healthy controls • Similar adherence to Dutch Dietary Guidelines for patients and the reference population
3	Patients with colorectal cancer (N=95)	• Sniffin' Sticks • Taste Strips • Appetite, Hunger and Sensory Perception questionnaire • Macronutrient and Taste Preference Ranking Task	• Worse subjective olfactory and gustatory function in patients undergoing chemotherapy compared to patients undergoing merely surgery; no differences in objective olfactory and gustatory function • No effect of chemotherapy treatment on food preferences • Positive correlation between preference for protein and objective gustatory function
Neurobiological changes in morphology and function of olfactory-related brain areas			
4	Patients with clinically diagnosed changes in olfactory function (N=105)*	• Manual measurements of OB** volume • Automated measurements of OB volume by applying convolutional neural networks	• Successful localization and segmentation of OB as well as subsequent calculation of OB volume • Faster measurements of OB volume compared to manual measurements
5	Patients with clinically diagnosed changes in olfactory function (N=257)*	• Sniffin' Sticks • Voxel-based morphometry analysis	• Reduced density in gyrus rectus but increased density of OFC*** in patients with congenital anosmia compared to patients with acquired changes in olfactory function • Positive relation between density of OFC and olfactory function
6	Patients with clinically diagnosed changes in olfactory function (N=48)*	• Sniffin' Sticks • General linear model analysis • Group independent component analysis	• Activation of piriform cortex during odor stimulation • Correlation between olfactory function and recruitment of a sensory processing network

* (Part of) this population was recruited within the Smell and Taste Center of Hospital Gelderse Vallei in Ede, the Netherlands; ** OB = olfactory bulb; *** OFC = orbitofrontal cortex

Main findings

We first investigated the effect of changes in olfactory and gustatory function on eating behavior by investigating food preferences and food intake in patients with changes in olfactory and gustatory function **(chapter 2 and 3)**. In an online survey, we found that patients with self-reported smell loss, acquired at later age, showed the lowest preference for high-carbohydrate foods and highest preference for low-energy foods, which was similar to the preference pattern of a control group with healthy individuals. In contrast, patients with congenital anosmia showed an aberrant pattern, with a higher preference for high-fat foods. Adherence to the recommendations on dietary intake by the Dutch Dietary Guidelines was similar for patients with self-reported smell loss and the reference population, but patients did show a reduced food enjoyment **(chapter 2)**. In colorectal cancer patients undergoing chemotherapy, despite a worse subjective olfactory and gustatory function during treatment, there were no differences in objective olfactory and gustatory function compared to control patients undergoing merely surgery. We found no effect of chemotherapy treatment on food preferences, but preference for protein was positively correlated with objective gustatory function **(chapter 3)**.

Secondly, we aimed to get more insight in the possible neurobiological changes in morphology and function of olfactory-related brain areas in patients with changes in olfactory function **(chapter 4-6)**. We first focused on the olfactory bulb and applied convolution neural networks (CNNs) to allow automated volume measurements. These measurements are faster than current manual measurements and may lead to more insight in the role of the olfactory bulb in diagnosis, prognosis and treatment of changes in olfactory function for both research and health care. Localization and segmentation of the olfactory bulb as well as subsequent calculation of olfactory bulb volume were performed successfully and with a high accuracy, displayed by a high overlap in measurements performed by the CNNs and a manual reference method **(chapter 4)**. Next step was investigating the morphology of olfactory-related brain regions

of patients with changes in olfactory function. Patients with congenital anosmia showed reduced density in the gyrus rectus compared to patients with acquired changes in olfactory function, while the density of the orbitofrontal cortex (OFC) of patients with congenital anosmia was increased compared to the other patient groups. Moreover, there was a positive relation between density of the OFC and olfactory function **(chapter 5)**. Lastly, we aimed to determine how changes in olfactory functioning affect neural activation patterns and networks in the olfactory system of the brain. We found that patients with olfactory loss showed piriform cortex activation during odor stimulation compared to pure sniffing, even in anosmic patients. Moreover, olfactory function was correlated with the recruitment of a sensory processing network **(chapter 6)**.

The effect of changes in olfactory and gustatory function on food preferences and intake

Smell and taste, and their combined perception in flavor of food, are important determinants for food preferences and intake. Changes in olfactory and gustatory function are therefore likely to affect eating behavior. However, we did not find an effect of changes in olfactory function on food preferences in patients with acquired changes in olfactory function **(chapter 2)** nor in colorectal cancer patients undergoing chemotherapy **(chapter 3)**. For cancer patients, this is in line with previous findings [1]. For patients who solely had changes in olfactory function, these results contrast with a previous study on food preferences, that did show changes in food preferences [2]. However, this study only reported self-reported changes within patients and did not compare preferences of patients with changes in olfactory function to healthy controls. Research on food preferences in patients with changes in olfactory function is scare; related studies support our findings, showing that changes in olfactory function also do not affect liking of food odors [3] or sensory-specific satiety, i.e. declining satisfaction derived from a certain food with its consumption relative to other,

unconsumed, foods [4]. Hence, it seems that changes in olfactory function do not directly affect food preferences.

In contrast, in colorectal cancer patients, we found a correlation between objective gustatory function and food preferences **(chapter 3)**. This is consistent with previous findings on the association between gustatory function and preference for high-protein foods in other populations of cancer patients [1,5]. Cancer patients experiencing changes in gustatory function often report the perception of a constant bitter or a metallic taste [6,7]. Although we did not measure the prevalence of metallic taste among patients undergoing chemotherapy, they did report a lower subjective gustatory ability than the control group **(chapter 3)**. Due to aberrant taste perception, patients with a worse gustatory function might shift towards food products that are more neutral in taste, which is the case for many low-energy foods, like fruits and vegetables [8]. Previous studies showed an association between both reduced energy and protein intake, and changes in gustatory function in cancer patients undergoing chemotherapy [9,10]. Therefore, we propose that the correlation between gustatory function and changes in food preferences is related to the change in flavor perception that is likely to occur in patients experiencing changes in gustatory function.

While there were no differences in food preferences between patients and healthy controls **(chapter 2)**, there were differences in patterns in food preferences among patients. Patients with acquired olfactory loss displayed the same pattern as healthy controls, while patients with congenital anosmia showed a different pattern, that was more taste or nutrient oriented **(chapter 2)**. In patients with congenital anosmia the absence of olfactory function and the subsequent changes in flavor perception might therefore impact the formation of food preferences from the start of their life onwards already. In contrast, in both colorectal cancer patients preference for high-protein foods was highest **(chapter 3)**, which was not displayed in any of the other populations. In cancer patients, the mostly subjective changes in olfactory and gustatory perception

are a transient side-effect of treatment. However, during treatment, they highly impact food enjoyment and food liking, and can lead to food aversions [11,12], probably also affecting food preferences. Therefore, we conclude that the cause of changes in olfactory and gustatory function affect subsequent food preferences.

We did not find an effect of changes in olfactory function on food intake in patients with self-reported changes in olfactory function **(chapter 2)**. To measure food intake, we used an online questionnaire that scores adherence to the Dutch Dietary Guidelines, based on dietary intake during the past month [13]. Patients did have a lower adherence to three of the separate dietary guidelines compared to the reference population, but this did not result in a lower overall adherence to the Dutch Dietary Guidelines. Previous studies on food intake among older adults with changes in olfactory function showed contrasting results. Elderly women who suffered from smell loss showed reduced adherence to dietary guidelines and an increased risk for poorer diet quality over time [14,15], while others found no relation between olfactory function and nutritional status [16,17]. While it is possible that changes in olfactory function affect food intake, there are also other (external) factors that are known to play a role in eating behavior in general [18,19]. For example, the context in which choices for food intake are made are important, like the social context of eating with others [19] or the context of time of the day and foods that are appropriate for that moment [20]. These factors are learned already early in life [21,22]. Therefore, they might overrule the effects of changes in olfactory function on food intake; this is supported by the fact that we did not find a different food intake in patients with congenital anosmia compared to healthy controls, while they did show an aberrant pattern in food preferences.

KEY MESSAGE

Changes in olfactory and gustatory function seem less important for actual measures of eating behavior, such as food preferences and intake, but more relevant for changes in subsequent flavor perception and food enjoyment.

Alterations in the neurobiology of olfaction in relation to changes in olfactory function

Olfactory perception in the brain starts when signals are excited by binding of odor molecules to the olfactory epithelium and are transferred to the olfactory bulb. The volume of the olfactory bulb is related to olfactory function in both healthy individuals [23,24] and in patients with changes in olfactory function [25,26]. However, current results are based on manual measurements, which limits the possibilities for comparisons among studies or over time. In **chapter 4**, we successfully applied convolutional neural networks to automatically measure olfactory bulb volume. This method can be utilized in both research and health care and may lead to more insight in the role of the olfactory bulb in diagnosis, prognosis and treatment of olfactory loss, as volume of the olfactory bulb can be a predictor for recovery in patients with changes in olfactory function [26]. Moreover, the olfactory bulb is important for the transduction of olfactory signals to other olfactory-related regions in the brain. Changes in olfactory bulb volume are not only related to peripheral olfactory input, but also to changes in other olfactory-related brain regions [27]. In depressed patients with a reduced olfactory bulb volume, also a reduction in the volume of several olfactory-related brain regions, like the amygdala and the insula, was found [28]. In addition, in patients with changes of olfactory function due to neurodegenerative diseases, changes in olfactory bulb volume might be related to alterations in morphology and function at central nervous level [29]. Follow up of

olfactory bulb over time is needed to gain more insight in the relation between olfactory bulb volume, olfactory function and the morphology of olfactory-related areas in the brain.

After the olfactory bulb, olfactory signals are transferred to primary olfactory-related brain regions, like the piriform cortex. This brain region plays a major role in the cortical processing of olfactory signals [30]. We observed piriform cortex activation in response to olfactory stimulation in both anosmic and hyposmic patients. Moreover, during odor administration, a sensory processing network, including the piriform cortex, was recruited **(chapter 6)**. This indicates that, despite loss of olfactory function, the brain still responded to odors. Previous studies showed reduction of volume of the piriform cortex in both anosmic [31,32] and hyposmic [33] patients, as well as activation of the piriform cortex in patients with changes in olfactory function [34,35]. Therefore, it would be highly relevant to investigate if activation of the piriform cortex is depending on its volume and how this is related to olfactory function in patients with changes in olfactory function.

Next, olfactory signals reach secondary olfactory-related brain regions. These regions are important determinants of olfactory function: there is a positive relationship between density of the OFC and olfactory function in healthy individuals [23]. In patients with changes in olfactory function, both morphology and function of secondary olfactory-related brain regions were related to olfactory function. In **chapter 5**, density of the OFC was correlated to olfactory function, while in **chapter 6**, activation of the sensory processing network was dependent on olfactory function. This is in line with previous research in patients with acquired changes in olfactory function [36,37]. Moreover, individuals with an outstanding olfactory function ('super smellers') [38] and individuals who trained their olfactory function, like perfumers or wine tasters [39–41] had increased density in secondary olfactory-related areas in the brain compared to controls with a normal olfactory function. As a next step, we would recommend to apply both morphological and functional analysis in one study. This will yield

more insight in the processes that underly the neuroplasticity of olfactory-related brain regions, which can be triggered by losing the sense of smell, but also by following olfactory training [42] in patients with changes in olfactory function.

While we did not find a direct effect of duration of changes in olfactory function on density of olfactory-related regions, we did show that that olfactory function was lower in patients with a longer duration of changes in olfactory function **(chapter 5)**. This decreased olfactory function alters peripheral input to central olfactory-related brain regions over the time course of change in olfactory function, which was previously shown to be related to reduced responses to odors in both primary and secondary olfactory-related brain regions [34]. In patients with congenital anosmia, who had poorest olfactory function, we showed an increased density of the orbitofrontal cortex, while density of the gyrus rectus was decreased compared to the other patient groups **(chapter 5)**. This indicates that lifelong changes in olfactory function trigger changes in olfactory-related regions in the brain, as was also postulated by a recent study on morphological changes in patients with congenital anosmia [43]. In contrast, increased exposure to peripheral input due to olfactory training improved olfactory function as well as functional connectivity of an olfactory network in patients with acquired changes in olfactory function [44], similar to the sensory network we found in **chapter 6**. This confirms that olfactory-related brain regions show neural plasticity as a response to changes in peripheral input and subsequent changes in central processing of odors, which provides lead for the development of treatment strategies for changes in olfactory function.

KEY MESSAGE

Changes in olfactory function affect olfactory processing in both primary and secondary olfactory-related brain regions. The relation between changes in olfactory function and morphology and function of olfactory-related brain regions depends on the degree of olfactory function.

Connecting eating behavior and the neurobiology of changes in olfactory function

While smell and taste have a differential role in eating behavior and different processing pathways, they interact when it comes to flavor perception [45]. Therefore, changes in olfactory function and related brain networks will subsequently affect flavor perception as well.

In this thesis, we showed that activation of a sensory processing network in patients with changes in olfactory function was dependent on olfactory function **(chapter 6)**. This network involved several regions that are known to play a role in flavor perception, like the insula and the anterior cingulate cortex [45,46]. It is postulated that brain regions related to flavor perception show greater activation in response to congruent odor-taste stimuli compared to incongruent odor-taste stimuli [47]. Thus, when the perception of odors and subsequent brain responses to these odors change, this probably also results in different brain responses to the flavor of foods during consumption. However, we did not find an effect of changes in olfactory function on food preferences **(chapter 2 and 3)** and intake **(chapter 2)**. In contrast, we did find a reduced food enjoyment in patients with changes in olfactory function **(chapter 2)**. This might relate to changes in the orbitofrontal cortex; in **chapter 5**, we showed a relation between olfactory function and density of the orbitofrontal cortex. While this brain region was not represented in the sensory processing network we

identified in **chapter 6**, the orbitofrontal cortex plays an important role in flavor processing and is known to play a role in the perceived pleasantness of foods during consumption [48–50]. Therefore, this confirms our previous conclusion that changes in olfactory function seem less important for actual measures of eating behavior, but more relevant for changes in subsequent flavor perception and food enjoyment.

Most patients acquire changes in olfactory function at later age. However, a specific subgroup of patients suffers from congenital anosmia: they are born without the ability to smell. In this thesis, we found that patients with congenital anosmia differ from patients with acquired changes in olfactory function. This was not only reflected in brain morphology **(chapter 5)**, but also in food preferences **(chapter 2)**. Previous research confirms this, reflected by both morphological [51] and functional differences [52] compared to individuals with a normal sense of smell. Moreover, one study demonstrated higher liking during prolonged exposure to a sweet food in patients with congenital anosmia compared to healthy controls [53]. This indicates that life-long deprivation of olfactory input can affect the development of the brain and consequently can also affect the processing of chemosensory stimuli.

As this thesis did not directly study the relation between eating behavior and the neurobiology of changes in olfactory function, it is not possible to draw conclusions based on the current results. However, the results of the individual chapters point toward an important role of learned responses to flavors in early life in relation to food preferences in patients with changes in olfactory function. Moreover, the decrease in food enjoyment in patients with changes in olfactory function seems to have a neurobiological foundation. These assumptions give directions for future research on the relation between eating behavior and the neurobiology of changes in olfactory function. These studies should be conducted in longitudinal cohorts to follow possible changes over time, as repeated exposure might be needed before changes arise.

Methodological considerations

To investigate the effect of changes in olfactory and gustatory function on eating behavior and the neurobiology of olfactory-related brain regions, this thesis includes different study designs and patient populations. While we aimed to overcome limitations in study design, some methodological considerations should be taken into account when interpreting the results.

Study populations

In this thesis, we included two different groups of patients to study the effect of changes in olfactory and gustation function on eating behavior. For **chapter 2**, we recruited participants among members of the Dutch Anosmia Foundation. This allowed us to include a broad sample of patients, representative to the different causes of changes in olfactory function. However, olfactory function was self-reported in these patients. We recommend a replication of this study in a population of clinically diagnosed patients to confirm our results. In **chapter 3**, we included colorectal cancer patients undergoing chemotherapy. Recruitment took place for a period of 2.5 years in 8 different hospitals. Despite our recruitment efforts, we were able to include only 15 patients undergoing chemotherapy to take part in the study during treatment. In the Netherlands, the participation rate to the nationwide screening for early detection of colorectal cancer is high [54]. In many patients, chemotherapy is not needed due to this early detection. However, the results we found were in line with previous findings in colorectal cancer patients on self-reported changes in olfactory and gustatory function, thereby increasing representativeness of our results for this patient group.

In **chapter 4-6**, we were able to include large samples of clinically diagnosed patients with changes in olfactory function thanks to our collaboration with the Smell and Taste Center of Hospital Gelderse Vallei in Ede, the Netherlands. In **chapter 4**, we also included a dataset collected at the Smell and Taste Center in Dresden, Germany. This allowed us to test performance of our me-

thod on datasets derived from different scan sites. However, we aim to expand our dataset with additional MRI data of various patient groups and healthy individuals to increase the variability of the dataset on which the method is trained. The datasets in **chapter 5** and **chapter 6** solely included patients who visited the Smell and Taste Center in Ede, the Netherlands. This allowed us to perform a voxel-based morphometry study in the largest sample of patients with changes in olfactory function for so far **(chapter 5)** and to study brain function and connectivity in a homogenous population of patients **(chapter 6)**. However, in these studies, we did not include a control group. In a follow-up, inclusion of a group of healthy control participants would be warranted to further investigate morphology and function of olfactory-related brain regions in patients with changes in olfactory function.

Measuring eating behavior

When studying eating behavior, there are different methods and outcomes measures that can be applied. In this thesis, we aimed to measure eating behavior by using validated tests which were easy, quick to apply and the best possible representation of eating behavior in daily life.

To measure food preferences, we used the Macronutrient and Taste Preference Ranking Task (MTPRT) [55] **(chapter 2 and 3)**. This task uses a quantitative ranking procedure to measure food preferences and does not focus on separate foods, as was done previously in patients with changes in olfactory function [2,14], but includes taste and macronutrient categories. This makes the task applicable in several patient populations and allows comparisons between groups **(chapter 2 and 3)**. Other tasks that include macronutrient and taste categories are the Macronutrient Preference Checklist, in which participants are asked to check off all items on the list they would like to eat at that moment [56] and the Leeds Food Preference Questionnaire, which is comparable to the MTPRT but only includes two macronutrient categories [57]. Therefore, the MTPRT seems to be the best suited method to study the effect of relation be-

tween food preferences and other factors like olfactory and gustatory function. However, food preferences are also influenced by other factors, like time of the day [58], while the MTPRT askes for what a participant would like to eat at the moment the task is performed. This might have an effect on measuring changes in food preferences over time. In **chapter 3**, we tried to control for this effect by performing repeated measurements within patients on the same time of the day. Nevertheless, this effect should be taken into account when generalizing results.

To investigate whether food preferences represent actual food intake, further measures are needed. As a proxy measure for food intake, we used an online questionnaire that scores diet quality by calculating adherence to the Dutch Dietary Guidelines, using dietary intake during the past month as reference [13] **(chapter 2)**. This questionnaire only includes a limited number of categories. Other methods, like food frequency questionnaires or a 24 hour recall, give a complete overview of total dietary intake and are therefore be more representative for total intake. However, these methods are also more time-consuming. In our study, we chose to use the current questionnaire as it was less time-consuming for participants. Moreover, the questionnaire was previously validated by correlating the results to the outcomes of a 180-item food frequency questionnaire combined with a 24-hour urinary natrium excretion value, demonstrating that the questionnaire was appropriate to measure dietary intake on group level [13]. As our results are in line with previous findings based on food frequency questionnaires in older adults with changes in olfactory function [14,15], we therefore consider the questionnaire used in this thesis as representative for food intake in daily life in our patient population.

Neurobiology of olfactory function

In our studies on the neurobiology of olfaction **(chapter 4-6)**, we included both measures of morphology of the brain (olfactory bulb volume; voxel-based morphometry) and function of the brain (general linear model based on

BOLD activation and group independent component analysis), which gives a complete overview of the neurobiology of the olfactory system. However, our studies included cross-sectional datasets, while for a better understanding of the relation between changes in the olfactory system and changes in olfactory function also longitudinal studies are needed. In collaboration with the Smell and Taste Center, these studies can be designed, which will allow the follow-up of several measures of olfactory function over time to for example track disease progression or recovery after olfactory training. To allow follow-up of the morphology and function of olfactory-related regions in the brain, it is important to use objective methods, like the algorithm we developed to measure olfactory bulb volume **(chapter 4)** and voxel-based morphometry **(chapter 5)**, which is a well-documented method [59]. This will allow not only comparisons over time, but also comparisons with results from other studies.

In our studies on neurobiological changes in patients with changes in olfactory function in **chapter 5** and **chapter 6**, we only included patients and no healthy controls. Therefore, we applied a region of interest (ROI) analysis including a priori ROIs that are known to play a role in olfactory processing from meta-analyses [30,60] in **chapter 5**. Moreover, in **chapter 6**, we applied a functional mask for the piriform cortex from a meta-analysis [23] in our ROI analysis for piriform cortex activation.

Objective versus subjective olfactory and gustatory function

To investigate changes in olfactory and gustatory function, both objective and subjective testing can be applied. In **chapter 2**, patients self-reported their olfactory function, while in **chapter 3** we used both objective and subjective scores for olfactory and gustatory function. Moreover, in **chapter 5** and **chapter 6**, we only used objective scores for olfactory function.

As the perception of smell and taste are closely intertwined as flavor, patients are often not able to distinguish a smell disorder from a taste disorder [61–63]. On population level, patients are able to recognize an improvement

in olfactory functioning when the improvement is 5.5 points or more on the Sniffin' Sticks test (scale: 1-48) [64]. However, therapies like smell training can have a smaller effect on olfactory function [65], but at the same time still lead to changes in brain morphology [66]. Therefore, it is recommended to use objective testing to relate other measures to olfactory function, like changes in brain morphology or function.

In contrast, in the effect of changes in olfactory and gustatory function on eating behavior, subjective changes do play an important role. Patients often report a reduced food enjoyment **(chapter 2)**, most likely due to the fact that changes in olfactory and gustatory function impact subsequent flavor perception. Therefore, we recommend to apply both objective and subjective testing when investigating the effect of changes in olfactory and gustatory function on eating behavior, to get a complete overview of the effect of these changes and subsequent flavor perception on eating behavior.

Clinical implications

Changes in olfactory and gustatory function occur more frequent than expected in several patient populations. Therefore, health care providers should be educated on the occurrence and importance of changes in olfactory and gustatory function in patients. This can be supported by the development of clinical guidelines on the management of changes in olfactory and gustatory function. This will create more awareness about changes in olfactory and gustatory among health care providers, as patients often do not mention these changes themselves. These guidelines should be developed in collaboration with health care providers and researchers, and should include recommendations for olfactory and gustatory testing as well as for imaging in these patients.

So far, there are little possibilities available for treatment of changes in olfactory and gustatory function. Interventions for these patients should therefore not solely be focused on the treatment, but should also target nutritional

recommendations to improve quality of life for patients. Although we did not find significant changes in adherence to Dutch Dietary Guidelines **(chapter 2)**, patients with changes in olfactory and gustatory function often report a lower food enjoyment (**chapter 2**, [2,11,67]). As recovery of olfactory function is not always possible in patients with changes in olfactory function, nutritional recommendations for these patients should be focused on the long term and mainly on increasing food enjoyment. In contrast, changes in gustatory function are mostly due to the use of medication or treatment and are often temporary. For these patients, nutritional recommendations should specifically be aimed at interventions that can be applied during treatment, such as eating smaller portions sizes more often [68]. These interventions should focus on flavor enhancement of foods as well as on education on nutritional intake, as combined interventions were found to be more effective [21,69].

Moreover, health care providers should consider the role that the neurobiology of olfaction can play in diagnostics and prognostics. In the context of daily health care, most promising is the application of measures of olfactory bulb volume, for example to allow prediction of recovery of olfactory ability [26] or treatment success [70–73]. Moreover, olfactory bulb volume can be an early indicator of the onset of disease or for being at risk of a wide variety of diseases, like depression [74,75], schizophrenia [76,77] or neurodegenerative diseases [29,78]. In **chapter 4**, we developed a tool that provides fast and reliable measures of olfactory bulb volume. Incorporating the scan protocol to obtain images suitable for olfactory bulb volume measurements will add only a few extra minutes to currently used standard whole-brain scanning protocols. It will enable the follow-up of olfactory bulb volume over time, which can be applied to follow progression olfactory bulb volume over time or to monitor the effect of treatment for changes in olfactory function, like olfactory training, on olfactory bulb volume. For the application of other morphological and functional changes in the olfactory system in daily health care, as shown in **chapter 5** and **chapter 6**, more research is needed to develop tools that allow easy and quick

assessment of structure and function. Current methods are time-consuming and not suited to yield results on the level of the individual patient.

Recommendations for future research

The results from this thesis indicate several directions for future research. Our collaboration with the Smell and Taste Center of Hospital Gelderse Vallei will allow us to perform these in a large population of clinically diagnosed patients.

It would be interesting to investigate the role of trigeminal perception. Many odors also have a trigeminal component, for example menthol or eucalyptus. While the trigeminal stimuli have a distinct processing pathway [79], changes in olfactory function can lead to a reduced trigeminal sensitivity [80]. Moreover, olfactory, gustatory and trigeminal stimuli share central processing areas like the orbitofrontal cortex and the insula [80,81], which also play a role in flavor processing. A study in healthy participants showed that increasing spiciness of a food can increase satiation and food enjoyment [82]. Also in patients with changes in olfactory and gustatory function, the use of spices can increase food enjoyment and food intake [2,83,84]. Therefore, studying the relation between trigeminal function and eating behavior in patients with changes in olfactory and gustatory function will give more insight in how trigeminal signals affect flavor processing and subsequent eating behavior in these patients.

In addition, we found that sensory networks in response to odors were still activated in anosmic patients **(chapter 6)**. This indicates that peripheral input does not necessarily need to be perceived consciously for the activation of olfactory-related brain regions in patients with acquired changes in olfactory function. While it is known that olfactory training can improve olfactory function, the underlying mechanism is so far not resolved [65]. More longitudinal studies in relation to different causes of changes in olfactory function will yield further insight in the processes underlying the relation between peripheral olfactory input and the neurobiology of the olfactory system. This will lead to a fundamental understanding of how the olfactory system works and might lead to better

predictions on prognosis for patients with changes in olfactory and gustatory function.

Lastly, while we studied changes in morphology and function of olfactory-related brain regions, data on neurobiological changes in patients with changes in gustatory function is limited. One study found that in patients with changes in gustatory function, the OFC demonstrated higher activation in response to taste stimuli compared to healthy controls [85]. As the integration of smell and taste into flavor is partly located in brain regions that are also involved in gustatory processing, like the OFC [48,86], these regions are of interest for further research. However, as the number of patients with solely changes in gustatory function is limited [87], recruitment of participants for such a study will be challenging.

Conclusion

Within this thesis, we aimed to gain more insight in the effect of changes in olfactory and gustatory function on eating behavior and the neurobiology of smell and taste. We can conclude that changes in olfactory and gustatory function seem less important for actual measures of eating behavior, such as food preferences and intake. We propose that these changes are more relevant for alterations in subsequent flavor perception and food enjoyment. Moreover, changes in olfactory function affect olfactory processing in both primary and secondary olfactory-related brain regions. The effect of these changes on morphology and function of olfactory-related brain regions is dependent on the degree of olfactory function. Morphological and functional changes were reflected in several brain regions that are known to play a role in flavor perception and in the perceived pleasantness of foods during consumption, which aligns with our proposition on the effect of changes in olfactory and gustatory function on eating behavior.

As changes in olfactory and gustatory function occur in several patient populations, health care providers should be educated on the occurrence and

consequences of these changes. Moreover, results from this thesis suggest that the neurobiology of olfaction, like volume of the olfactory bulb, can play an important role in diagnostics and prognostics of disease. Future research should focus on more longitudinal studies combining several outcome measures of eating behavior and the neurobiology of smell, taste, and their integration in flavor perception. This will lead to further insights in treatment possibilities and effective strategies to optimize eating behavior in patients with changes in olfactory and gustatory function.

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Summary

Summary

Changes in olfactory function are a widespread problem: 3% up to 20% of the general population suffers from changes in olfactory function. Changes in gustatory function are less common: of all patients seeking clinical assistance for changes in olfactory and gustatory function, less than 4% is diagnosed with changes in gustatory function. Smell and taste, and their combined perception in flavor of food, are important determinants for food intake and subsequently nutritional status. However, the effect of changes in olfactory and gustatory function on eating behavior and the neurobiology of smell and taste is not well enough understood. Hence, within this thesis, we aimed to gain more insight in the effect of these changes on eating behavior and the neurobiology of smell and taste, in order to ultimately provide patients with sufficient health care and nutritional recommendations.

In **chapter 2**, we used an online survey to investigate the effect of changes in olfactory function on food preferences and adherence to dietary guidelines in a population of Dutch patients with self-reported changes in olfactory function. Patients with acquired changes in olfactory function displayed the lowest preference for high-carbohydrate foods and highest preference for low-energy foods, which was similar to the preference pattern of a control group with healthy individuals. In contrast, patients with congenital anosmia showed an aberrant pattern, with a higher preference for high-fat foods. Adherence to the dietary guidelines was similar for the patients with changes in olfactory function and the reference population, but patients did show a reduced food enjoyment. Thus, changes in olfactory function seem less important for actual measures of eating behavior, such as food preferences and intake, but more relevant for changes in subsequent flavor perception and food enjoyment.

Next, in **chapter 3**, we followed a group of patients with colorectal cancer during and after chemotherapy treatment to assess possible changes in olfactory and gustatory function and food preferences. Here, no differences in objective olfactory and gustatory function compared to control patients were shown. However, during treatment, subjective olfactory and gustatory function were rated significantly worse by patients undergoing chemotherapy than the control patients. We found no effect of undergoing chemotherapy treatment on food preferences, but preference for protein was positively correlated with objective gustatory function. This correlation is presumably related to the change in flavor perception that is likely to occur in patients experiencing changes in gustatory function.

Chapter 4 describes the application of convolution neural networks to allow automated volume measurements of olfactory bulb volume. The olfactory bulb is the first receptor of olfactory signals in the human brain and therefore of importance to study in the context of changes in olfactory function. Localization and segmentation of the olfactory bulb as well as subsequent calculation of olfactory bulb volume were performed successfully. This method can be utilized in both research and health care and may lead to more insight in the role of the olfactory bulb in diagnosis, prognosis and treatment of olfactory loss

In **chapter 5**, we investigated the morphology of primary and secondary olfactory-related brain regions of patients with primary changes in olfactory function. Patients with congenital anosmia showed reduced density in the gyrus rectus compared to patients with acquired changes in olfactory function, while the density of the orbitofrontal cortex of patients with congenital anosmia was increased compared to the other patients groups. Moreover, there was a positive relation between density of the orbitofrontal cortex and olfactory function. This brain region is related to flavor perception and is known to play a role in the perceived pleasantness of foods during consumption.

Lastly, in **chapter 6**, we aimed to determine how changes in olfactory functioning affect neural activation patterns and networks in the olfactory system of the brain and how this is related to olfactory function. We found that patients with olfactory loss showed piriform cortex activation during odor stimulation compared to pure sniffing, even in anosmic patients. Moreover, olfactory function was correlated with the recruitment of a sensory processing network. This network involved several regions that are known to play a role in flavor perception, like the insula and the anterior cingulate cortex.

From this thesis, we conclude that changes in olfactory and gustatory function seem less important for actual measures of eating behavior, such as food preferences and intake, but more relevant for changes in subsequent flavor perception and food enjoyment. Moreover, we found that changes in morphology and function of olfactory-related brain regions were dependent on olfactory function. These changes were reflected in several brain regions that are known to play a role in flavor perception and in the perceived pleasantness of foods during consumption. Interventions for patients with changes in olfactory and gustatory function should therefore include nutritional recommendations and take food enjoyment in consideration. Moreover, health care providers should consider the role that the neurobiology of olfaction, like volume of the olfactory bulb, can play in diagnostics and prognostics of disease. Future research should focus on more longitudinal studies combining several outcome measures of eating behavior and the neurobiology of smell, taste and their integration in flavor perception. This will lead to further insights in treatment possibilities that target the neuroplasticity of olfactory-related brain regions and effective strategies in regard to eating behavior in patients with changes in olfactory and gustatory function.

Dankwoord

Abouth the author

List of publications

Overview of completed training activities

Dankwoord

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onderzoek gaat, komt het altijd mijn kant op, en als ik op TV of radio kom, heb jij gekeken of geluisterd.

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Heit: samen achter de computer schreef ik mijn eerste verhalen. Met z'n tweeën naar het archief voor een werkstuk op de middelbare school, en nu hebben we samen de laatste puntjes op de i gezet voor mijn proefschrift. Wat ben ik blij dat we dat samen konden doen!

Mem: toen ik naar de universiteit ging, kon jij me uitleggen hoe de webmail en de printer werkten. Niet alleen deze, maar veel meer waardevolle levenswijsheden heb ik van jou meegekregen. Dankjewel hiervoor!

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About the author

Elbrich Marije Postma was born on the 30th of October 1990 in Leeuwarden, the Netherlands. After completing secondary school at 'Lauwers College' in Buitenpost, she enrolled in the BSc program Biology at the University of Groningen in 2008. Her major specialization was Behavioral and Neurosciences, while she followed a minor specialization in Education. This minor included teaching Biology at a secondary school. After finishing her bachelor, she took a year off to be the head of the board of study association GLV Idun. In 2012, Elbrich enrolled in the MSc program Biology, in which she specialized in Science, Business and Policy. Her graduation internship was at the Nutrition and Healthcare Alliance in Ede and was focused on setting up a Smell and Taste Center to facilitate both health care for patients and research on changes in olfactory and gustatory function.

After her graduation in 2014, Elbrich started working for the Nutrition and Healthcare Alliance to execute the plans that were developed for the Smell and Taste Center. This center was opened at Hospital Gelderse Vallei in 2015. For finetuning of the logistics of the center, Elbrich was appointed at the hospital as coordinator of the center. Moreover, she was appointed as a PhD candidate at the Department of Human Nutrition and Health of Wageningen University & Research in 2016. Her project focused on changes in olfactory and gustatory function and their effects on eating behavior and possible neurobiological alterations in diverse patient populations.

During her PhD project, Elbrich was involved in several MRI studies. Moreover, two studies were conducted in collaboration with patients associations, namely Reuksmaakstoornis.nl and HungerNdThirst. Elbrich attended several courses

and presented her work at international conferences. In 2016, she won the Young Professional Award at the Voeding Nederland conference. Furthermore, Elbrich was involved in teaching and supervising BSc and MSc students and was part of the organizing committee of the PhD tour to East Canada in 2019. Currently, Elbrich is still involved in the Smell and Taste Center at Hospital Gelderse Vallei. Moreover, she is appointed as a postdoctoral researcher at the Department of Human Nutrition and Health. In 2020 she received a grant to perform a new MRI study to measure brain activation in response to odors in healthy participants.

List of publications

Publications in peer-reviewed journals

Postma EM, Kok DE, De Graaf C, Kampman E, Boesveldt S. Chemosensory perception and food preferences in colorectal cancer patients undergoing adjuvant chemotherapy. Clinical Nutrition ESPEN. 2020 Sept 29. doi: 10.1016/j.clnesp.2020.09.012.

De Vries RJ, Morquecho Campos P, De Vet EWML, De Rijk MG, **Postma EM**, De Graaf C, Engel B, Boesveldt S. Human spatial memory implicitly prioritizes high-calorie foods. Scientific Reports. 2020 Oct 8. doi: 10.1038/s41598-020-72570-x.

Peter MG, Fransson P, Mårtensson G, **Postma EM**, Nordin LE, Westman E, Boesveldt S, Lundström JN. Normal Olfactory Functional Connectivity Despite Lifelong Absence of Olfactory Experiences. Cereb Cortex. 2020 Aug 19:bhaa217. doi: 10.1093/cercor/bhaa217.

Peter MG, Mårtensson G, **Postma EM**, Nordin LE, Westman E, Boesveldt S, Lundström JN. Morphological changes in secondary, but not primary, sensory cortex in individuals with life-long olfactory sensory deprivation. NeuroImage. 2020 Sep;218:117005. doi: 10.1016/j.neuroimage.2020.117005. Epub 2020 May 30.

Noothout JMH, De Vos BD, Wolterink JM, **Postma EM**, Smeets PAM, Takx RAP, Leiner T, Viergever MA, Isgum I. Deep Learning-Based Regression and Classification for Automatic Landmark Localization in Medical Images. IEEE Trans Med Imaging. 2020 Jul 13;PP. doi: 10.1109/TMI.2020.3009002.

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population of Dutch individuals with self-reported smell loss: An online survey. Food Quality and Preference. 2019 May;79:103771. https://doi.org/10.1016/j.foodqual.2019.103771

Van der Want E, **Postma EM**, Holverda M, Postema H, Menkveld-Beukers M, Witteman BJM. Hoe ervaren patiënten voedingsadvies tijdens de behandeling van kanker? Voeding & Visie jaargang 33, lente 2020. [Dutch]

Postma EM, Reichert JL, Smeets PAM, Boek WM, de Graaf K, Schöpf V, Boesveldt S. Severity of olfactory deficits is reflected in functional brain networks-An fMRI study. Hum Brain Mapp. 2018 Aug;39(8):3166-3177. doi: 10.1002/hbm.24067.

Boesveldt S, **Postma EM**, Boak D, Welge-Luessen A, Schöpf V, Mainland JD, Martens J, Ngai J, Duffy VB. Anosmia-A Clinical Review. Chem Senses. 2017 Sep 1;42(7):513-523. doi: 10.1093/chemse/bjx025.

Van Belzen L, **Postma EM**, Boesveldt S. How to quench your thirst. The effect of water-based products varying in temperature and texture, flavour, and sugar content on thirst. Physiol Behav. 2017 Oct 15;180:45-52. doi: 10.1016/j.physbeh.2017.08.007.

Postma EM, de Vries YC, Boesveldt S. Tasty food for cancer patients: the impact of smell and taste alterations on eating behaviour. Ned Tijdschr Geneeskd. 2017;160:D748. [Dutch]

Submitted for publication

Postma EM, Smeets PAM, Boek WM, Boesveldt S. Morphological changes in the brain due to olfactory loss: a voxel-based morphometry study on etiology and duration of olfactory dysfunction.

Van Noort HHJ, **Postma EM**. Relieve of thirst in hospitalized patients with a mini popsicle of 10cc: a feasibility study.

Hontelez S, Stobernack T, Pelsser LM, Van Baarlen P, Frankena K, Groefsema MM, Kleerebezem M, Rodrigues Pereira R, **Postma EM**, Smeets PAM, Stopyra MA, Zwiers MP, Aarts, E. Clinical symptom decrease in children with ADHD following a few-foods diet is associated with increased brain activation.

In preparation for submission

Postma EM, Noothout JMH, Boek WM, Joshi A, Herrmann T, Hummel T, Smeets PAM, Išgum I, Boesveldt S. Automatic quantification of olfactory bulb volume in MRI scans using convolutional neural networks: the potential for clinical application.

Abstracts and presentations

Postma EM, Kok DE, Kampman E, Boesveldt S (2019). Chemosensory perception and food preferences in colorectal cancer patients undergoing chemotherapy. British Feeding and Drinking Group annual meeting, Swansea, UK. (oral presentation) (bursary to support attendance)

Postma EM, van der Want E (2019). Voedingsadvies in het zorgpad: de ervaring van oncologiepatiënten in een patient journey. Voeding Nederland conference, Utrecht, NL. (poster presentation)

Postma EM, Kok DE, Kampman E, Boesveldt S (2019). Changes in smell, taste and food preferences in colorectal cancer patients. Masterclass Nutrition and Cancer, Wageningen, NL. (poster presentation)

Postma EM, Van Amerongen L, Boek WM, De Graaf C, Boesveldt S (2018). Size Does Matter: Comparing Two Manual Methods of Measuring Olfactory

Bulb Volume. Association for Chemoreception Sciences XL, Bonita Springs, USA. (poster presentation) (AChemS Student Housing Award; ECRO Travel Grant)

Postma EM, Boesveldt S, Van der Werf B, Kok DE, Kampman E (2018). Changes in smell, taste and food preferences in colorectal cancer patients undergoing chemotherapy. Voeding Nederland Conference, Utrecht, NL. (poster presentation)

Postma EM (2017). Reuk- en smaakveranderingen bij oncologische patiënten. Studiedag Voeding, Bewegen en Kanker. Bussum, NL. (oral presentation)

Postma EM, Boesveldt S, Kok DE, Kampman E (2017). Changes in smell, taste and food preferences in colorectal cancer patients. European Society for Clinical Nutrition and Metabolism Congress, Den Haag, NL. (poster presentation)

Postma EM, Reichert JL, Smeets PAM, Boek WM, De Graaf C, Schöpf V, Boesveldt S (2017). Human Olfaction Conference, Nijmegen, NL. (poster presentation)

Postma EM, Jonker LK, Boesveldt S (2017). Dietary Patterns and Food Preferences in a Population of Dutch Patients Suffering From Smell Loss. Association for Chemoreception Sciences XXXIX, Bonita Springs, USA. (poster presentation)

Postma EM (2016) Eten met je neus: het belang van reuk en smaak voor een optimale voedingsstatus. Voeding Nederland conference, Utrecht, NL. (oral presentation) (Young Professional Award)

Overview of completed training activities

Discipline specific courses and activities	Organizer and location	Year
NutriScience: A Multifaceted Approach to Nutrition Research	VLAG; Wageningen, NL	2015
Fifth Sense Symposium	Fifth Sense; University of Surrey, UK	2015
SPM Course for fMRI and MRI/VBM	Wellcome Trust Centre for Neuroimaging; University College London, UK	2016
Sensory and Liking symposium	TiFN; Groningen, NL	2016
Voeding Nederland conference	AVZ, NAV, DCN, NVD, NVVL; Utrecht, NL	2016
AChemS annual meeting	Association for Chemoreception Sciences; Bonita Springs, USA	2017
Human Olfaction Conference	Centre for Language Studies, Radboud University; Nijmegen, NL	2017
Summer School on Human Olfaction	Smell & Taste Clinic, University of Dresden Medical School; Dresden, DE	2017
ESPEN Congress	European Society for Clinical Nutrition and Metabolism; Den Haag, NL	2017
Studiedag Voeding, Bewegen en Kanker	Mark Two Academy; Bussum, NL	2017
Sensory Perception & Food Preference	VLAG; Wageningen, NL	2018
Voeding Nederland conference	AVZ, NAV, DCN, NVD, NVVL; Utrecht, NL	2018
AChemS annual meeting	Association for Chemoreception Sciences; Bonita Springs, USA	2018
Masterclass Nutrition and Cancer	VLAG, WCRF, WKOF; Wageningen, NL	2019
Voeding Nederland conference	AVZ, NAV, DCN, NVD, NVVL; Utrecht, NL	2019
Annual meeting BFDG	Swansea University; Swansea, UK	2019
WIOS meeting	WIOS; Wageningen, NL	2019

General courses and activities	Organizer and location	Year
Good Clinical practice course	Profess Academy; Wageningen, NL	2016
VLAG PhD week	VLAG; Baarlo, NL	2017
Scientific Writing	WGS; Wageningen, NL	2018
Philosophy and Ethics of Food Science and Technology	VLAG, WGS; Wageningen, NL	2018
Project and Time Management	WGS; Wageningen, NL	2018
Good Clinical practice course	Tapas Group; Apeldoorn, NL	2019
Career assesment	WGS; Wageningen, NL	2019
FameLab Talking Science Masterclass	KNAW; Amsterdam, NL	2019
Nutritional Leadershop Workshop Debating	Young NAV; Den Haag, NL	2019

Optional courses and activities	Organizer and location	Year
Preparation of research proposal	Wageningen, NL	2016
Organizing PhD tour	Wageningen UR; Wageningen, NL	2019
PhD tour to East Canada	Wageningen UR; CA	2019
Chair group meetings	Wageningen UR; Wageningen, NL	2016-2020
Food for Thought meetings	AVZ; Ede, NL	2016-2020

Colophon

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