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Direktor: Herr Prof. Dr. Thomas Zahnert

Investigations of the gustatory system: from peripheral saliva parameters to central neuroimaging

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List of Abbreviations

TBs	Taste buds
TRCs	Taste receptor cells
CNS	Central nervous system
CN	Cranial nerves
GERPs	Gustatory Event-Related Potentials
MRI	Magnetic Resonance Imaging
fMRI	functional Magnetic Resonance Imaging
CaVI	Carbonic anhydrase VI
TAC	Total antioxidative capacity
GC	Gustatory cortex
IC	Insular cortex
OFC	Orbital frontal cortex
NaCl	Sodium chloride
HCl	Hydrochloric acid
MVPA	Multivariate pattern analysis
GG	Geniculate ganglion
NST	Nucleus of the solitary tract
BDI	Beck Depression Inventory
TE	Echo time
TR	Repetition time
FCA	Functional connectivity analysis
ROIs	Regions of interests
GLM	General Linear Model

BOLD	Blood-oxygenation level detection
PFC	Piriform cortex
ENT	Ear, nose and throat
PVC	Polyvinyl chloride
SPM	Statistical parametric mapping
MNI	Montreal Neurological Institute
HRF	Hemodynamic Response Function
SPSS	Statistical Package for the Social Sciences
poCG	Postcentral gyrus
lFP	Left side of frontal pole
lSFG	Left side of superior frontal gyrus
RRC	ROI-to-ROI connectivity
CER	Cerebellum
rSFGdl	Right side of dorsolateral superior frontal gyrus
lMTG	Left side of middle temporal gyrus
CCAS	Cerebellar cognitive affective syndrome
BA	Brodmann area
TRPV1	Transient receptor potential channels for vanilloid
ANOVA	Analysis of Variance

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Introduction of the topic

The gustatory system

Definitions of "taste" and "flavour"

"Taste sensation" or "gustation" is defined as the sensation that results from the direct stimulation of the gustatory receptors residing in taste buds (TBs) (Spence et al., 2015). Sweet, sour, salty, bitter and umami are recognized as the five basic taste qualities (Fábián et al., 2015). In clinics it is common that, for people who claim they lost their sense of "taste", most of them are eventually diagnosed with olfactory deficits instead of the five basic tastes being affected (Spielman, 1998; Pribitkin et al., 2003). This phenomenon arises because, "taste" is often used synonymously with "flavour" (Landis & Heckmann, 2004) and the latter is a word used in multiple ways in daily lives. Generally, it refers to the complex feel that we perceive during eating food combining the multi-sensory inputs from the gustatory, the oral somatosensory, and the olfactory systems (Spence et al., 2015). The olfactory components, especially for the retronasal olfactory part, contribute a lot to the flavour of food (Bartoshuk et al., 2019). Hence, it is no wonder why people having olfactory impairments claim that they cannot perceive the "taste", which is actually the flavour, of the food. However, in most of the academic literature and in this thesis, "taste sensation" or "gustation" refers to the oral sensation resulting from the activation of taste receptors in taste buds and is confined to the five basic taste qualities (sweet, sour, salty, sweet and bitter) (Spence et al., 2015).

The robust gustatory system

The gustatory system is robust. It maintains a very stable whole-mouth taste sensation (Bartoshuk, 1989). The peripheral sensory units for taste perception, known as taste buds (TBs), are distributed throughout the oral cavity and surrounding regions. Each person has roughly 2000 to 5000 TBs (Lalonde & Eglitis, 1961; Miller, 1986; Roper, 2013). Most TBs reside in three types of gustatory papillae (circumvallate, foliate and fungiform) which respectively locate at the posterior part, bilateral sides and tip of the tongue (lingual TBs) (Gutierrez & Simon, 2021). Extralingual TBs are just embedded in the surrounding oral epithelium of the mucosa of the palate, and to a lesser extent the oropharynx, epiglottis and the upper esophagus. Each TB is a cluster of approximately 100 neuroepithelial cells including three types of taste cells (Type I, II and III) characterized by their morphological phenotypes, and basal cells (Type IV). The latter are immature taste cells which can

differentiate into various types of taste cells (Gutierrez & Simon, 2021). Taste cells are continually renewed with a fast turnover rate ranging from approximately 8 to 22 days (Gutierrez & Simon, 2021).

The function of different types of taste cells has not been studied as thoroughly. Although, many authors referred to all these cells as “taste receptor cells”, it is now thought that only half or fewer of all these cells expressing receptors work as “receptors” (Chaudhari, 2014), known as taste receptor cells (TRCs). When receptors detect chemicals involved in the perception of tastes (e.g., sweetness), some TRCs release neurotransmitters that activate the afferent fibres which convey taste information to the central nervous system (CNS) (Gutierrez & Simon, 2021). There are three cranial nerves (CN) conveying taste information (facial [CN VII], glossopharyngeal [CN IX] or vagal [CN X] nerves) (Roper, 2013). One branch of the facial nerve, the chorda tympani nerve, innervates the TBs in fungiform papillae located in the anterior tongue and part of the TBs in the foliate papillae in lateral sides of the tongue. Another branch of the facial nerve, the greater superficial petrosal nerve, innervates the TBs in the palate. The glossopharyngeal nerve innervates the TBs of the posterior tongue in the circumvallate papillae and part of TBs in foliate papillae at the lateral sides of the tongue, whereas the vagal nerve innervates the TBs located in the epiglottis, pharynx, and larynx (Roper, 2013).

It has been hypothesized that activation of one nerve inhibits the function of other taste nerves (Bartoshuk, 1989). For example, anesthetization of the chorda tympani nerve of the tongue could produce an increase in perceived taste intensity of the whole mouth (Ostrom et al., 1985). This means when a single taste nerve is injured, the whole-mouth taste perception tends to be maintained due to the loss of the inhibition from the injured nerve to other taste nerves. Apart from that, there is a phenomenon called “taste localization illusion” (Bartoshuk, 1989; Todrank & Bartoshuk, 1991). During eating, taste sensations are not referred to the locations of taste buds, but seem to originate from the entire inner surface of the mouth. In experiments, local taste stimulation of the tongue also tends to be projected into the whole mouth (Bartoshuk, 1989; Todrank & Bartoshuk, 1991). This explains why there is little or no change in taste experience of the whole mouth even if localized damage of taste buds occurs.

It has been suggested that there is a certain degree of “redundancy” in the gustatory system to maintain whole mouth taste sensation (Bartoshuk, 1989). Hence, serious gustatory dysfunctions such as complete taste loss are rare with 0.10 – 0.85% individuals being affected compared to 32% who exhibit profound olfactory dysfunction (Deems et al., 1991; Pribitkin et al., 2003; Welge-Lüssen et al., 2011).

Gustatory dysfunction (taste disorder) and taste assessment

Taste disorder

Taste disorders can be classified as two types, quantitative disorders and qualitative disorders, which often coexist (Landis & Heckmann, 2004). “Qualitative taste disorder” refers to the distortion of taste sensations, characterized by mostly bothersome complaints that cannot be measured by available techniques. “Quantitative taste disorder” includes hypogeusia – a partial taste loss and ageusia, a complete taste loss, which can be assessed with a number of methods. Hence, taste assessments are mainly aimed to evaluate quantitative taste disorders. Qualitative taste disorders are often accompanied by psychological issues, such as depression (Deems et al., 1996).

Taste disorders change our lives in many ways. On the one hand, patients with taste disorders have an increased risk to ingest rotten or spoiled foods and, on the other hand, they may lose enjoyment of foods which could further result in significant changes in eating habits (Clark, 1998) and mental health, e.g., depression and anxiety (Bergdahl & Bergdahl, 2002; Han et al., 2018; Hur et al., 2018). Changes in eating habits may promote malnutrition (Schiffman, 1983), metabolic and cardiovascular disease (Sergi et al., 2017; Xue et al., 2020), or obesity (Nasser, 2001). Overall, this may result in an impaired quality of life (Risso et al., 2020).

Because of the lack of a universal standard/test for diagnosing taste loss, estimates of the prevalence of taste dysfunction in the general population vary. They range from 0.85% to 20% (Deems et al., 1991; Pribitkin et al., 2003; Vennemann et al., 2008; Welge-Lüssen et al., 2011; Khil et al., 2015) and older people are more vulnerable (Cowart et al., 1997). A population-based study, conducted on 3,005 community-dwelling U.S. older adults (aged from 57 to 85 years), showed that 74% of them had taste impairments (Correia et al., 2016). However, this high percentage of “taste impairment” among older people might be overestimated. At first, the method employing only 4 strips, respectively for sweet, sour, salty and bitter taste, to evaluate taste ability used in the study was not a validated taste test. Participants with only one error in identifying the taste quality were classified with “taste impairment”. However, this criterion is not entirely convincing because people with normal taste function can also make a few errors in taste identification test (Pilková et al., 1991; Soter et al., 2008) and the study did not recruit participants under 57 years of age for comparison. Second, whether these older people with “taste impairments” also had subjective complaints/symptoms of taste disorder was not mentioned in the study. It is also not mentioned whether the strip was put on one specific region of the tongue (localized test)

or used as a whole mouth test. As mentioned earlier, people could have localized taste loss with normal whole mouth taste function so that they would not have symptoms (Bartoshuk, 1989).

One study based on 408 patients complaining of diminished taste capacity (quantitative taste disorders) in a smell and taste clinic showed that the most frequent causative factor for taste loss was the administration of drugs, 32% of the patients exhibiting drug-induced taste loss. For people over 65 years old, the proportion rose to 47% (Ikeda et al., 2008), suggesting that aging is also an important factor for taste loss. The percentage also depends on the investigated sample. In patients presenting themselves to another smell and taste clinic with taste loss (quantitative taste disorders) and/or taste distortions (qualitative taste disorders) only 4% of 491 patients had medication-induced taste disorders (Fark et al., 2013), but idiopathic factor being the first reason. Other causative factors include systemic diseases (Schelling et al., 1965; Solomons et al., 1977; Atkin-Thor et al., 1978; Burch et al., 1978), zinc deficiency (Yoshida et al., 1991; Sakai et al., 2002), glossitis and stomatitis (Itoh et al., 2002), inflammation of the upper respiratory tract (Henkin et al., 1975), and disorders of the central nervous system (Lang et al., 2006; Shah et al., 2009; Theys et al., 2009; Nakajima et al., 2010). Ikeda et al. believed that zinc deficiency was a key factor for the following reasons: 1. In drug-induced taste disorder, a drug-related chelation of zinc might cause zinc deficiency; 2. In patients with idiopathic taste disorder, “marginal zinc deficiency may be present despite a normal serum zinc level”; 3. In cases of systemic disease, hepatic dysfunction, renal dysfunction and diabetes affect zinc absorption or enhance zinc excretion; 4. Fever induced by upper respiratory tract inflammation may lead to the consumption of zinc. All in all, in their study, subjects associated with the above four etiological factors plus zinc deficiency per se responded to zinc treatment with response rates ranging from approximately 65 to 75%. In other studies zinc has also been shown to be an effective therapy of taste disorders (Henkin et al., 1999b; Heckmann et al., 2005; Takaoka et al., 2010)

Taste Assessment

Many people are inaccurate in self-evaluating their own taste functions (Soter et al., 2008), reflected by the fact that hypogeusia could exist without the patients’ awareness (Landis et al., 2005), particularly if other symptoms are present (Welge-Lussen et al., 2011). People with taste complaints could have normal taste function (Soter et al., 2008). Therefore, standard taste assessments are necessary when evaluating taste function.

Psychophysical taste testing is most widely applied in clinic and in research. For example, the “taste strips” test is a standard and validated identification test based on filter papers (Landis et al., 2009) with a length of 8 cm and a tip area of 2 cm^2 being impregnated with tastants. Sucrose, citric acid, sodium chloride and quinine hydrochloride are employed as solute with distilled water as solvent. For each taste quality, there are four concentrations: sweet: 0. 4, 0. 2, 0. 1, 0. 05 g/ml sucrose; sour: 0. 3, 0. 165, 0. 09, 0. 05 g/ml citric acid; salty: 0. 25, 0. 1, 0. 04, 0. 016 g/ml sodium chloride; bitter: 0. 006, 0. 0024, 0. 0009, 0. 0004 g/ml quinine hydrochloride, resulting in a total of 16 trials. Taste strips can be placed regionally on right or left side of the anterior third of the extended tongue, resulting in 32 trials. Between several trials, the mouth is rinsed with water. For each taste quality, tastes are presented in increasing concentrations with the different taste qualities being randomized in their order of presentation. Patients are instructed to identify the taste from a list of four descriptors, i.e., “sweet”, “sour”, “salty”, and “bitter” in a forced choice manner. The number of correctly identified tastes is added up to a “taste score” representing the general taste identification ability. Advantages of the "taste strips" are their long shelf-life, the option of lateralized testing, and availability of a number of concentrations of the tastants. Normative data have been established (Landis et al., 2008).

However, psychophysical methods strongly rely on the cooperation of the patients. In cases of an inadequate ability to cooperate, e.g., in children or patients with cognitive impairment, or potential malingering concerning medicolegal contexts, psychophysical taste testing is unreliable (Hummel et al., 2004). Gustatory Event-Related Potentials (GERPs) are less biased by the individuals’ beliefs and motives but due to its relatively complex technical prerequisites, the method is not widely used (Hummel et al., 2010). Histological investigations or contact endoscopy could help to examine morphological abnormalities of taste papillae/taste buds on the tongue (Srur et al., 2011; Walliczek-Dworschak et al., 2017b) but they are not standardized for diagnostic purposes. Magnetic Resonance Imaging (MRI) provides visualization of structural lesions of brain regions related to taste processing (Abolmaali, 2004). However, even in the absence of visible peripheral or central lesions (Just et al., 2006; Pavlidis et al., 2013), taste loss may still persist and bother patients. Gustatory functional MRI (fMRI) provides a non-invasive way to examine gustatory function without a major bias in terms of cooperation from the patients. In addition, MRI scanners are widely available so that the technique could be easily applied in many different places. Hence, fMRI has the potential to be used as a relatively objective method to diagnose taste loss. Hummel et al. employed fMRI to compare the brain responses to taste stimulations between patients with taste disorders and healthy controls (Hummel et al.,

2007). However, group comparisons did not reveal obvious differences between patients' group and healthy control group but a little bit stronger activations in the gustatory cortices in patients' group. This fMRI study investigated the brain responses in isolated brain regions, lacking a functional network perspective.

More recent findings suggest that the gustatory system is separated into interacting taste areas, consisting networks of feedforward and feedback pathways from other brain regions, e.g., forebrain areas (Katz et al., 2002). Importantly, recent advances in analysis methods of functional neuroimaging data have provided new tools to investigate the functional connections between anatomically separated brain regions with fMRI (van den Heuvel & Hulshoff Pol, 2010). Although the significant differences of brain activations of isolated brain regions between patients' group and control group was not observed by fMRI, the functional connections between different brain regions of patients with taste loss might be significant distinct from that of healthy controls. Our publication 2 confirmed this speculation, although further studies are needed.

Taste perception and saliva-related parameters

Food chemicals are strong stimuli for the secretion of saliva (Matsuo, 2000). When the "ductal saliva" of the salivary glands enters the mouth, it blends with other constituents originating from mucosal cells, immune cells, and oral microorganism, known as "mixed saliva" or "whole saliva", which much determines the environment of the oral mucosa including TBs (Fábián et al., 2015). Whole saliva protects taste receptors from desiccation and bacterial infection (Matsuo, 2000). Carboanhydrase VI (caVI), one of the salivary enzymes, plays a role in the maintenance of the taste papillae (Shatzman & Henkin, 1981; Melis et al., 2013). CaVI deficiency was found in gustatory dysfunction, and was accompanied by morphological abnormalities of TBs (Henkin et al., 1999a).

Saliva is a solvent for tastants because taste molecules need to be in solution to interact with taste receptors (Matsuo, 2000). Taste sensitivity depends on the concentration of taste molecules in the saliva. Hence, alterations in salivary flow rate and salivary chemical compositions can disturb taste perception. People with a tendency of higher saliva proteolysis are more sensitive for bitter taste (Morzel et al., 2014). The anti-oxidative enzymes in the whole saliva, e.g., catalase, protect taste cells from damages of oxidative process. The level of the salivary total antioxidative capacity (TAC) has been linked to the catalase level in saliva (Walliczek-Dworschak et al., 2017b), which also relate taste

sensitivity. The buffering capacity of saliva, which can modulate the H^+ ions present in saliva (salivary pH), contributes to the sensation of sour taste (Lugaz et al., 2005).

In one cross-sectional research (Walliczek-Dworschak et al., 2017b), 81 patients with taste disorders and 40 healthy controls were recruited. Their taste functions were evaluated with "taste strips" test and their saliva-related parameters were also measured. They found that scores of "taste strips" tests correlated negatively with the salivary flow rate and proteolysis, and positively with caVI and catalase values. Compared to healthy controls patients with taste disorders exhibited a higher salivary total protein concentration, TAC, proteolysis and salivary flow rate, indicating that explorations on saliva-related parameters might be helpful for assessments of taste disorders. Could the improvement or deterioration of taste disorders be reflected by changes on salivary parameters? This question was investigated in publication 1.

Taste coding in the brain

How exactly a taste quality, e.g., sweet, is coded in the brain, is not clear. According to animal studies, the primary gustatory cortex (GC) refers to the area in the insular cortex (IC) that receives direct taste projections from the thalamus and neurons in that area are tuned to the five basic taste qualities (Rolls, 2019). An area in the orbito-frontal cortex (OFC) is defined as the secondary GC because it receives direct inputs from the primary GC (Rolls, 2019). In studies on primates, the firing rate (spikes/s) of single neurons in the insula can be recorded by microelectrodes during oral taste stimulations. It has been found that an insular taste cortex neuron (bo139c2, the name of the neuron) responded to different taste stimuli (including glucose - sweet, quinine - bitter, sodium chloride (NaCl) - salty, hydrochloric acid (HCl) - sour, monosodium glutamate - umami) with significantly different levels of firing rates (Verhagen et al., 2004), suggesting that the insula plays a role in the identification of taste qualities. Like the insula, neurons in the secondary taste cortex – the OFC – also respond to different taste qualities and other types of oral stimulations, e.g., capsaicin and oils, with significantly different firing rates (Rolls et al., 2003).

At a higher level beyond the single-neuron level, spatial coding strategies are mainly investigated within the GCs. Generally, there are two models. "Topographic model" proposed a "taste topographic map" (Chen et al., 2011) within the GCs analogous to somatotopy in the somatosensory system, wherein a specific spatial area selectively responds to a specific taste, such as sweet. One study in rodents (mice) supported this model, in which the neurons of the posterior insular cortex (IC) specifically responded to

bitter taste, whereas the anterior IC mainly responded to sweet taste. However, findings from recent studies are more prone to a “population coding model”, wherein taste quality information is signalled by a pattern of activity across a population of neurons (Avery et al., 2020; Chen et al., 2021). Within the population coding model, the different activity pattern of ensembles of cortical neurons represents different taste qualities without clear spatial preference for one specific taste. Several studies in mice found that broadly tuned neurons that could respond to several taste qualities were spatially distributed across the IC. Avery et al. examined the spatial representation of multiple tastes (sweet, sour, and salty) within the human brain using ultra-high-resolution fMRI at high magnetic field strength (7 Tesla) (Avery et al., 2020). With a multivariate pattern analysis (MVPA) technique, they identified that quality-selective activity patterns, shown as multiple distributed voxels, were present within the primary taste cortex - insula. That means, with MVPA technique, it was possible to classify sweet, salty and sour tastes within the insula with an accuracy of 62%, significantly greater than chance. This finding supports the “population coding model” within the insula as a strategy of taste coding.

Both the “topographic model” or the “population coding model” are static spatial models of taste coding within isolated brain regions. By analysing of response dynamics to gustatory stimuli in single-neuron ensembles in awake rats, Katz et al. found that during gustatory stimulation, the firing rates of GC neurons change with time (Katz et al., 2002). The timings of firing rate changes (time-courses) are specific for different tastants, suggesting that tastant-specific time-courses might be a coding strategy for representing different taste qualities in the brain. They also found that the time-courses were driven by distinct contributions at different times from both the gustatory system and the oral somatosensory system.

Gustatory and oral somatosensory systems are intimately related in terms of their anatomy. Peripherally, taste buds are surrounded by epithelia containing various somatosensory receptors such as mechanoreceptors, thermoreceptors and nociceptors (Green, 2003). While intra-oral stimuli activate taste receptors, concurrent somatosensory information is simultaneously sent to the central nervous system (Simon et al., 2008). Gustatory information is delivered by special sensory branches of the facial, glossopharyngeal or vagal (Huang & Xu, 2021) nerves. Somatosensory information is mainly transmitted by the trigeminal nerve as well as by general sensory branches of the glossopharyngeal or vagal (Huang & Xu, 2021) nerves (Simon et al., 2008). Updated research shows that gustatory neurons of geniculate ganglion (GG, a part of facial nerve) might also transmit oral mechanosensory information (Gutierrez & Simon, 2021). Gustatory and somatosensory

pathways converge in the nucleus of the solitary tract (NST) and then the thalamus (Ogawa et al., 1987) where taste and somatosensory information might have early crosstalk (Simon et al., 2008; Gutierrez & Simon, 2021). Gustatory and oral somatosensory inputs are also integrated in the anterior insula (Cerf-Ducastel et al., 2001; De Araujo & Rolls, 2004; Rudenga et al., 2010), which contains multimodal neurons responding to both somatosensory and gustatory stimuli in primates (Rolls, 2019). Some authors argue that gustatory perception is inherently linked to the concurrent somatosensory processing (Simon et al., 2008). Fact is that the same stimuli can activate both the gustatory system and the somatosensory system (Simon et al., 2008). For example, oral astringent sensation is described as a feeling of puckering, rough and drying sensation plus a slight bitter taste on the tongue and membranes of the oral cavity (Critchley & Rolls, 1996; Huang & Xu, 2021). Rhesus monkeys' selection of food depends much more on the level of astringency of the plant rather than the level of nutrition (Marks et al., 1988). Tannic acid is one of the common chemicals which produces astringency (Ashok & Upadhyaya, 2012) and it could activate both chorda tympani nerves (gustatory nerve) and lingual nerves (trigeminal nerve) (Schiffman et al., 1992; Schöbel et al., 2014). How oral astringency is processed in the human brain was investigated in publication 3.

Methods

Method 1: Publication 1 - The association between changes of gustatory function and changes of salivary parameters: A pilot study

Subjects

A total of 14 patients with taste disorders (6 males, 8 females; age range 40-70, mean age = 58.6 ± 8.6 years) participated in both the first session (baseline) and the return visit session. The diagnosis of taste dysfunction was based on taste strips (Landis et al., 2009) and the patients' self-reports. The study was approved by the local Ethics Committee. Written informed consent from all subjects was obtained before the experiment. The participants were asked not to eat for 3 hours before the experiment.

Experimental design

We investigated in patients with taste disorders after one-year oral zinc therapy whether there were changes in gustatory function as well as in saliva-related parameters, and if so,

whether the change of gustatory function is correlated with changes in saliva-related parameters, such as salivary compositions, salivary pH and salivary flow rate.

Before zinc therapy, taste function (evaluated by taste strips) of patients was measured as their baseline taste function. At the same time, baseline salivary parameters (flow rate, total proteins, proteolysis, catalase, total anti-oxidative capacity [TAC], carbonic anhydrase VI [caVI], and pH) were recorded before zinc therapy.

Patients started oral zinc for one year aiming to relieve their symptoms of taste disorders. After one year, patients' taste function and salivary parameters were re-evaluated with the same methods as return visit data. Thus, the changes of data represented by "Δ" indicating return visit data minus baseline were acquired. After we got the Δ taste function (Δ taste strip scores) of patients, patients were divided into two groups based on their Δ taste function for further data analysis. Patients whose Δ taste strip scores were less than 2 points, meaning their taste function decreased after zinc therapy (von Grundherr et al., 2019), were labelled as not-improved group (no-group). Those whose Δ taste strip scores were more than or equal to 2 points were labelled as improved group (im-group).

In addition, to investigate the relation between taste function and patients' mental and psychological state as well as the association between patients' subjective ratings and objective taste function, we also assessed the subjective symptom ratings and Beck Depression Inventory (BDI) scores before and after the zinc therapy. An additional question related to the investigation of the relation between changes of taste function and changes of smell function which was also evaluated before and after zinc therapy.

Examinations and measurements

Visual analogue scales

We used 10 cm rating scales, anchored with "not any symptoms" (lowest score "0") at the left end and "very intense" (highest score "10") at the right end, to record the intensity of patients' symptoms of taste disorders at the time of the visit and during the week prior to that. The questions matched with the scales are "How is your symptom at the moment?" and "How was your symptom during the last week?". For example, if a patient's chief complaint was an ongoing bitter taste even in absence of a bitter stimulant (phantogeusia), the patient sued the rating scale to describe this symptom. In essence, the symptoms mentioned in the scales aimed at the patients' chief complaints, the main reason why they came to see a doctor in the smell and taste clinic.

Gustatory and olfactory function

Gustatory function of all participants was evaluated by taste strips (Landis et al., 2008). Orthonasal olfactory function was measured using the extended “Sniffin’ Sticks” test (Hummel et al., 1997). This test consists of 3 subtests: odour threshold, odour discrimination, and odour identification test. The scores of the olfactory subtests were then summed up building an overall score.

Saliva collection and biochemical analysis

Participants were asked to chew a piece of Parafilm® laboratory film (American National Can) during 5 min and spit out saliva regularly (Feron et al., 2014). The collected saliva sample was weighed first and then stored at –80°C until biochemical analysis (Morzel et al., 2015). Salivary flow rate (ml/min) equals the weight of saliva (assuming that 1 g = 1mL) divided by time. Saliva samples were defrosted and then centrifuged 30 min at 10 000 G (Poette et al., 2014) and the resulting supernatants were used to do the biochemical analysis.

Total protein concentration (mg/mL) was quantified using a Quick Start Bradford protein assay (Bio-Rad, France). Proteolysis (IU) was measured with a Pierce Fluorescent Assay Kit (Pierce Biotechnology). CaVI (ng/mL) was assessed by Enzyme-Linked Immunosorbent Assay kits from USCN Life Science Inc. and Cusabio, respectively. Catalase activity was measured using a Catalase Fluorescent Activity Kit (Arbor Assay). TAC status was determined with an ORAC Assay kit (Zen-Bio) (Walliczek-Dworschak et al., 2017a). Salivary pH was measured by indicator paper (Rebasit; Dr.Welte Pharma, Geislingen, Germany).

Beck Depression Inventory (BDI)

Participants were asked to complete the BDI, which is a widely used, standardised, and validated tool for assessment of depressive symptoms (Beck et al., 1961).

Statistical analysis

Statistics were performed using SPSS (IBM SPSS Statistics for Windows, version 25.0 (IBMCorp., Armonk, N.Y., USA)). Paired t tests were used to analyse the differences between the baseline and the return visit data. Independent t tests were used to compare the mean Δ values between groups. For correlation analysis of Δ values, Spearman statistics were used. The level of significance was set at $p < .05$.

Method 2: Publication 2 - Exploring brain functional connectivity in patients with taste loss – a pilot study

Participants

Seven patients with hypogeusia or ageusia (5 women, 2 men, mean age 56 years, age range: 38-73 years, Table 1, Publication 2) and 12 healthy controls with normal taste function were included (6 women, 6 men, mean age 30 years, age range: 21-51 years). All investigations were conducted in accordance with the Guidelines for Biomedical Studies Involving Human Subjects (Helsinki Declaration). The study protocol was approved by the Ethics Committee at the University Clinic “Gustav-Carl-Carus” of the “Technische Universitaet Dresden”. Written informed consent from all subjects was obtained before the experiment.

These patients subjectively complained of taste loss and they were diagnosed with hypogeusia or ageusia based on a validated and reliable psychophysical taste test, the “taste strips” (Landis et al., 2009). The duration of their taste loss varied between 6 and 86 months. In three patients, taste loss was reported after trauma, two after infections, and the remaining patient had no specific cause. Structural MR scans did not show any lesions of the brain related to the taste loss in any of the patients. Twelve healthy controls, who reported normal taste function, were ascertained as normogeusic with the identical “taste strips” test.

Taste stimulation

Two taste qualities were used for taste stimulation: sweet and sour. Stimulants were administered in liquid form. The sweet stimulant was presented as a 2.92 mol/l sucrose solution, the sour one as a 0.21 mol/l citric acid solution. The solvent of the sweet/sour solution was tasteless water (Evian®, Danone Waters, Wiesbaden, Germany), which was also used as a control stimulation. Taste solutions were freshly prepared prior to each investigation.

Stimulants were delivered to the subject’s mouth using dedicated Teflon® tubing fed through a small outlet in the wall of the scanner room. Three separate tubes for the respective stimulants (sweet, sour solution and tasteless water) were connected to one common mouthpiece which could easily be held by the subject’s lips and teeth. The other end of the tubing was connected to a three-way valve, which linked syringes, enabling the delivery and replenishment of the liquids, and blockage of flow from either end. Prior to the experiment, the tubes were filled with the respective stimulants by means of syringes. Stimulation was performed by releasing 0.1 ml liquid onto the subject’s tongue. Preliminary experiments on a small group of expert observers had ascertained that this amount (0.1ml) of stimulant in the specific concentration produced a clear gustatory sensation and did not

immediately evoke swallowing. Neither significant mechanical stimulation nor thermal stimulation was perceived in this amount (0.1ml). Stimulants were presented at room temperature. In between stimulations, the subject's mouth was rinsed with 2 ml of water. Subjects were instructed through message on a screen only to swallow during the "rinse" condition.

Experimental design

Each participant had one functional imaging investigation compromising four sessions (Table 2, Publication 2). In each session, there were three experimental conditions: 1. "Water" condition - tasteless water (0.1 ml) was presented; 2. "Rinse" condition - tasteless water (2ml) was presented and subjects were only allowed to swallow in this condition; 3. "Taste" condition – sweet or sour solution (0.1 ml) was presented. The "Rinse" condition was established in order to prevent smearing effects on the tongue and enhance distinction of the taste/no-taste sensations. The "Rinse" condition was performed after each of the two main conditions ("Water" and "Taste" conditions), resulting in a basic sequential module of four conditions: Water (water, 0.1ml) - Rinse (water, 2ml) – taste (sweet/sour solution, 0.1ml) - Rinse (water, 2ml) (Figure 1, Publication 2). This sequence of four conditions was repeated three times within each session, yielding a succession of 12 conditions (Figure 1, Table 2, Publication 2). For each condition, 10 functional imaging scans were performed. With a repetition time of 3s for each scan, the total scanning time of one complete fMRI investigation was 24 min. Within one session, only one type of taste quality was presented in "Taste" condition, either sweet or sour. Sweet and sour stimulants were presented in a randomized and alternating manner.

Data acquisition

Brain scans were obtained by a Siemens-Sonata 1.5 T scanner (Siemens, Erlangen, Germany) with an eight-channel head coil. For functional imaging, a spin echo/echo planar imaging sequence, with echo time (TE) = 35 ms, repetition time (TR) = 3000 ms, flip angle = 90°, and 1 average. Slice thickness was 3 mm, slice spacing 3.75 mm. Ten scans were taken during each of the 12 conditions of any session, yielding 120 scans per session, and a total of 480 scans in one run. Structural images were recorded using a T1 weighted sequence, with TR = 5.98s, TE = 2.91 ms, 2 mm slice thickness, and 3 averages. One set consisted of 104 slices. In each subject, anatomy scans were acquired first, followed by the complete functional imaging run.

Data analysis

ROI-to-ROI functional connectivity analysis (FCA) was computed using the CONN toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012), (http://www.nitrc.org/projects/conn), implemented in MATLAB. Preprocessing steps including realignment, coregistration/normalization, segmentation, outlier identification and smoothing, and de-noising steps which aim to remove possible confounds in the BOLD signal, including motion, physiological and other noise sources were all done using the CONN toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012).

After pre-setting region of interests (ROIs), a General Linear Model (GLM) was used to calculate correlations of the mean BOLD time-series between each two different ROIs at the single-subject level, resulting ROI-to-ROI functional connectivity matrices consisting Fisher-transformed bivariate correlation coefficients (z-scores) between each two different ROIs (https://web.conn-toolbox.org/fmri-methods/connectivity-measures/roi-to-roi). Both sweet and sour taste stimulants were evaluated as one common “taste condition”. These correlations were computed for the taste condition as well as for the water condition. Group analysis was then performed using a two-sample t-test to uncover differences in functional connections between the patient and control groups in both conditions. Connection threshold $p < 0.05$ (p-FWE corrected) was regarded as significant.

The pre-set ROIs in the present study included right and left IC (Small et al., 2003; Hummel et al., 2007; Veldhuizen et al., 2011; Rolls, 2019), operculum (Small et al., 2003; Veldhuizen et al., 2011), OFC (Small et al., 2003; Veldhuizen et al., 2011), cingulate (Small et al., 2003; Veldhuizen et al., 2011), amygdala (Small et al., 2003; Hoogeveen et al., 2015), thalamus (Veldhuizen et al., 2011; Yeung et al., 2016), cerebellum (Small et al., 2003), temporal pole (Small et al., 2003) and putamen (Small et al., 2003), identified as relevant regions with respect to taste cerebral processing by previous studies. We also added ROIs related to frontal cortices considering their roles in modulating gustatory processing (Jones et al., 2006). Because of the close relation between gustation and olfaction we added the piriform cortex (PFC), which is considered to be a significant part of the primary olfactory cortex (Rolls, 2019). The ROIs of OFC and PFC were provided by Fjaeldstad et al (Fjaeldstad et al., 2017). The remaining ROIs were chosen from the FSL Harvard-Oxford Atlas and the AAL atlas provided by the software (Tzourio-Mazoyer et al., 2002), resulting in a total of 52 ROIs (26 pairs) in the FCA (Table 3, Publication 2).

Method 3: Publication 3 - Processing of Sweet, Astringent and Pungent Oral Stimuli in the Human Brain

Participants

Twenty-four healthy participants (age range: 20 – 37 years, mean 26.0 ± 3.8 years, 10 men, 14 women) without ENT (ear, nose and throat) disease and history of neurological or psychiatric disorder were included. All of them had normal taste functions ascertained with a standardized, validated taste test kit “taste strips” (Landis et al., 2009). None of the participants had been taking medication at the time of the study. To verify the reproducibility of the present fMRI study, seven of the participants (age range: 24 – 37 years, mean 28.6 ± 4.6 years, 4 men, 3 women) were asked to visit again and complete the identical experiment procedures with an average interval of 18 days between two visits.

All participants were able to recognize the differences among astringent (tannin), sweet (sucrose), and pungent (capsaicin) solutions before the fMRI test. The participants were asked not to eat at least for 2 hours before the experiment.

All investigations were conducted in accordance with the Guidelines for Biomedical Studies Involving Human Subjects (Helsinki Declaration). The study protocol was approved by the Ethics Committee at the University Clinic “Gustav-Carl-Carus” of the “Technische Universitaet Dresden” (ethics protocol number EK 389102017). Consent to participate and publication: Written informed consent was obtained from all participants prior to their inclusion.

Stimuli

Three types of stimuli (sweet - sucrose, astringent - tannin and pungent - capsaicin) were administered in liquid form: sucrose (order number: S9378; Sigma-Aldrich, Deisenhofen, Germany) for sweet taste stimuli (10g dissolved in 100mL distilled water, 100g/L); a wine tannin (ordered from a wine making supplies and commercial winery business “Presque Isle Wine Cellars”, www.piwine.com) derived from European chestnuts as astringent stimuli (1g dissolved in 100mL distilled water, 10g/L). The capsaicin (analytical standard of $\geqslant$ 99.0% by HPLC; Sigma-Aldrich, Steinheim, Germany; order number 12084) was dissolved with 95% ethanol first. Then, as a pungent stimulus 10ml capsaicin-ethanol solution (90µmol/L) was diluted with 60ml distilled water. The respective stimuli were iso-intense as established in pilot experiments in a small group of experienced observers.

Stimulus solutions were delivered into the subject’s mouth via tubing, a combination of four separate sterile PVC tubes (Type: IV-Standard - PVC, Original Perfusor® Line, B.Braun Melsungen AG, Melsungen, Germany, Figure 1, Publication 3) for the respective stimulants plus water. One end of the tubing was connected to a mouthpiece (Figure 1, c, d,

Publication 3) which could be easily placed between the lips, held by the subject's teeth. The other end of the tubing went through a small outlet in the wall of the scanner room and was connected to three-way valves and syringes (Figure 1, a, b, Publication 3), enabling the delivery and replenishment of the liquids, and blockage of flow from either end. The outer diameter of the tubes was 3 mm, their inner diameter was 2 mm, and the total length of the tubing was approximately 10 m.

Importantly, these tubes needed to be filled with the respective stimulus solutions without bubbles using syringes before starting the experiment. For stimulations, 0.1 ml of the corresponding liquid (room temperature) were given into the subject's mouth. In between stimulations, 2 ml of water were given to the subjects' mouth as rinse. Subjects were instructed to swallow only during the "rinse" condition (please see "*Experimental design*" below).

Experimental design

We employed the fMRI event-block mixed design for three types of stimuli (sucrose, tannin and capsaicin). For each type of stimulus, there were eight cycles (320s). One cycle lasted 40s in total as shown schematically in Figure 2 (Publication 3). In each cycle, the subject was first asked to stay still in the scanner for 5-s without any movement (this period was used as "baseline condition" when setting contrasts in data analysis). Then, 0.1mL stimuli were given onto the tongue of the subject within 2-s (BC). For the following 3-s, via a screen with language instructions the subject moved their mouth and tongue to perceive the given stimuli. Then, the subject was instructed to keep still again without any movement for 10-s. This period was the "task condition" when setting contrasts in data analysis. At the end of this cycle, 2 mL of water were given to the subject for rinsing and the subject was allowed to swallow during this period of 20 s. This cycle was repeated eight times forming a session (320s in total). Within one session, only one type of stimulus, which could be either sucrose, tannin or capsaicin solutions, was presented to the subject. Immediately after each session, the subject rated the intensity and pleasantness of the stimulus using analogue scales (intensity: 0 (no sensation) to 10 (very strong sensation); pleasantness: −5 (very unpleasant), 0 (neutral), +5 (very pleasant); intensity and pleasantness ratings were added as covariates into the analyses. The order of the given sessions was randomized. Before each scan, the participant was instructed how to perform when he/she saw the corresponding language instructions on the screen during the scan.

Following fMRI scanning, each participant completed a questionnaire regarding their eating/drinking habits (for details please see supplementary materials in Publication 3), which were also used as covariates added into analysis.

Functional MRI data acquisition

The system used for both functional and structural imaging was a 3.0 T scanner (Prisma; Siemens, Erlangen, Germany). For functional imaging the following parameters were used: echo time (TE) = 37ms, repetition time (TR) = 800ms, flip angle = 52°, voxel size: 2.0×2.0×2.0 mm, gap = 0 mm; 403 measurements in one run. Structural images were recorded using a T1 weighted sequence, with TR = 2300ms, TE = 2.29ms, 0.94 mm slice thickness, and 1 average. One slab consisted of 176 slices. In each subject, anatomical scans were acquired first, followed by the complete functional imaging runs.

Functional MRI data analysis

The imaging data were analyzed by means of the software package statistical parametric mapping (SPM) 12 (The Wellcome Centre for Human Neuroimaging, UCL Queen Square Institute of Neurology, London, UK) within MATLAB R2018b (The MathWorks, Inc., Natick, MA, USA). Preprocessing included motion correction (realignment and unwarping), co-registration of individual anatomical and functional data, normalization to the Montreal Neurological Institute (MNI) coordinate system (Collins et al., 1994), and smoothing with an 8-mm full width Gaussian kernel.

As mentioned in "*Experimental design*", in total there were three runs (sessions), each run included two types of condition: baseline (AB, Figure 2, Publication 3) and task (DE, Figure 2, Publication 3) condition. There were three types of "task condition", i.e., "sucrose", "tannin" and "capsaicin". The type of "task condition" was consistent within one run but different among runs. In first-level analysis, contrasts were calculated for "task condition" versus "baseline". Three kinds of contrasts were calculated in first level: sucrose – baseline, tannin – baseline, and capsaicin – baseline. The Canonical Hemodynamic Response Function (Canonical HRF) was applied.

In second-level analysis, one-sample t-test was used to show activations across the whole brain in response to each of the three stimuli, separately with a threshold of $p < 0.05$ (FWE corrected, cluster size > 50). To analyze the co-activated regions by three kinds of stimuli, conjunction analysis (Friston et al., 1999) was used with a threshold of $p < 0.05$ (FWE corrected, cluster size > 50). One-way within-subject ANOVA was employed to test the differences of activations among the three types of stimuli with a threshold of $p < 0.001$ (p-

uncorrected, cluster size > 50). Covariates including, 1. pleasantness ratings, 2. intensity ratings, 3. taste strips scores representing taste identification ability and 4. consumption habits of beverages corresponding to the three types of stimuli, were introduced into the one-way ANOVA. Marsbar toolbox was used to extract the BOLD values of brain regions that showed significantly different responses to the three types of stimuli. A further one-way repeated measures ANOVA was performed based on the extracted values using Statistical Package for the Social Sciences (SPSS, IBM SPSS Statistics for Windows, version 19.0 (IBMCorp., Armonk, N.Y., USA)) to investigate the explicit difference among the three types of stimuli. For the seven subjects who participated in the identical experiment twice, paired t-tests with a threshold of $p < 0.001$ (p-uncorrected, cluster size > 50) was used to compare the brain activations between two visits. Contrasts were calculated for the “first visit” minus the “second visit” and also the “second visit” minus the “first visit”, respectively for three stimulants. At the individual level, the BOLD values were extracted using Marsbar toolbox from the primary taste cortex – insula and the primary somatosensory cortex – postcentral gyrus (poCG) for each participant (the result of this part is in supplementary materials in Publication 3).

Contributions in the Publications

Publication 1: Conceptualization, Formal analysis, Writing of the manuscript

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

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

List of Published Papers

Zhu Y, Feron G, Von Koskull D, Neiers F, Brignot H, Hummel T. The association between changes of gustatory function and changes of salivary parameters: A pilot study. *Clin Otolaryngol*. 2021;46(3):538-545. doi:10.1111/coa.13705. Impact Factor: 2.729

Zhu Y, Joshi A, Thaploo D, Hummel T. Exploring brain functional connectivity in patients with taste loss – a pilot study. *European Archives of Oto-Rhino-Laryngology* (accepted). Impact Factor: 3.236.

Zhu Y, Thaploo D, Han P, Hummel T. Processing of Sweet, Astringent and Pungent Oral Stimuli in the Human Brain [published online ahead of print, 2023 Mar 24]. *Neuroscience*.

2023;S0306-4522(23)00128-8. doi:10.1016/j.neuroscience.2023.03.011. Impact Factor: 3.708.

Publication 1 (First study) The association between changes of gustatory function and changes of salivary parameters: A pilot study

Abstract of Publication 1

Objective: The aim of the pilot study was to explore which of the salivary parameters best reflect improvement or deterioration of taste function.

Methods: A total of 14 patients were included. Taste ability was measured using taste strips and patients rated their symptom strength using visual analogue scales. Salivary parameters (flow rate, total proteins, proteolysis, catalase, total anti-oxidative capacity [TAC], carbonic anhydrase VI [caVI], and salivary pH) were determined and the Beck Depression Inventory (BDI) was administered. All these parameters were measured twice with a one-year interval to acquire the changes of data.

Results: Patients with decreased taste function exhibited a decrease in salivary proteolysis and caVI, and an increase in salivary total protein. Patients with increased taste function also showed an increase in salivary total protein. Δ Salivary flow rate was negatively correlated with Δ taste strip scores. Δ Salivary pH was significantly lower in patients with increased taste function compared to patients with decreased taste function. Δ BDI was positively correlated with both Δ symptoms ratings. Across all patients, symptom ratings decreased while salivary total protein increased; salivary flow rate, proteolysis and caVI decreased significantly compared with baseline.

Conclusions: The present longitudinal results suggest that changes of both taste function and taste complaints were accompanied by changes in salivary parameters, indicating that salivary parameters have the potential to be useful in the diagnosis of patients with qualitative taste disorders.

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ORIGINAL ARTICLE

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The association between changes of gustatory function and changes of salivary parameters: A pilot study

Yunmeng Zhu[1] | Gilles Feron[2] | Dorothee Von Koskull[1] | Fabrice Neiers[2] | Helene Brignot[2] | Thomas Hummel[1]

[1]Smell and Taste Clinic, Department of Otorhinolaryngology, TU Dresden, Dresden, Germany

[2]Centre des Sciences du Goûtet de l'Alimentation, CNRS, INRA, Université Bourgogne Franche -Comté, Dijon, France

Correspondence
Yunmeng Zhu, Mathilden str.24 zim.502, 01069, Dresden, Germany.
Email: zhym182@hotmail.com

Abstract

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Conclusions: The present longitudinal results suggest that changes of both taste function and taste complaints were accompanied by changes in salivary parameters, indicating that salivary parameters have the potential to be useful in the diagnosis of patients with qualitative taste disorders.

1 | INTRODUCTION

Taste is a strong stimulant for saliva secretion.[1] In turn, saliva controls the release, transport and adsorption of taste molecules, as well as their metabolism by enzymatic modification.[2] It also plays a role in the maintenance of taste-sensing cells,[3] and therefore appears to be a key variable in taste perception. Taste perception is important for the differentiation of essential nutrients from harmful and potentially toxic substances.[4] Taste disorders can cause severe health problems, eg malnutrition or impaired immunity,[5] and is also associated with impaired mental health and quality of life.[6] Taste disorders can be classified as two types, quantitative disorders and qualitative disorders.[7] Quantitative disorders, a diminished or a completely loss of taste perception, can be assessed psychophysically whereas qualitative taste disorders are characterised by mostly bothersome, completely

subjective complaints that cannot be measured by any technique. In this context, explorations on saliva-related parameters might help investigations on both pathogenesis and assessments of taste disorders.

In our previous cross-sectional research,[6] we examined 81 patients with taste disorders on their taste function and saliva-related parameters and found that scores of a taste function tests correlated negatively with the salivary flow rate and proteolysis, and positively with carbonic anhydrase VI (caVI) and catalase values. Compared to healthy controls patients with taste disorders exhibited a higher salivary total protein concentration, total anti-oxidative capacity (TAC), proteolysis and salivary flow rate, indicating that assessment of saliva is of high importance in research on taste dysfunction.

For the present longitudinal study, we tracked a small part of these patients to explore how changes of salivary parameters correlate with changes of taste function after a year of zinc therapy, as treatment with zinc has been shown to be the effective therapy of taste disorders.[8-11] The question was which of these salivary parameters would best reflect the improvement/deterioration of taste function. In addition, it is shown that zinc therapy could improve depression,[12] and mood state also has close relation with complaints of qualitative taste disorders.[13] Hence, changes of Beck Depression Inventory (BDI) scores were also investigated in the current study.

Key points

- This is a longitudinal study to explore the changes of parameters from same subjects.
- Associations of changes were found between taste function and salivary parameters.
- The increased taste sensitivity was accompanied by a decreased salivary pH level.
- The dissociation between patients' subjective complaints about taste disorders symptoms and their objective taste capacities were found.
- Changes of self-ratings about taste disorders symptoms were found to be related to changes of BDI scores.

2 | MATERIALS AND METHODS

2.1 | Overall design

We investigated in patients with taste disorders after one-year oral zinc therapy whether there were changes in gustatory function as well as in saliva-related parameters, and if so, whether the change of gustatory function is correlated with changes in saliva-related parameters, such as salivary composition, salivary pH and salivary flow rate.

Before zinc therapy, taste function (evaluated by taste strips) of patients was measured as their *baseline* taste function. At the same time, *baseline* salivary parameters (flow rate, total proteins, proteolysis, catalase, total anti-oxidative capacity [TAC], carbonic anhydrase VI [caVI], and pH) were recorded before zinc therapy.

Patients started oral zinc for one year aiming to relieve their symptoms of taste disorders. After one year, patients' taste function and salivary parameters were re-evaluated with the same methods as *return visit data*. Thus, the changes of data represented by "Δ" indicating *return visit data* minus *baseline* were acquired (Table 1). After we got the Δ taste function (Δ taste strip scores) of patients, patients were divided into two groups based on their Δ taste function for further data analysis. Patients whose Δ taste strip scores were less than 2 points, meaning their taste function decreased after zinc therapy,[14] were labelled as *not-improved group (no-group)*. Those whose Δ taste strip scores were more than or equal to 2 points were labelled as *improved group (im-group*; Table 1).

In addition, to investigate the relation between taste function and patients' mental and psychological state as well as the association between patients' subjective ratings and objective taste function, we also assessed the subjective symptom ratings and Beck Depression Inventory (BDI) scores before and after the zinc therapy.

An additional question related to the investigation of the relation between changes of taste function and changes of smell function which was also evaluated before and after zinc therapy.

2.2 | Participants

A total of 14 patients with taste disorders (6 males, 8 females; age range 40-70, mean age = 58.6 ± 8.6 years, see Table 2) participated in both the first session (*baseline*) and the *return visit* session. The diagnosis of taste dysfunction was based on gustatory testing using taste strips[15] and the patients' self-report. The study was approved by the local Ethics Committee. Written informed consent from all subjects was obtained before the experiment. The participants were asked not to eat for 3 hours before the experiment.

2.3 | Examinations and measurements

2.3.1 | Visual analogue scales

We used 10 cm rating scales, anchored with "not any symptoms" (lowest score "0") at the left end and "very intense" (highest score "10") at the right end, to record the intensity of patients' symptoms of taste disorders at the time of the visit and during the week prior to that. The questions matched with the scales are "How is your symptom at the moment?" and "How was your symptom during the last week?". For example, if the patient's chief complaint was an ongoing bitter taste even in absence of a bitter stimulant (phantogeusia), patients sued the rating

TABLE 1 Overall design

Baseline		Return visit data	Δ Values
Taste function 1	Zinc therapy for one year	Taste function 2	Taste function 2 - Taste function 1
Saliva parameters 1		Saliva parameters 2	Saliva parameters 2 - Saliva parameters 1
Symptoms ratings 1		Symptoms ratings 2	Symptoms ratings 2 - Symptoms ratings 1
BDI 1		BDI 2	BDI 2 - BDI 1
Smell function 1		Smell function 2	Smell function 2 - Smell function 1
Group division			
Not-improved group		ΔTaste strips score < 2	
Improved group		ΔTaste strips score ≥ 2	

scale to describe this symptom. In essence, the symptoms mentioned in the scales aimed at the patients' chief complaints, the main reason why they came to see a doctor in the smell and taste clinic.

2.3.2 | Gustatory and olfactory function

Gustatory function of all participants was evaluated by taste strips,[15] details of which are described in our previous study.[6] Orthonasal olfactory function was measured using the extended "Sniffin' Sticks" test.[16] This test consists of 3 subtests: odour threshold, odour discrimination, and odour identification test. The scores of the olfactory subtests were then summed up building the overall TDI score.[17]

2.3.3 | Saliva collection and biochemical analysis

Salivary parameters including flow rate, total proteins, proteolysis, catalase, total anti-oxidative capacity (TAC), carbonic anhydrase VI (caVI), and pH were selected as they are known to be associated with taste function based on our previous cross-sectional studies.[6] The details of how saliva samples were collected and biochemically analysed were exactly the same as in our previous study.[6]

2.3.4 | Beck Depression Inventory (BDI)

Participants were asked to complete the BDI, which is a widely used, standardised, and validated tool for assessment of depressive symptoms.[18]

2.4 | Statistical analysis

Statistics were performed using SPSS Version 25.0 (IBM). Paired t tests were used to analyse the differences between the *baseline* and the *return visit data*. Independent *t* tests were used to compare the mean *Δ values* between groups. For correlation analysis of *Δ values*, Spearman statistics were used. The level of significance was set at $P < .05$.

3 | RESULTS

3.1 | Changes after one-year therapy of oral zinc (Return visit data versus Baseline)

Paired t test was used to compare the mean values of parameters from the *baseline* and from the *return visit*. Table 3 shows the parameters whose mean values have significant differences compared with the baseline data.

3.1.1 | All patients

Total patients' taste and smell function based upon the measurements of taste strips and Sniffin' Sticks did not change significantly after a year of therapy. Other parameters that did not change were BDI scores, salivary pH, salivary TAC and catalase.

Symptom ratings decreased significantly, indicating improvement of the subjective feelings about the present disease symptoms and symptoms during the week prior to the visit (present: $P = .021$, during the last week: $P = .017$; see Table 3).

Salivary total protein increased ($P = .001$; see Table 3) while salivary flow rate ($P = .021$), proteolysis ($P = .007$) and caVI ($P = .024$) decreased (see Table 3).

3.1.2 | Not-improved group (*no-group*) and improved group (*im-group*)

In the present study, 9 patients with decreased taste function (Δ Taste strips score <2) after a year of zinc therapy were thus labelled

as *not-improved group* (*no-group*, see Table 2). Five patients with improved taste function (Δ Taste strips score ≥2) were labelled as *improved group* (*im-group*, see Table 2).

The increased level of salivary total protein could be observed in both groups (*no-group*: $P = .017$; *im-group*: $P = .005$; see Table 3) while the level of salivary proteolysis ($P = .036$; see Table 3) and caVI ($P = .048$; see Table 3) decreased in the *no-group*.

No significant changes of other parameters (smell function, salivary pH, flow rate, TAC, catalase, symptom ratings and BDI scores) were found in *im-* and *no-* subgroups using the paired *t* test.

3.2 | Δ values: no-group versus im-group

Independent t test was used to compare the Δ value between *no-group* and *im-group*. The mean Δ salivary pH of *no-group* (0.2 ± 0.2) was positive and higher than that of *im-group* (-0.4 ± 0.4) which was negative ($P = .003$). The data of salivary pH and taste strips scores of each participants was shown in Table 4. No significant differences on any other Δ values between groups were observed.

3.3 | Δ values- correlations

Spearman correlations were used to investigate the correlations between Δ values. Δ BDI was positively correlated with both Δ symptoms ratings (present: $P = .002$, $r = .76$; during the last week: $P = .0018$, $r = .62$). Δ total taste strip score was negatively correlated with Δ salivary flow rate ($P = .039$, $r = -.56$). No other significant correlation pertaining to Δ value was found between other parameters.

4 | DISCUSSION

We observed no significant changes on taste and smell function after one-year zinc therapy using paired *t* tests. However, the sample size (n = 14) was too small to evaluate the curative effect of oral zinc therapy, also, we were unable to receive a precise documentation of the dose of zinc treatment from each patient. However, zinc therapy was not the primary interest in the present study. We selected subjects with taste disorders treated with zinc because taste function is more likely to change under zinc therapy.[9,10] The focus of the present study was to explore the associations between changes of taste function and changes of salivary parameters instead of how zinc therapy would affect taste function or saliva parameters.

In our study, we found that Δ salivary flow rate was negatively correlated with Δ taste strip scores, indicating that when the salivary flow rate increased, the taste strips would decrease, and vice versa. This is in accordance with our previous cross-sectional research, that is, the taste strip score correlated negatively with the salivary flow rate, and patients with taste disorders exhibited a higher salivary flow rate compared to healthy controls.[6]

TABLE 2 Description of subjects and the changes of taste function

Group	Self-report	Age	Diagnosis	TS 1	TS 2	Δ TS
im-	Taste loss after surgery	47	Hypogeusia	18	22	4
im-	Salty dysgeusia	68	Idiopathic dysgeusia + hypogeusia	8	10	2
im-	Metal dysgeusia	58	Idiopathic dysgeusia + hypogeusia	4	9	5
im-	Sweet, salty dysgeusia	58	Idiopathic dysgeusia	13	23	10
im-	Taste loss	70	Idiopathic hypogeusia	11	17	6
no-	Salty dysgeusia	40	Idiopathic dysgeusia	20	18	−2
no-	Sweet dysgeusia	56	Idiopathic dysgeusia + hypogeusia	11	2	−9
no-	Salty dysgeusia	50	Idiopathic dysgeusia	15	4	−11
no-	Bitter dysgeusia	56	Idiopathic dysgeusia	14	12	−2
no-	Salty dysgeusia	62	Idiopathic dysgeusia	17	7	−10
no-	Taste loss	66	Idiopathic hypogeusia	14	13	−1
no-	Bitter dysgeusia	68	Idiopathic dysgeusia	26	22	−4
no-	Bitter dysgeusia	61	Idiopathic dysgeusia	26	18	−8
no-	Sour, metal dysgeusia	61	Idiopathic dysgeusia	25	20	−5

Abbreviations: Im, Improved group; No, Not-improved group; TS 1, Taste strips scores of baseline; TS 2, Taste strips scores of return visit.

When we reviewed other studies investigating the relationship between taste function and salivary flow rate, we did not find an uniform picture. Gustatory loss can be accompanied by increased, decreased, or unchanged salivary flow rates[6] and the correlations has been reported to vary for different taste qualities. For example, one study[19] showed that there was a negative correlation between salt perception and salivary flow rate, whereas no correlation was found for bitterness or sweetness, and contradictory results were reported for sourness. Other studies showed that bitter taste sensitivity correlates positively with unstimulated saliva flow rate.[20] In addition, also a negative correlation has been observed for sourness and salivary flow rate.[19]

One reason for this inconsistency of results may relate to the different methods used in the respective studies. For example, results may differ when taste function was evaluated by self-ratings or chemosensory tests, whether salivary flow rate was assessed as stimulated or unstimulated, or whether participants were healthy or patients with taste disorders. Hence, when considering the relationship between salivary flow rate and taste function, individual taste qualities (sweet, salt, sour, bitter) could be studied and discussed separately using comparable techniques in future studies (unfortunately, individual taste qualities could not be analysed in a meaningful way in the present study). Moreover, considering that the salivary pH and the salivary buffer capacity are highly dependent on the salivary flow rate (they increase when the salivary flow rate increases and vice versa),[21-24] there could be also an optimal range of salivary flow rate for the best taste sensitivity.

Salivary pH is maintained at a relatively constant level, ie 6.5-7.4, buffering acids and thereby diminishing the rate of dental demineralisation.[23,25] Several previous investigations showed that salivary pH interacts with the salivary flow rate and is important for sour[26-28] and sweet taste perception.[29,30] In the present study, Δ salivary pH was found to be significantly different between two groups - salivary pH tended to increase in *no-group* while it decreased in the *im-group* (Table 4), indicating that the increased taste sensitivity might be accompanied by decreased salivary pH during the zinc therapy. However, more research is needed to confirm this tendency.

In some previous investigations, proteolytic activity of human saliva plays a role in the perception of bitter, fatty, and salty stimuli[31-33] and enhanced in-mouth proteolysis is a key peri-receptor

TABLE 3 The mean values of parameters of both return visit session and baseline session

	Improved group (n = 5)		Not-improved group (n = 9)		Total (n = 14)	
	Mean ± SD	*P* value	Mean ± SD	*P* value	Mean ± SD	*P* value
Taste strips						
Baseline	10.8 ± 5.3		18.7 ± 5.8		15.9 ± 6.7	
Return	16.2 ± 6.5		12.9 ± 7.2		14.1 ± 6.9	
Flow rate (mL/min)						
Baseline	0.8 ± 0.5		0.6 ± 0.3		0.6 ± 0.4	.021
Return	0.3 ± 0.1		0.4 ± 0.2		0.4 ± 0.2	
Total protein (mg/mL)						
Baseline	0.6 ± 0.2	.005	0.7 ± 0.5	.017	0.7 ± 0.4	.001
Return	1.2 ± 0.2		1.5 ± 0.7		1.4 ± 0.6	
Proteolysis (IU)						
Baseline	11.6 ± 12.9		13.2 ± 14.5	.036	12.6 ± 13.4	.007
Return	1.1 ± 0.2		1.1 ± 0.4		1.1 ± 0.3	
CaVI (ng/mL)						
Baseline	1.6 ± 1.4		4.1 ± 4.0	.048	3.2 ± 3.5	.024
Return	0.9 ± 0.3		1.5 ± 0.5		1.3 ± 1.2	
Current symptoms						
Baseline	7.4 ± 2.1		6.8 ± 2.1		7.0 ± 2.1	.021
Return	6.1 ± 3.7		4.7 ± 3.3		5.2 ± 3.4	
Last week symptoms						
Baseline	7.8 ± 1.6		7.2 ± 1.4		7.4 ± 1.5	.017
Return	6.0 ± 3.9		4.6 ± 3.3		5.1 ± 3.5	

Note: Paired *t* test was used to compare the mean values of parameters from the baseline and from the return visit, both in the total sample and in improved and not-improved group. Significant *P* values which means there were significant differences between baseline and return visit on the mean values of each group or total patients are given in the table.

TABLE 4 The changes of salivary pH and taste strips scores compared with baseline of each participants

Group		no	no	no	no	no	no	no	no	im	im	im	im	im	im
Baseline	pH	7.20	6.90	6.70	7.00	6.70	7.20	6.50	6.70	7.20	6.30	7.40	7.00	7.20	6.90
Return visit		7.40	7.20	7.00	6.80	6.90	7.40	6.40	7.20	7.20	6.00	7.20	6.30	6.30	6.90
Δ pH		+0.2	+0.3	+0.3	−0.2	+0.2	+0.2	−0.1	+0.5	0.0	−0.3	−0.2	−0.7	−0.9	0.0
Δ taste strips		−2	−9	−11	−2	−10	−1	−4	−8	−5	+6	+5	+10	+4	+2
Baseline	Taste strips	20	11	15	14	17	14	26	25	11	4	13	13	18	8
Return visit		18	2	4	12	7	13	22	18	20	17	9	23	22	10

factor associated with higher gustatory sensitivity.[33] One *hypothesis* is that the mucosal pellicle forms a barrier that controls the accessibility of tastants to the receptors.[33] A thinner or looser pellicle due to higher proteolytic activity would then be associated with a facilitated tastant-taste receptor interaction.[33] In the present study, for the *no-group* whose taste strip scores decreased, their salivary proteolytic activity also decreased combined with increased salivary total protein (Table 3), which support this *hypothesis*.[33] However, we also observed an increased salivary total protein without concomitantly decreased salivary proteolysis in the *im-group* whose taste strip scores increased (Table 3). This difference might be explained by the small sample size in the *im-group* (n = 5) which did not reflect the significant changes in proteolysis. The overall contradictory results on the relation between taste function and salivary proteolysis might also be interpreted in light of the differences between correlations with individual taste qualities (sweet, salt, sour, bitter). In our study, the taste strip score represents the combined function of the four basic tastes, but the negative correlation may exist only between salivary proteolysis and a specific taste quality, such as bitter as shown in previous studies.[33]

CaVI (gustin) in saliva has been associated with the growth and development of taste buds[10] and a lower caVI concentration is associated with lower levels of total parotid salivary zinc in subjects with reduced taste function. Our previous cross-sectional study also found there was a positive correlation between the caVI concentration and taste scores.[6] What we found in present study, patients in *no-group* showed a significant decreased caVI concentration, is also in accordance to these findings.

Although, for total patients, there was no significant change in taste function after one-year zinc therapy, the improvements of symptoms of total patients were significant. It is not unusual that the patients' subjective complaints about taste disorders symptoms are not paralleled by their objective taste capacities. Qualitative taste disorders which can only be diagnosed by self-report[34] do not have to coexist with quantitative taste disorders and for quantitative taste disorders, many individuals do not even notice their taste deficiency.[35] A study on 48 patients with qualitative dysgeusia showed that two thirds experienced spontaneous resolution of the dysgeusia (evaluated by self-ratings), with an average duration of 10 months and mood state (evaluated by BDI scores) related to resolution rates.[13] In the present study, we found that Δ BDI scores were positively correlated with Δ taste disorder symptom ratings, ie, when taste disorder symptoms improved, patients' depressive symptoms also improved and vice versa. This suggests that, psychotherapy might help these patients to feel better, without necessarily improving their gustatory sensitivity.

One study investigating subjects with oral sensory complaints (OSC) including burning mouth syndrome, idiopathic taste aberrations and xerostomia indicated that salivary and taste analyses were helpful in distinguishing healthy subjects from subjects with complaints.[36] In the present study, we could observe both improvement of symptoms of taste disorders and changes of saliva-related

parameters of total patients (Table 3, Total). However, the sample size was small and a healthy control group was missing. Hence we cannot conclude that the improvement of complaints could be reflected by saliva-related parameters. Still, the results suggest that salivary parameters may be useful in the distinction between healthy subjects and patients with qualitative taste disorders and thus call for more investigations on saliva testing as an objective measurement to evaluate either taste dysfunction or taste complaints.

Because of the high rate of dropouts, there are only 14 samples in the present study and the etiologies and subtypes of their taste disorders are heterogeneous. As is shown in Table 2, the first patient's hypogeusia is reported after a surgery while other patients' taste disorder (either dysgeusia or hypogeusia or both) are idiopathic. Theoretically, saliva parameters could change in order to compensate for the recovery of taste function (secondary change). However, changes of salivary parameters could also be the primary cause of taste disorders (primary change). Samples in current study exhibit heterogeneity but they are too small to be divided into subgroups which could be analysed separately. Thus, based on the present work, several directions can be suggested for future studies to investigate the association of changes between saliva-related parameters and taste function. At first, larger sample sizes are needed. The presently observed changes in saliva-related parameters such as total protein, proteolysis, salivary flow rate, and pH, could be the priorities to be studied. More patients with taste disorders with different etiologies and subtypes should be included. Healthy controls plus a group of untreated patients would also be desirable. As mentioned above, studies found that individual taste qualities could be influenced by saliva differently.[19,20] Hence, the suggestion would be to not only measure general function, but also measure sensitivity for specific taste qualities (sweet/sour/salty/bitter) using validated assessment tools. At last, when measuring taste function with psychophysical tests, it is also important to record taste complaints with symptom scales so that the association between taste complaints and salivary parameters could be studied in greater detail.

5 | CONCLUSION

The present longitudinal results suggest that changes of both taste function and taste complaints were accompanied by changes in salivary parameters, indicating that salivary parameters have the potential to be useful in the diagnosis of patients with qualitative taste disorders and that assessment of saliva is of importance in research on taste dysfunction.

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CONFLICT OF INTEREST

None to declare.

AUTHOR CONTRIBUTIONS

YZ and TH drafted the article. VKD collected the data and saliva samples. GF, FN and HB analysed the saliva samples. YZ analysed the data. All authors revised it critically for intellectual content and approved the final version of the manuscript.

ETHICS APPROVAL

All investigations were conducted in accordance with the Guidelines for Biomedical Studies Involving Human Subjects (Helsinki Declaration). The study protocol was approved by the Ethics Committee at the University Clinic "Gustav-Carl-Carus" of the "Technische Universitaet Dresden" (ethics protocol number EK320082014). Consent to participate and publication: Written informed consent was obtained from all participants prior to their inclusion.

DATA AVAILABILITY STATEMENT

All data and material are available upon request.

ORCID

Yunmeng Zhu https://orcid.org/0000-0001-9542-2609

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Publication 2 (Second study) Exploring brain functional connectivity in patients with taste loss – a pilot study.

Abstract of Publication 2

Background: In a previous neuroimaging study, patients with taste loss showed stronger activations in gustatory cortices compared to people with normal taste function during taste stimulations. The aim of the current study was to examine whether there are changes in central-nervous functional connectivity in patients with taste loss.

Methods: We selected 26 pairs of brain regions related to taste processing as our regions of interests (ROIs). Functional magnetic resonance imaging (fMRI) was used to measure brain responses in 7 patients with taste loss and 12 healthy controls as they received taste stimulations (taste condition) and water (water condition). The data was analysed using ROI-to-ROI functional connectivity analysis (FCA).

Results: We observed weaker functional connectivity in the patient group between the left and right orbitofrontal cortex in the taste condition and between the left frontal pole and the left superior frontal gyrus in the water condition.

Conclusions: These results suggested that patients with taste loss experience changes of functional connectivity between brain regions not only relevant to taste processing but also to cognitive functions. While further studies are needed, fMRI might be helpful in diagnosing taste loss as an additional tool in exceptional cases.

Publication2: Exploring brain functional connectivity in patients with taste loss – a pilot study.

Abstract:

Purpose: In a previous neuroimaging study, patients with taste loss showed stronger activations in gustatory cortices compared to people with normal taste function during taste stimulations. The aim of the current study was to examine whether there are changes in central-nervous functional connectivity in patients with taste loss. Methods: We selected 26 pairs of brain regions related to taste processing as our regions of interests (ROIs). Functional magnetic resonance imaging (fMRI) was used to measure brain responses in 7 patients with taste loss and 12 healthy controls as they received taste stimulations (taste condition) and water (water condition). The data was analysed using ROI-to-ROI functional connectivity analysis (FCA). Results: We observed weaker functional connectivity in the patient group between the left and right orbitofrontal cortex in the taste condition and between the left frontal pole and the left superior frontal gyrus in the water condition. Conclusion: These results suggested that patients with taste loss experience changes of functional connectivity between brain regions not only relevant to taste processing but also to cognitive functions. While further studies are needed, fMRI might be helpful in diagnosing taste loss as an additional tool in exceptional cases.

Key words: taste loss; gustation; fMRI; functional connectivity

1. Introduction:

The human sense of taste is important for the enjoyment of food and making food choices [1-3]. Taste loss often leads to negative effects on eating behavior and nutritional status causing damages on human health [1,4-6]. Clinically, the diagnosis of hypogeusia (partial taste loss) or ageusia (complete taste loss) largely depends on subjective complaints and psychophysical taste testing [7], which strongly relies on the cooperation of the patients. In cases of an inadequate ability to cooperate, e.g., in children or patients with cognitive impairments, or potential malingering concerning medicolegal contexts, psychophysical taste testing is unreliable [8]. Gustatory Event-Related Potentials (GERPs) are less biased by the individuals' beliefs and motives but due to the relatively complex technical prerequisites, the method is not widely used [9]. Histological investigations or contact endoscopy could help to examine morphological abnormalities of taste papillae/taste buds on the tongue [10,11], but they are not standardized for diagnostic purposes. Magnetic Resonance Imaging (MRI) provides visualization of structural lesions of brain regions related to taste processing [12]. However, even in the absence of visible peripheral or central lesions [13-15], taste loss may persist and bother patients. Gustatory functional MRI (fMRI) provides a non-invasive way to examine gustatory function without a major bias in terms of cooperation from the patients. In addition, MRI scanners are widely available so that the technique could be easily applied in many different places.

To explore the potential use of fMRI in the diagnosis of taste loss, a previous gustatory fMRI study [16] was performed by our research group. In this study, eight patients with hypogeusia or ageusia and twelve healthy controls with normal taste function were recruited. The functional images of their brains when they were receiving taste stimulations were recorded using a 1.5 T scanner. We observed that the recognized primary and secondary taste cortices – insula cortex (IC) and orbital frontal cortex (OFC)[17] were activated by taste stimulations not only for most healthy participants but also for most patients with taste loss. There were considerable individual variations regarding the overall degree of activations and the sites of maximum activations. These results suggested that it is problematic to differentiate patients with taste loss from healthy controls based on gustatory functional MRI at an individual level. Interestingly, when doing group comparison, the patient group tended to show stronger activations in the IC and OFC, compared to the control group. This result was interpreted as patients with taste loss putting more efforts than controls into the processing of gustatory information.

In human brain, gustatory information is processed and transported in forms of neural networks of pathways arranged in series, in parallel and recurrently [18-21]. The temporal correlation of neuronal activation patterns of anatomically separated brain regions is defined as functional connectivity [22]. In the past years, increasing researchers have started to explore functional connectivity by calculating the correlation of time-series from different brain regions using fMRI [13,14,22]. The aim of the present study was to examine whether brain functional connectivity of patients with taste loss is different from that of healthy controls. We re-analyzed the fMRI data collected in the previous study [16] using ROI-to-ROI (ROI: region of interest) Functional Connectivity Analysis (FCA), which allows us to see the functional connections between ROIs. So far, no study has investigated the functional connectivity of the brains of patients with taste loss. Hence, we took advantage of our previous data to explore this. Compared to healthy controls, in patients with taste loss we predicted significantly weaker functional connections between some ROIs, e.g. the primary and secondary taste cortices, IC and OFC [17].

2. Methods

2.1 Subjects

Seven patients with hypogeusia or ageusia (5 women, 2 men, mean age 56 years, age range: 38-73 years, **Table 1**) and 12 healthy controls with normal taste function were included (6 women, 6 men, mean age 30 years, age range: 21-51 years). All investigations were conducted in accordance with the Guidelines for Biomedical Studies Involving Human Subjects (Helsinki Declaration). The study protocol was approved by the Ethics Committee at the University Clinic "Gustav-Carl-Carus" of the "Technische Universitaet Dresden". Written informed consent from all subjects was obtained before the experiment.

Table 1. Patients and clinical status

Patient no.	Gender	Age (years)	Gustatory function	Onset (months) prior to fMRI	Cause
2[a]	Woman	73	Hypogeusia	6	Infection of URT[b]
3	Woman	52	Hypogeusia	17	Head trauma
4	Man	50	Ageusia	54	Infection of URT
5	Woman	38	Hypogeusia	86	Unknown
6	Man	64	Hypogeusia	8	Head trauma
7	Woman	57	Ageusia	16	Infection of URT
8	Woman	59	Ageusia	12	Head trauma

a. In the previous study, there were in total 8 patients with taste loss. However, the data of one participant was damaged so that only 7 patients were included in the present study. b. URT = upper respiratory tract.

These patients subjectively complained of taste loss and they were diagnosed with hypogeusia or ageusia based on a validated and reliable psychophysical taste test, the "taste strips" [23]. The duration of their taste loss varied between 6 and 86 months. In three patients, taste loss was reported after trauma, two after infections, and the remaining patient had no specific cause. Structural MR scans did not show any lesions of the brain related to the taste loss in any of the patients. Twelve healthy controls, who reported normal taste function, were ascertained as normogeusic with the identical "taste strips" test [23].

2.2. Taste stimulation

Two taste qualities were used for taste stimulation: sweet and sour. Stimulants were administered in liquid form. The sweet stimulant was presented as a 2.92 mol/l sucrose solution, the sour one as a 0.21 mol/l citric acid solution. The solvent of the sweet/sour solution was water (ordered from Evian®, Danone Waters,

Wiesbaden, Germany), which was also used as a control stimulation. Taste solutions were freshly prepared prior to each investigation.

Stimulants were delivered to the subject's mouth using dedicated Teflon® tubing fed through a small outlet in the wall of the scanner room. Three separate tubes for the respective stimulants (sweet, sour solution and water) were connected to one common mouthpiece which could easily be held by the subject's lips and teeth. The other end of the tubing was connected to a three-way valve, which linked syringes, enabling the delivery and replenishment of the liquids, and blockage of flow from either end. Prior to the experiment, the tubes were filled with the respective stimulants by means of syringes. Stimulation was performed by releasing 0.1 ml liquid onto the subject's tongue. Preliminary experiments on a small group of expert observers had ascertained that this amount (0.1ml) of stimulant in the specific concentration produced a clear gustatory sensation and did not immediately evoke swallowing. Neither significant mechanical stimulation nor thermal stimulation was perceived in this amount (0.1ml). Stimulants were presented at room temperature. In between stimulations, the subject's mouth was rinsed with 2 ml of water. Subjects were instructed through message on a screen only to swallow during the "rinse" condition.

2.3. Experimental design

Each participant had one functional imaging investigation compromising four sessions (**Table 2**). In each session, there were three *experimental conditions*: 1. "Water" condition - water (0.1 ml) was presented; 2. "Rinse" condition - water (2ml) was presented and subjects were only allowed to swallow in this condition; 3. "Taste" condition – sweet or sour solution (0.1 ml) was presented. The "Rinse" condition was established in order to prevent smearing effects on the tongue and enhance distinction of the taste/no-taste sensations. The "Rinse" condition was performed after each of the two main conditions ("Water" and "Taste" conditions), resulting in a basic sequential module of four conditions: Water (water, 0.1ml) - Rinse (water, 2ml) – taste (sweet/sour solution, 0.1ml) - Rinse (water, 2ml) (**Figure 1**). This sequence of four conditions was repeated three times within each session, yielding a succession of 12 conditions (**Figure 1, Table 2**). For each condition, 10 functional imaging volumes were obtained. With a repetition time of 3s for each volume, the total scanning time of one complete fMRI investigation was 24 min. Within one session, only one type of taste quality was presented in "Taste" condition, either sweet or sour. Sweet and sour stimulants were presented in a randomized and alternating manner.

Table 2. Experimental design

Experimental condition	Scans (volumes)			
	Session 1 (sweet*)	Session 2 (sour)	Session 3 (sweet)	Session 4 (sour)
Water	0-9	120-129	240-249	360-369
Rinse	10-19	130-139	250-259	370-379
Taste	20-29	140-149	260-269	380-389
Rinse	30-39	150-159	270-279	390-399
Water	40-49	160-169	280-289	400-409
Rinse	50-59	170-179	290-299	410-419
Taste	60-69	180-189	300-309	420-429
Rinse	70-79	190-199	310-319	430-439
Water	80-89	200-209	320-329	440-449
Rinse	90-99	210-219	330-339	450-459
Taste	100-109	220-229	340-349	460-469
Rinse	110-119	230-239	350-359	470-479

Table 2 The number of volumes in one run. *Sweet and sour stimulants were presented in a randomized and alternating manner. Water was applied as both control stimulus and rinse fluid.

Fig.1. Experimental design

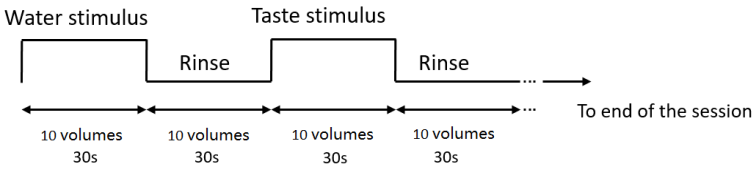

Fig.1 Experimental design. The first four conditions of one session are shown in the figure with 10 volumes (3 seconds for each volume) recorded for each condition.

2.4. Data acquisition

Brain images were obtained by a Siemens-Sonata 1.5 T scanner (Siemens, Erlangen, Germany) with an eight-channel head coil. For functional imaging, a spin echo/echo planar imaging sequence, with echo time (TE) = 35 ms, repetition time = 3000 ms, flip angle = 90°, and 1 average. Slice thickness was 3 mm, slice spacing 3.75 mm. A total of 480 volumes were obtained in one run. Structural images were recorded using a T1 weighted sequence, with TR = 5.98s, TE = 2.91 ms, 2 mm slice thickness, and 3 averages. One set consisted of 104 slices. In each subject, anatomy scans were acquired first, followed by the functional imaging run.

2.5. Data analysis

ROI-to-ROI FCA was computed using the CONN toolbox [24], (http://www.nitrc.org/projects/conn), implemented in MATLAB. Preprocessing steps including realignment, coregistration/normalization, segmentation, outlier identification and smoothing, and de-noising steps which aim to remove possible confounds in the BOLD signal, including motion, physiological and other noise sources were all done using the CONN toolbox [24].

After pre-setting region of interests (ROIs), a General Linear Model (GLM) was used to calculate correlations of the mean BOLD time-series between each two different ROIs at the single-subject level, resulting ROI-to-ROI functional connectivity matrices consisting Fisher-transformed bivariate correlation coefficients (z-scores) between each two different ROIs (https://web.conn-toolbox.org/fmri-methods/connectivity-measures/roi-to-roi) in two different task conditions. Task conditions include “Taste condition” and “Water condition” (**Figure 1, Table 2**). Both sweet and sour taste stimulants were evaluated as one common “taste condition”. Group analysis was then performed using a two-sample t-test to uncover differences in functional connections between the patient and control groups in both conditions. Age and sex of participants were introduced as covariates into the analysis. Connection threshold $p < 0.05$ (p-FWE corrected) was regarded as significant.

The pre-set ROIs in the present study included right and left IC [16,17,25,26], operculum [25,26], OFC [25,26], cingulate [26,25], amygdala [25,27], thalamus [26,28], cerebellum [25], temporal pole [25] and putamen [25], identified as relevant regions with respect to taste cerebral processing by previous studies. We also added ROIs related to frontal cortices considering their roles in modulating gustatory processing [19]. Because of the close relation between gustation and olfaction we added the piriform cortex (PFC), which is considered to be a significant part of the primary olfactory cortex [17]. The ROIs of OFC and PFC were provided by Fjaeldstad et al [29]. The remaining ROIs were chosen from the FSL Harvard-Oxford Atlas and the AAL atlas provided by the software [30], resulting in a total of 52 ROIs (26 pairs) in the FCA (**Table 3**).

Table 3. Selected ROIs for FCA.

ROIs (Region of interests)
ROIs provided by the CONN (the FSL Harvard-Oxford atlas and the AAL atlas)
*Insular Cortex (r & l)**
Frontal Operculum Cortex (r & l)
Parietal Operculum Cortex (r & l)
Cingulate Gyrus, anterior division
Cingulate Gyrus, posterior division
Amygdala (r & l)
Thalamus (r & l)
Putamen (r & l)
Cerebellum Crus 1-10 (r & l)
Temporal Pole (r & l)
Postcentral Gyrus (r & l)
Frontal Pole (r & l)
Superior Frontal Gyrus (r & l)
Middle Frontal Gyrus (r & l)
Inferior Frontal Gyrus, pars triangularis (r & l)
Inferior Frontal Gyrus, pars opercularis (r & l)
ROIs provided by Fjaeldstad et al.
Orbitofrontal cortex (r & l)
Prefrontal cortex (r & l)

Table 3 *r & l: right and left.

3. Results

3.1 Group level:

In the taste condition, the patient group showed significantly weaker functional connectivity between left OFC (lOFC) and right OFC (rOFC) compared to the control group ($T(17) = 6.79$, connection threshold: $p < 0.05$, p-FWE corrected, **Figure 2** left panel).

For the water condition, the patient group showed significantly weaker functional connectivity between left frontal pole (lFP) and left superior frontal gyrus (lSFG) in comparison to the control group ($T(17) = 5.16$, connection threshold: $p < 0.05$, p-FWE corrected, **Figure 2** right panel).

The functional connectivity of ROIs was also compared between taste and water conditions but there were no significant differences for all participants.

Fig.2 Differential functional connections in healthy controls vs. patients with taste loss (controls > patients)

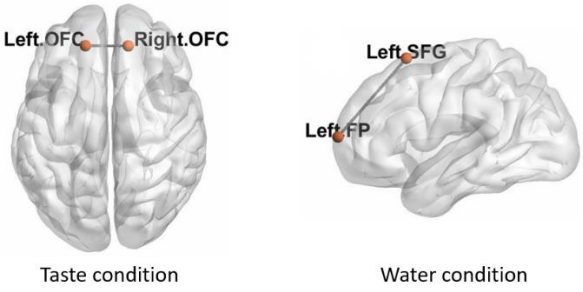

Fig.2 Two functional connections in the patient group were significantly weaker than in the control group. One was between the right and the left orbital frontal cortex (OFC) in the taste condition (T (17) = 6.79, connection threshold: p < 0.05, p-FWE corrected, left panel), another one was between the left Frontal Pole (FP) and the left Superior Frontal Gyrus (SFG) in the water condition (T (17) = 5.16, connection threshold: p < 0.05, p-FWE corrected, right panel).

3.2 Individual level:

On an individual level, each participant had a unique pattern of ROI-to-ROI connectivity (RRC) matrix containing Fisher-transformed bivariate correlation coefficients (z-scores) between every two different ROIs. **Figure 3** is an example of the RRC matrix of one single subject of taste condition. On the individual level, RRC can be thresholded based on z-scores but only for display purposes and it is not supported by any form of statistical inference as reported by the CONN forum (https://www.nitrc.org/forum/message.php?msg_id=5149).

Fig.3 An ROI-to-ROI connectivity (RRC) matrix of one single-subject

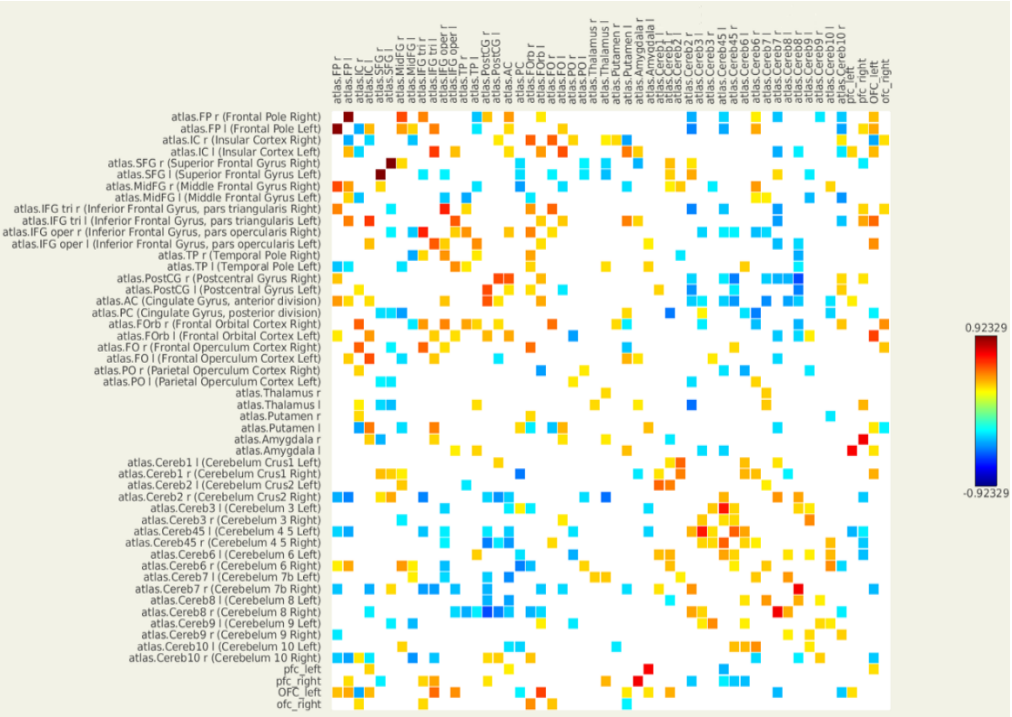

Fig.3 An ROI-to-ROI connectivity (RRC) matrix of one single-subject of taste condition created by the CONN toolbox. Each element in an RRC matrix is defined as the Fisher-transformed bivariate correlation coefficient (z-score) between a pair of ROI BOLD time series. We pre-defined 52 regions of interest (ROIs) and the calculated z-scores form a 52-by-52 matrix (the z-score was not calculated between one ROI and itself). The squares are shown/colored when z-scores were above +0.25 or below -0.25.

As *group* analysis showed two pairs of functional connections (lOFC – rOFC and lFP – lSFG) that were significantly different between patient and control group, we checked these two pairs of connections in individual RRC matrix. We set z-score > 0.25 (0.25 is the default value set by the CONN toolbox) as a threshold to display the matrix. As shown in **Table 4**, in the water condition, z-scores corresponding to the lFP – lSFG connection were more than 0.25 in 9 out of 12 healthy controls but were less than or equal to 0.25 in all 7 patients. In taste condition, for all patients and most healthy controls (9 out of 12), the z-scores corresponding to the lOFC – rOFC connection were less than or equal to 0.25.

Table 4. Two functional connections in individual level.

	Taste condition lOFC and rOFC	Water condition lFP and lSFG
Patient no.		
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
Control no.		
1	-	+
2	+	-
3	-	+
4	-	+
5	+	-
6	-	+
7	-	+
8	-	-
9	-	+
10	-	+
11	-	+
12	+	+

Table 4 Two functional connections in individual level. "+" means the z-score is more than 0.25 while "-" means z-scores is less than or equal to 0.25. Z-score equals to Fisher-transformed bivariate correlation coefficients between the left and right orbital frontal cortex (OFC) in taste condition, or between the left frontal pole (lFP) and the left superior frontal gyrus (lSFG) in water condition.

4. Discussion

On a group level, a weaker functional connection between right and left OFC was observed in patients with taste loss compared to healthy controls during taste stimulations. In primates, OFC receives direct inputs from the primary taste cortex - IC [31] and neurons in the OFC can respond to the prototypical tastes [32,33]. Human neuroimaging studies also showed that the OFC can be activated by gustatory stimuli [34-36]. Hence, the OFC is considered to contain the secondary taste cortex in humans [17]. It also plays an important role in integrating gustation with retronasal olfaction and oral somatosensation into a "flavor" [37]. The right and left OFCs are anatomically separated in two hemispheres. In the previous study [16] of our laboratory, when participants receiving taste stimulations, activations of the right and left OFCs in the patient group tended to be stronger than that in control group. Interestingly, at the same situation, the functional connectivity between right and left OFCs was weaker in patient group compared to control group based on the FCA in the present study.

To our knowledge, no other study has investigated how brain functional connectivity changes after a long-term taste loss. However, there was a study investigating the effects of chronic *peripheral* olfactory loss on brain functional connectivity where patients with long-term peripheral olfactory loss and healthy controls were asked to do a sniffing task in the MRI scanner. The FCA revealed that compared to healthy controls, patients with olfactory loss showed a decrease in functional connectivity [38] involving the anterior prefrontal cortex, the anterior cingulate cortex, the entorhinal cortex and the cerebellum [38]. In other words, long-term

peripheral olfactory loss is associated with decreased functional connectivity among the brain regions relevant to olfactory processing. This is consistent with what we have found on our patients with long-term taste loss.

Generally, there were two types of task conditions in the present study that is taste condition and water condition. Taste condition is a condition that participants were receiving sweet or sour taste stimulations (0.1ml sweet/sour solutions). Water condition is a condition that participants were perceiving purely water (0.1ml water). Importantly, water has been mentioned as an independent taste modality [39]. The functional connectivity of ROIs has been compared between taste and water conditions but there were no significant differences between two conditions at a group level. This might be because the sample size was too small to show the significant difference; or the difference between cerebral processing of taste solutions and that of pure water does not manifest itself in the level of functional connectivity.

Interestingly, we observed, when participants perceiving purely water (in water condition), a weaker functional connectivity between the left frontal pole (lFP) and the left superior frontal gyrus (lSFG) in the patient group compared to the control group. FP contains areas associated with many higher cognitive functions such as drawing analogies and making plans [40,41]. The SFG is generally thought as a core brain region in cognitive control systems [42]. Cognitive functions are expected to modulate taste-related activations in gustatory cortices [19]. The decrease in functional connectivity between brain regions relevant to cognition may contribute to the perceived taste loss. Several studies [43-45] have demonstrated that people with cognitive impairments or with dementia in the early stage exhibited significant impairments of taste sensitivity in comparison to age-matched healthy controls. These findings suggest a close relation between taste function and cognition. Unfortunately, the cognitive abilities of our participants were not evaluated at that time. Nevertheless, the finding seems to emphasize the association between taste function and cognition. Cognitive functions should receive more attention when patients complain about taste loss, especially in idiopathic taste loss. In this perspective, fMRI could be an available tool to follow up patients and evaluate brain changes at the level of functional connectivity.

At an individual level, each participant exhibited a unique pattern of ROI-to-ROI functional connectivity (RRC) matrix. This was true for patients and controls. This individual variation was expected because sensory systems are highly plastic [38,46] at both cellular [47] and cognitive levels [48], which relates to learning/training experiences [49,50]. As mentioned above, at a group level, we found a weaker functional connectivity between the lFP and the lSFG in the patient group compared to the control group. In individual level, we found that z-scores corresponding to the lFP – lSFG connection were more than 0.25 in 9 out of 12 healthy controls but were less than or equal to 0.25 in all patients with taste loss (**Table 4**). This provides an impression that there might be a useful criterion regarding the z-scores that could differentiate patients with taste loss from healthy controls. This impression suggests the possibility that fMRI might help diagnosing taste loss as an additional tool in future by focusing on specific functional connections.

However, the shortcoming of the present study is not only the small sample size but also the uncertainty and inconsistency regarding the etiology of taste loss. Regarding the seven patients in our study, three patients claimed that their taste losses started after head traumas. However, their structural MR scans did not show any lesions that could be related to the taste loss in the brain. Three patients claimed that their taste loss began after infections of the upper respiratory tract (also with no lesions visible in structural brain imaging). The remaining patient had idiopathic taste loss. The common characteristic of these patients was the long-term taste loss and the impaired ability to identify taste qualities based on psychophysical taste testing. So far, we could only suggest that symptoms of taste loss as well as impaired taste identification ability are associated with decreased central functional connectivity between some brain regions. We cannot make certain whether the symptoms of taste loss cause the decreased functional connectivity of the brain regions or the other way around. In addition, the sample size of the current study was too small. Introducing age and gender as covariates further reduced the power of analysis. Hence, we framed the study as exploratory and hope these observations might provide some useful references for other researchers to design related studies. Future studies with a larger sample size, age/gender-matched controls and with subgroups, e.g., 1) a subgroup

including patients with long-term peripheral taste loss and 2) a subgroup including patients with idiopathic taste loss (possibly related to earlier central cognitive damages), should elucidate the possible causal relationship of the decreased functional connectivity of brain regions and the symptom of taste loss.

5. Conclusion

Patients with taste loss appear to have central functional changes in terms of decreased functional connectivity between brain regions not only relevant for taste processing but also for cognition. While further studies are needed, fMRI might be helpful in diagnosing taste loss as an additional tool in some exceptional cases.

Declarations

Ethics approval: All investigations were conducted in accordance with the Guidelines for Biomedical Studies Involving Human Subjects (Helsinki Declaration). The study protocol was approved by the Ethics Committee at the University Clinic "Gustav-Carl-Carus" of the "Technische Universitaet Dresden". Consent to participate and publication: Written informed consent was obtained from all participants prior to their inclusion.

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Publication 3 (Third study) Processing of Sweet, Astringent and Pungent Oral Stimuli in the Human Brain.

Abstract of Publication 3

Background: Taste and oral somatosensation are intimately related to each other from peripheral receptors to the central nervous system. Oral astringent sensation is thought to contain both gustatory and oral somatosensory components.

Methods: In the present study, we compared the cerebral response to an astringent stimulus (tannin), with the response to one typical taste stimulus (sweet – sucrose) and one typical somatosensory stimulus (pungent – capsaicin) using functional magnetic resonance imaging (fMRI) of 24 healthy subjects.

Results: Three distributed brain subregions responded significantly different to the three types of oral stimulations: lobule IX of the cerebellar hemisphere, right dorsolateral superior frontal gyrus, and left middle temporal gyrus.

Conclusions: Sub-regions of the cerebellum, frontal cortex and temporal cortex might play a major role in the discrimination of sucrose, tannin and capsaicin solutions.

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Processing of Sweet, Astringent and Pungent Oral Stimuli in the Human Brain

Yunmeng Zhu, [a]* **Divesh Thaploo,** [a] **Pengfei Han** [b] **and Thomas Hummel** [a]

[a] *Smell & Taste Clinic, Department of Otorhinolaryngology, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany*

[b] *Faculty of Psychology, Southwest University, Chongqing, China*

Abstract—Taste and oral somatosensation are intimately related to each other from peripheral receptors to the central nervous system. Oral astringent sensation is thought to contain both gustatory and somatosensory components. In the present study, we compared the cerebral response to an astringent stimulus (tannin), with the response to one typical taste stimulus (sweet – sucrose) and one typical somatosensory stimulus (pungent – capsaicin) using functional magnetic resonance imaging (fMRI) of 24 healthy subjects. Three distributed brain subregions responded significantly different to the three types of oral stimulations: lobule IX of the cerebellar hemisphere, right dorsolateral superior frontal gyrus, and left middle temporal gyrus. This suggests that these regions play a major role in the discrimination of astringency, taste, and pungency.

Key words: taste, gustation, astringency, fMRI, tannin, capsaicin.

INTRODUCTION

Gustation (taste sensation) and oral somatosensation (e.g., texture, pungency and temperature) are intimately related and both help to distinguish nutritive components from toxic and harmful substances (Green, 2003; Simon et al., 2006; Simon et al., 2008; Rudenga et al., 2010; Gutierrez and Simon, 2021). For example, sweet, salty and umami tastes as well as oral fatty texture are appetizing, while bitter taste, hot or sharp oral sensations (such as a small piece of bone in food) are aversive and likely to be rejected (Simon et al., 2008; Barlow, 2022).

Gustation generally refers to the sensation that results from the direct stimulation of the gustatory receptors residing in taste buds (Spence et al., 2015). Taste buds are surrounded by mucosa containing various somatosensory receptors such as mechanoreceptors, thermoreceptors and nociceptors (Green, 2003). During eating, multisensory inputs from those receptors plus retronasal olfactory inputs are transmitted to the central nervous system (CNS), and humans then consciously perceive the complex flavors including mouthfeel. Gustatory information is delivered by special sensory branches of the facial (VII), glossopharyngeal (IX) or vagal (X) (Huang and Xu, 2021) nerves. Somatosensory information is transmitted by the trigeminal (V) nerve as well as by general sensory branches of glossopharyngeal (IX) or vagal (X) (Huang and Xu, 2021) nerves (Simon et al., 2008; Gutierrez and Simon, 2021). Gustatory and somatosensory pathways converge in the nucleus of the solitary tract (NST) and the thalamus (Ogawa et al., 1987) where taste and somatosensory information might have early crosstalk (Simon et al., 2008; Gutierrez and Simon, 2021). Then, taste and somatosensory inputs reach their cortical targets respectively in the primary gustatory cortex – insula and the primary somatosensory cortex – postcentral gyrus (DiGuiseppi and Tadi, 2022). These two types of inputs are also integrated in the primary gustatory cortex – the insula (Cerf-Ducastel et al., 2001; De Araujo and Rolls, 2004; Rudenga et al., 2010).

Oral astringent sensation is described as a feeling of puckering, rough and drying sensation plus a slight bitter taste on the tongue and membranes of the oral cavity (Critchley and Rolls, 1996; Fleming et al., 2016; Huang and Xu, 2021). The potential aversiveness of that has been shown for rhesus monkeys' selection of food which depends more on the level of astringency of the plant than on its nutritional value (Marks et al., 1988). Tannic acid is one of the common chemicals which produces astringency (Ashok and Upadhyaya, 2012). Plants containing high levels of tannin are avoided by rodents

*Corresponding author. Address: Fetscherstrasse 74, 01307 Dresden, Germany.
E-mail addresses: zhym182@hotmail.com (Y. Zhu), taploodivesh4@gmail.com (D. Thaploo), p.han@foxmail.com (P. Han), thomas.hummel@tu-dresden.de (T. Hummel).

Abbreviations: CER9, Lobule IX of cerebellar hemisphere (cerebellum 9); CNS, central nervous system; fMRI, functional magnetic resonance imaging; IMTG, the left side of the middle temporal gyrus; NST, nucleus of the solitary tract; rSFGdl, the right side of the dorsolateral superior frontal gyrus; TG, trigeminal ganglion; TRCs, taste receptor cells.

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(Shimada and Saitoh, 2003) and primates (Takemoto, 2003). This might be because tannic acid binds proteins in foods (Critchley and Rolls, 1996) and prevents absorption/digestion of nutritious proteins.

The peripheral processing of astringency is inconclusive. Whether astringency is a taste or an oral somatosensation remains in dispute (Schöbel et al., 2014; Kishi et al., 2017). On the one hand, taste receptors have been shown to interact with astringent compounds in animal studies (Schiffman et al., 1992); and taste nerves – chorda tympani (a branch of the facial nerve) (Schiffman et al., 1992) – have been shown to be activated by astringent compounds. On the other hand, 1. astringent perception has been reported to be dependent on lingual nerve (trigeminal) function (Schöbel et al., 2014); 2. some astringent compounds can stimulate responses in the primary trigeminal ganglion (TG) neurons in mice (Schöbel et al., 2014); and 3. the perceived astringent sensation increases when an astringent compound (tannic acid) is repeatedly sampled (Lyman and Green, 1990), which is a trigeminal feature, but not a characteristic of the gustatory system (Schöbel et al., 2014). Hence, some researchers postulated that the perception of astringency contains multiple sub-qualities (Lee and Lawless, 1991; Critchley and Rolls, 1996). In fact, a synergism between chemosensory and mechanosensory activations relates to oral astringency (Schöbel et al., 2014). As mentioned above, the astringent sensation is subjectively rated as an integration of the sub-qualities include 'roughing' and 'puckering' as well as the associated side tastes 'bitter' and 'sour' (Fleming et al., 2016). Tannins interact with salivary proteins and the precipitated complexes adhere to the mucosa, raising the friction coefficient among mucosal surfaces (Soares et al., 2020), which might underlie the 'roughing' and 'puckering' sub-qualities. For the bitter side taste, Soares and coworkers have found that some tannins directly activated human bitter taste receptors (Soares et al., 2013). Tannins are a series of phenolic compounds containing sufficient hydroxyl and carboxyl groups (Ashok and Upadhyaya, 2012), and the protons (H^+) ionized from carboxyl groups in the tannin solution are agonists of sour taste receptor cells (TRCs) (Chang et al., 2010), which might be the basis of the sour side taste.

How the oral astringent stimulus is encoded at the cortical level in humans has rarely been investigated. Actually, despite extensive researches on the coding and differentiation of basic taste qualities like sweet, sour, salty, bitter and umami, their representations in the human brains remain inconclusive. A **topographic model** proposed a "taste topographic map" (Chen et al., 2011) within the insula analogous to somatotopy of the somatosensory system, wherein a specific spatial area selectively responds to a specific taste, such as sweet. However, findings from recent studies are more prone to a "**population coding model**", wherein taste quality information is signaled by a pattern of activity across a population of neurons (Avery et al., 2020; Chen et al., 2021).

To our knowledge, only one study investigated astringent representation in the human brain using functional Magnetic Resonance Imaging (fMRI) (Kishi et al., 2017). In this study, brain activities of healthy people were measured in response to three types of taste solutions: astringent (tannic acid), sweet (sugar) and bitter (caffeine). They found that all the three types of stimuli could activate the insula and within the insula, overlapping sub-regions were activated by astringent and bitter stimuli. In conclusion, they suggested that the human brain might recognize astringency as a taste. However, this study only employed two typical taste stimuli (sweet and bitter) for comparison. We do not yet know whether in the same experimental condition, the response to astringent stimulus is also similar with that to an oral somatosensory stimulus. Actually, in another human fMRI study (Rudenga et al., 2010), overlapping activations in sub-regions of the insula were also co-activated by sweet, bitter stimuli and capsaicin stimuli, the latter being a typical somatosensory stimulus (Green, 2005; Leijon et al., 2019; Gutierrez and Simon, 2021). Therefore, in the present study, we aimed to compare the cerebral responses to astringent stimuli (tannin), with the responses to one prototypical taste stimulus (sweet) and one typical somatosensory stimulus (capsaicin). We aimed to investigate whether the human brain responds to these three oral stimuli differently within the same framework of experimental design.

EXPERIMENTAL PROCEDURES

Participants

Twenty-four healthy participants (age range: 20–37 years, mean 26.0 ± 3.8 years, 10 men, 14 women) without ENT (ear, nose and throat) disease and history of neurological or psychiatric disorder were included. All of them had normal taste functions ascertained with a standardized, validated taste test kit "taste strips" (Landis et al., 2009). None of the participants had been taking medication at the time of the study. To verify the reproducibility of the present fMRI study, seven of the participants (age range: 24 – 37 years, mean 28.6 ± 4.6 ye ars, four men, three women) were asked to visit again and complete the identical experiment procedures with an average interval of 18 days between two visits.

All participants were able to recognize the differences among astringent (tannin), sweet (sucrose), and pungent (capsaicin) solutions before the fMRI test. The participants were asked not to eat at least for 2 h before the experiment.

All investigations were conducted in accordance with the Guidelines for Biomedical Studies Involving Human Subjects (Helsinki Declaration). The study protocol was approved by the Ethics Committee at the University Clinic "Gustav-Carl-Carus" of the "Technische Universität Dresden" (ethics protocol number EK 389102017). Consent to participate and publication: Written informed consent was obtained from all participants prior to their inclusion.

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Stimuli

Three types of stimuli (sweet – sucrose, astringent – tannin and pungent – capsaicin) were administered in liquid form: sucrose (order number: S9378; Sigma-Aldrich, Deisenhofen, Germany) for sweet taste stimuli (10 g dissolved in 100 mL distilled water, 100 g/L); a wine tannin (ordered from a wine making supplies and commercial winery business "Presque Isle Wine Cellars", https://www.piwine.com) derived from European chestnuts as astringent stimuli (1 g dissolved in 100 mL distilled water, 10 g/L). The capsaicin (analytical standard of ≥99.0% by HPLC; Sigma-Aldrich, Steinheim, Germany; order number 12084) was dissolved with 95% ethanol first. Then, as a pungent stimulus 10 mL capsaicin-ethanol solution (90 μmol/L) was diluted with 60 mL distilled water. The respective stimuli were iso-intense as established in pilot experiments in a small group of experienced observers (please see supplementary materials – Table S1).

Stimulus solutions were delivered into the subject's mouth via tubing, a combination of four separate sterile PVC tubes (Type: IV-Standard – PVC, Original Perfusor® Line, B. Braun Melsungen AG, Melsungen, Germany, Fig. 1) for the respective stimulants plus water. One end of the tubing was connected to a mouthpiece (Fig. 1(**C,D**)) which could be easily placed between the lips, held by the subject's teeth. The other end of the tubing went through a small outlet in the wall of the scanner room and was connected to **three-way valves** and syringes (Fig. 1(**A,B**)), enabling the delivery and replenishment of the liquids, and blockage of flow from either end. The outer diameter of the PVC tubes was 3 mm, their inner diameter was 2 mm, and the total length of the tubing was approximately 10 m.

Importantly, these tubes needed to be filled with the respective stimulus solutions without bubbles using syringes before starting the experiment. For stimulations, 0.1 mL of the corresponding liquids (room temperature) were given into the subject's mouth. In between stimulations, 2 mL of water were given to the subjects' mouth as a rinse. Subjects were instructed to swallow only during the "rinse" condition (please see "Experimental design" below).

Experimental design

We employed the fMRI event-block mixed design for three types of stimuli (sucrose, tannin and capsaicin). For each type of stimulus, there were eight cycles (320 s). One cycle lasted 40 s in total as shown schematically in Fig. 2. In each cycle, the subject was first asked to stay still in the scanner for 5-s without any movement (this period was used as "baseline condition" when setting contrasts in data analysis). Then, 0.1 mL stimuli were given onto the tongue of the subject within 2-s (BC). For the following 3-s, via a screen with language instructions the subject moved their mouth and tongue to perceive the given stimuli. Then, the subject was instructed to keep still again without any movement for 10-s. This period was the "task condition" when setting contrasts in data analysis. At the end of this cycle, 2 mL of water were given to the subject for rinsing and the subject was allowed to swallow during this period of 20 s. This cycle was repeated eight times forming a session (320 s in total). Within one session, only one type of stimulus, which could be either sucrose, tannin or capsaicin solutions, was presented to the subject. Immediately after each session, the subject rated the intensity and pleasantness of the stimulus using analogue scales (intensity: 0 (no sensation) to 10 (very strong sensation); pleasantness: −5 (very unpleasant), 0 (neutral), +5 (very pleasant); intensity and pleasantness ratings were added as covariates into the analyses. The order of the given sessions was randomized. Before each scan, the participant was

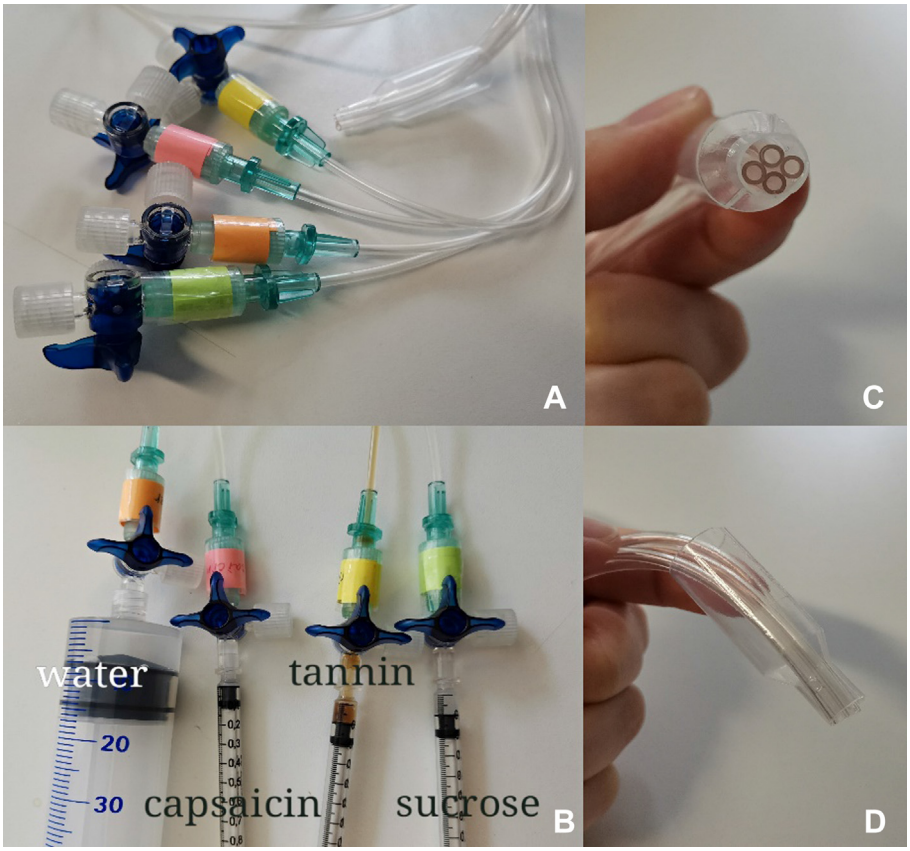

Fig. 1. (**A,B**) The PVC tubes are sterile with one end connected to **three-way valves** and syringes. (**C,D**) The other end of the tubing was connected to the mouthpiece, which could be easily placed between the subject's lips and held by the teeth.

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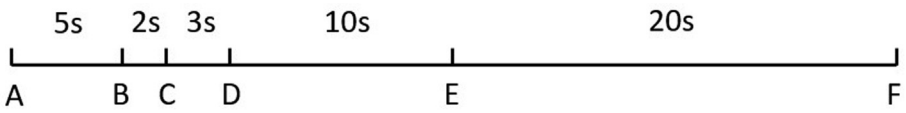

Fig. 2. One cycle **(AF)** of the fMRI paradigm lasted 40 s in total. **AB (5 s):** the subject was instructed (by watching the screen) to keep still in the scanner without any movement. **BC (2 s):** 0.1 mL stimuli (either sucrose/tannin/capsaicin) were presented on the tongue of the subject. **CD (3 s):** the subject was instructed to move his/her tongue to perceive the stimuli. **DE (10 s):** the subject was instructed to keep still in the scanner without any movement. **EF (20 s):** 2 mL water were presented on the tongue of the subject for rinse. The subject was allowed to swallow during the EF period.

instructed how to perform when he/she saw the corresponding language instructions on the screen during the scan.

Following fMRI scanning, each participant completed a questionnaire regarding their eating/drinking habits (for details please see supplementary materials), which were also used as covariates added into analysis.

Functional MRI data acquisition

The system used for both functional and structural imaging was a 3.0 T scanner (Prisma; Siemens, Erlangen, Germany). For functional imaging the following parameters were used: echo time (TE) = 37 ms, repetition time = 800 ms, flip angle = 52°, voxel size: 2.0 × 2.0 × 2.0 mm, gap = 0 mm; 403 measurements in one run. Structural images were recorded using a T1 weighted sequence, with TR = 2300 ms, TE = 2.29 ms, 0.94 mm slice thickness, and 1 average. One slab consisted of 176 slices. In each subject, anatomical scans were acquired first, followed by the complete functional imaging runs.

Functional MRI data analysis

The imaging data were analyzed by means of the software package statistical parametric mapping (SPM) 12 (The Wellcome Centre for Human Neuroimaging, UCL Queen Square Institute of Neurology, London, UK) within MATLAB R2018b (The MathWorks, Inc., Natick, MA, USA). Preprocessing included motion correction (realignment and unwarping), co-registration of individual anatomical and functional data, normalization to the Montreal Neurological Institute coordinate system (Collins et al., 1994), and smoothing with an 8-mm full width Gaussian kernel.

As mentioned in ‘‘Experimental design”, in total there were three runs (sessions), each run included two types of condition: baseline (AB, Fig. 2) and task (DE, Fig. 2) conditions. There were three types of ‘‘task” conditions, i.e., ‘‘sucrose”, ‘‘tannin” and ‘‘capsaicin”. The type of ‘‘task condition” was consistent within one run but different among runs. In first-level analysis, contrasts were calculated for ‘‘task condition” versus ‘‘baseline”. Three kinds of contrasts were calculated in first-level analysis: sucrose – baseline, tannin – baseline, and capsaicin – baseline. The Canonical Hemodynamic Response Function (Canonical HRF) was applied.

In second-level analysis, one-sample t-test was used to show activations across the whole brain in response to each of the three stimuli, separately with a threshold of $p < 0.05$ (Family Wise Error (FWE) corrected, cluster size >50). To analyze the co-activated regions by three kinds of stimuli, conjunction analysis (Friston et al., 1999) was used with a threshold of $p < 0.05$ (FWE corrected, cluster size >50). One-way within-subject ANOVA was employed to test the differences of activations among the three types of stimuli with a threshold of $p < 0.001$ (p-uncorrected, cluster size >50). Covariates including, 1. pleasantness ratings, 2. intensity ratings, 3. taste strips scores representing taste identification ability and 4. consumption habits of beverages corresponding to the three types of stimuli, were introduced into the one-way ANOVA. Marsbar toolbox was used to extract the BOLD values of brain regions that showed significantly different responses to the three types of stimuli. A further one-way repeated measures ANOVA was performed based on the extracted values using Statistical Package for the Social Sciences (SPSS, IBM SPSS Statistics for Windows, version 19.0 (IBMCorp., Armonk, N.Y., USA)) to investigate the explicit difference among the three types of stimuli. Shapiro-Wilk test was used to confirm normal distributions. Bonferroni-corrected was used for multiple comparisons of the ANOVA. For the seven subjects who participated in the identical experiment twice, paired t-tests using SPM12 with a threshold of $p < 0.001$ (p-uncorrected, cluster size >50) was used to compare the brain activations between two visits. Contrasts were calculated for the ‘‘first visit” minus the ‘‘second visit” and also the ‘‘second visit” minus the ‘‘first visit”, respectively for three stimulants. At the individual level, the BOLD values were extracted using Marsbar toolbox from the primary taste cortex – insula and the primary somatosensory cortex – postcentral gyrus (poCG) for each participant (the result of this part is in supplementary materials). To explore the possible difference of brain activations between male and female participants, we conducted a 2 (male, female) by 3 (sucrose, tannin, capsaicin) ANOVA using SPM12 with a threshold of $p < 0.001$ (p-uncorrected, cluster size >50). Considering the small sample size and the sex-difference is not the focus of the article, the results are only shown in supplementary materials (Table S2 and Fig. S2).

RESULTS

Behavioral results: The perceived intensity and pleasantness

The ratings of the intensity and pleasantness of three types of oral stimuli during the fMRI runs are summarized in Fig. 3. Participants rated the sucrose stimulus as more intense than tannin ($p = 0.030$) and capsaicin ($p = 0.022$) stimuli but there was no significant difference of intensity ratings between tannin and capsaicin ($p = 0.89$). Tannin and capsaicin stimuli were perceived as unpleasant and there was no significant difference of pleasantness ratings between

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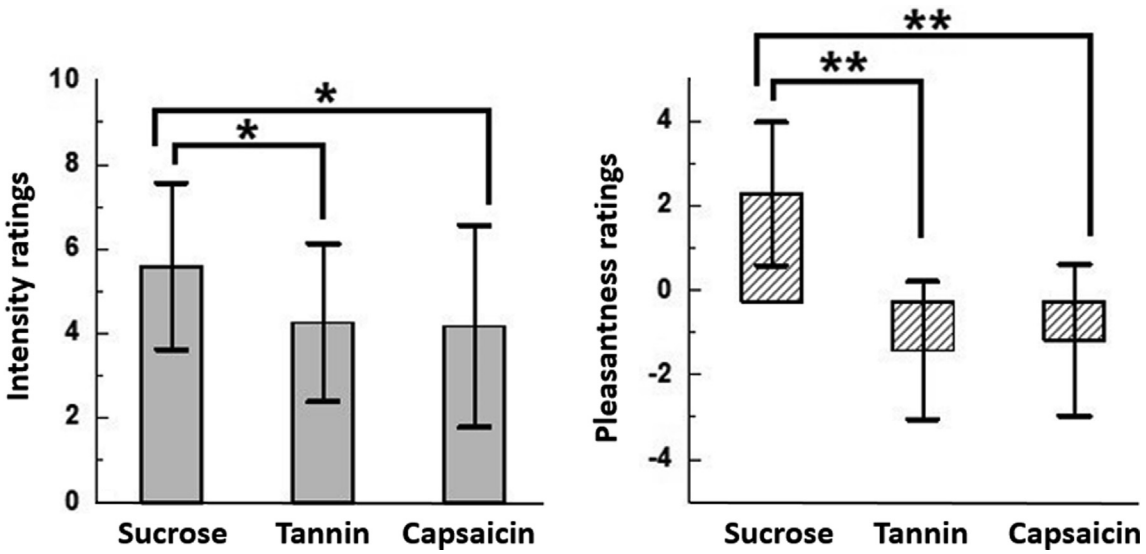

Fig. 3. Participant ratings of taste intensity and pleasantness respectively for sucrose, tannin and capsaicin solutions during functional magnetic resonance imaging. Data were analyzed with ANOVA and post-hoc testing. Data are presented as means ± SD. *$p < 0.05$, **$p < 0.001$. ANOVA: analysis of variance.

tannin and capsaicin ($p = 0.61$). However, sucrose solution was perceived as more pleasant than tannin ($p < 0.001$) and capsaicin solutions ($p < 0.001$) at the group level.

Functional neuroimaging

Brain activations for each stimulant (One-sample t-test). Sucrose solution activated the bilateral insula, bilateral postcentral gyrus, bilateral Lobule VI of cerebellar hemisphere (cerebellum 6, CER6), right supplementary motor area (SMA), left superior frontal gyrus, medial (SFGmedial), left caudate and left putamen ($p < 0.05$, FWE corrected, cluster size > 50, Table 1). Tannin solution activated the bilateral postcentral gyrus, bilateral precentral gyrus, bilateral CER6 and left insula. Capsaicin solution activated the bilateral postcentral gyrus, bilateral CER6, left precentral gyrus, right insula, right middle frontal gyrus (MFG) and bilateral inferior frontal gyrus pars orbitals (IFGorb), left inferior frontal gyrus, triangular part (IFGtriang), right inferior frontal gyrus, opercular part (IFGoperc), right SFGmedial, and middle cingulate (MCC).

Overlapping brain regions co-activated by three stimulants (Conjunction analysis). All three types of taste stimuli co-activated the bilateral postcentral gyrus and precentral gyrus, left insula, right rolandic operculum (ROL), bilateral CER6, bilateral inferior frontal gyrus pars orbitals (IFGorb), right middle frontal gyrus (MFG) and left inferior frontal gyrus, triangular part (IFGtriang) ($p < 0.05$, FWE corrected, cluster size > 50, Table 2).

Different brain activations in response to three stimulants (ANOVA analysis). Significant differences of the BOLD signals among the three types of oral stimuli were found in three distributed clusters respectively located in Lobule IX of cerebellar hemisphere (cerebellum 9, CER9), the right side of the dorsolateral superior frontal gyrus (rSFGdl) and the left side of the middle temporal gyrus (lMTG) ($p < 0.001$, p-uncorrected, cluster size > 50; Table 3, Figs. 5 and 6). Respectively in CER9, rSFGdl and lMTG, the averaged BOLD signals activated by tannin solution were all significantly stronger than that activated by sucrose ($p = 0.027$; $p = 0.033$; $p = 0.047$) and capsaicin ($p < 0.001$; $p < 0.001$; $p < 0.001$) solutions. Similarly, the averaged BOLD signals activated by sweet solution were significantly stronger than that activated by capsaicin ($p = 0.011$; $p = 0.011$; $p = 0.006$. Fig. 5).

The reproducibility of the present fMRI experiment (Paired t-test). For tannin, there was no suprathreshold cluster for both contrasts: "first visit – second visit" and "second visit – first visit". For sucrose, there was no suprathreshold cluster for the contrast "second visit – first visit". For the contrast "first visit – second visit", a cluster in the left inferior frontal gyrus pars orbitals (IFGorb) survived ($p < 0.001$, p-uncorrected, cluster size > 50, Table 4). For capsaicin, there was no suprathreshold cluster for the contrast "second visit – first visit", but a cluster survived in the right lingual gyrus for the contrast "first visit – second visit" ($p < 0.001$, p-uncorrected, cluster size > 50, Table 4). For more details regarding the changes of brain responses (BOLD signals) between two visits for individual participants, please see supplementary materials – Fig. S1.

DISCUSSION

The main aim of the present study was to investigate human brain responses to three types of oral stimuli, sweet (sucrose), astringent (tannin) and pungent (capsaicin) solutions within the same experimental framework. To achieve this, we measured brain

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Table 1. Brain activations for each stimulant

Cluster Size (in voxels)	T	Z	x	y	z	Side (L-left, R-right)	Location (AAL3*)
Sucrose – baseline							
6166	12.50	6.81	−62	−6	22	L	Postcentral gyrus, insula
5117	11.73	6.63	42	2	4	R	Insula, postcentral gyrus
1187	9.08	5.86	10	14	48	R	Supplementary motor area
	9.08	5.86	−6	34	36	L	Superior frontal gyrus (medial)
218	8.05	5.50	16	−62	–22	R	Cerebellum 6
250	7.85	5.42	−14	−60	–22	L	Cerebellum 6
60	7.90	5.44	−18	6	14	L	Caudate, putamen
Tannin – baseline							
1154	11.03	6.44	52	−6	26	R	Postcentral gyrus, precentral gyrus
1258	10.57	6.32	−56	−8	20	L	Postcentral gyrus, precentral gyrus
67	7.94	5.46	–32	−6	14	L	Insula
86	7.22	5.17	18	−62	–22	R	Cerebellum 6
55	7.04	5.09	−18	−66	–22	L	Cerebellum 6
Capsaicin – baseline							
1594	16.65	7.62	−62	−4	22	L	Postcentral gyrus, precentral gyrus
1411	9.12	5.88	40	−4	6	R	Postcentral gyrus
377	10.68	6.35	40	48	10	R	Middle frontal gyrus, inferior frontal gyrus pars orbitals
148	7.72	5.37	−44	40	12	L	Inferior frontal gyrus, triangular part
126	8.92	5.81	18	−64	−20	R	Cerebellum 6
142	7.88	5.43	−16	−62	−24	L	Cerebellum 6
83	8.64	5.71	40	6	−14	R	Insula
89	7.59	5.32	−48	36	−4	L	Inferior frontal gyrus pars orbitals
83	7.39	5.24	52	12	6	R	Inferior frontal gyrus, opercular part
50	7.35	5.22	6	26	40	R	Superior frontal gyrus (medial), middle cingulate

Table 1 One-sample t-test was used to compare "task condition" with oral stimulations versus "baseline" separately for three types of stimuli ($p < 0.05$, FWE corrected, cluster size > 50). T = t-value; Z = z value; x, y, z = Talairach coordinates. *The survived clusters were labelled according to Automated anatomical labelling atlas 3 (AAL3) (Rolls et al., 2020).

Table 2. Overlapping brain regions co-activated by three stimulants

Cluster Size (in voxels)	T	Z	x	y	z	Side (L-left, R-right)	Location (AAL3*)
2171	9.92	7.79	−60	−6	22	L	Postcentral gyrus, precentral gyrus
			−34	−6	12	L	Insula
1942	8.35	6.92	58	−10	24	R	Postcentral gyrus, precentral gyrus
			52	−6	28	R	Rolandic operculum
184	7.25	6.23	18	−62	–22	R	Cerebellum 6
167	6.68	5.85	−16	−64	–22	L	Cerebellum 6
264	5.97	5.34	34	42	8	R	Middle frontal gyrus Inferior frontal gyrus pars orbitals
135	5.80	5.22	−46	34	−2	L	Inferior frontal gyrus, triangular part Inferior frontal gyrus pars orbitals

Table 2 The clusters co-activated by all three types of oral stimulations in the whole brain ($p < 0.05$, FWE corrected, cluster size > 50). T = t-value; Z = z value; x, y, z = Talairach coordinates. *The survived clusters were labelled according to Automated anatomical labelling atlas 3 (AAL3) (Rolls et al., 2020).

Table 3. Brain regions showing different activations to three stimulants

Cluster Size (in voxels)	F	Z	x	y	z	Side (L-left, R-right)	Location (AAL3*)
50	16.16	4.38	−46	−20	−18	L	Middle temporal gyrus
90	11.72	3.76	22	16	40	R	Dorsolateral superior frontal gyrus
121	16.59	4.44	6	−48	−50	R	Cerebellum 9
						L	Cerebellum 9

Table 3 One-way ANOVA (within subject) was employed to test the differences of activations among three types of stimuli of the whole brain and there were three clusters respectively located in Lobule IX of cerebellar hemisphere (cerebellum 9), right side of dorsolateral superior frontal gyrus and left side of middle temporal gyrus responding significantly different to three types of oral stimulations ($p < 0.001$, p-uncorrected, cluster size > 50). F = f-value; Z = z value; x, y, z = Talairach coordinates. *The survived clusters were labelled according to Automated anatomical labelling atlas 3 (AAL3) (Rolls et al., 2020).

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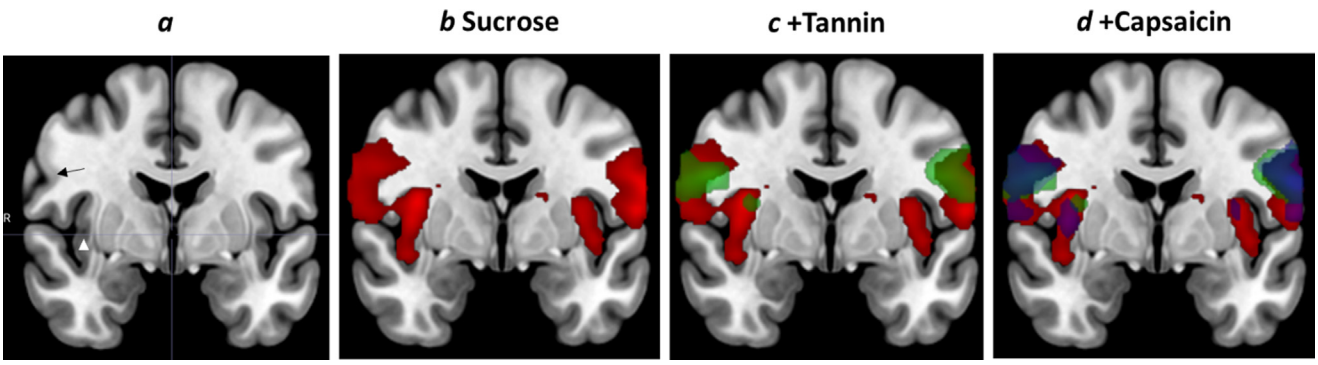

Fig. 4. Brain activations for each stimulant Fig. 4 The left picture (*a*) shows a coronal plane of the human brain where postcentral gyrus (black arrow) and insula (white triangle) can be seen. In the second picture (*b*), we overlaid the same coronal plane with red color representing the clusters activated by sucrose solutions; with green color to represent the clusters activated by tannin solutions (*c*) and blue color for capsaicin solutions (*d*).

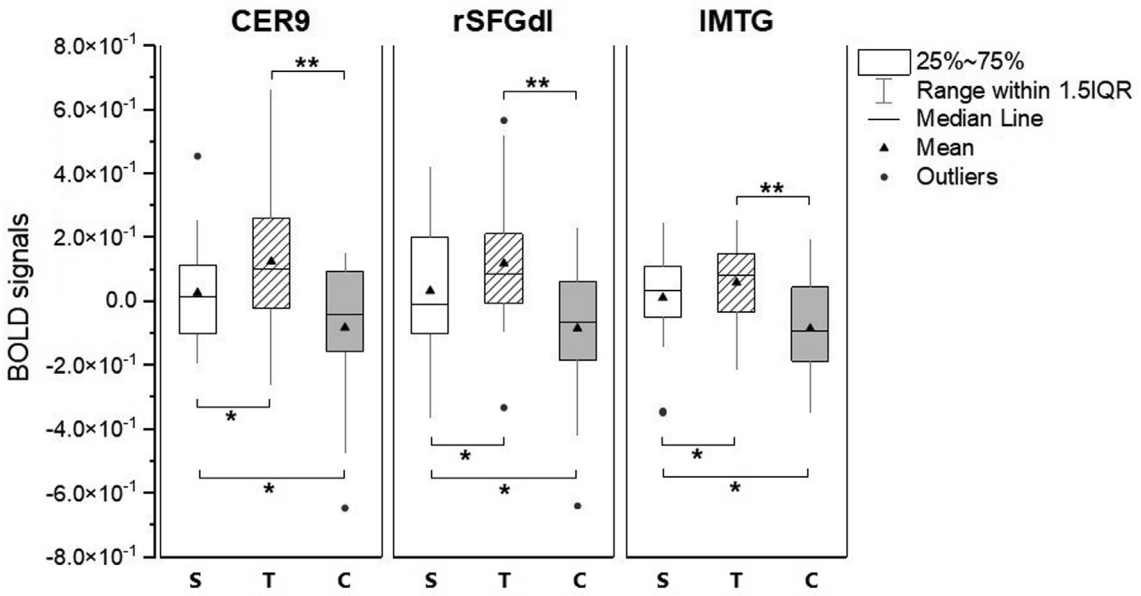

Fig. 5. The BOLD signals of three brain sub-regions (Lobule IX of cerebellar hemisphere, **CER9**; right side of dorsolateral superior frontal gyrus, **rSFGdl**; left side of middle temporal gyrus, **IMTG**) respectively during sucrose (**S**), tannin (**T**) and capsaicin (**C**) stimulations were extracted and a further one-way repeated measures ANOVA and post-hoc testing were done using SPSS. $*p < 0.05$, $**p < 0.001$.

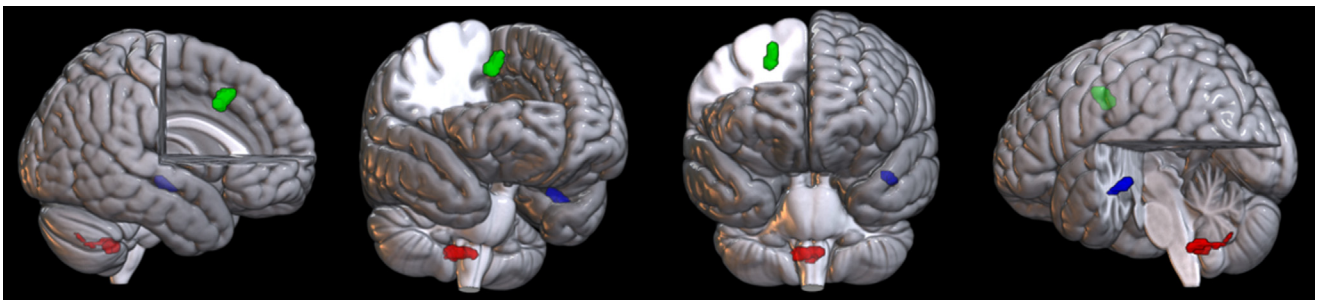

Fig. 6. Three distributed clusters listed in Table 3 were respectively located in Lobule IX of cerebellar hemisphere (CER9, **red color**), right side of dorsolateral superior frontal gyrus (rSFGdl, **green color**), left side of middle temporal gyrus (IMTG, **blue color**).

activations using fMRI during sweet, astringent, and pungent oral stimulations. We found that activation levels (BOLD signals) for the three types of oral stimuli within the primary taste cortex, the insula, were similar to each other. In contrast, significant differences of BOLD signals to the three stimuli were found in three separated brain regions located respectively in cerebellum_9 (Lobule IX of cerebellar hemisphere,

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Table 4. The reproducibility of the present fMRI experiment

Cluster Size (in voxels)	T	Z	x	y	z	Side (L-left, R-right)	Location (AAL3*)
Second visit – first visit							
Sucorse	No suprathreshold cluster						
Tannin	No suprathreshold cluster						
Capsaicin	No suprathreshold cluster						
First visit – second visit							
Sucrose							
251	12.91	4.36	−52	40	−8	L	Inferior frontal gyrus pars orbitals
Tannin	No suprathreshold cluster						
Capsaicin							
84	10.24	4.05	14	−54	6	R	Lingual gyrus

Table 4 Paired t-test was used to compare brain activations between two visits (first visit" minus ‘‘second visit" and ‘‘second visit" minus ‘‘first visit") separately for three types of stimuli ($p < 0.001$, p-uncorrected, cluster size > 50). T = t-value; Z = z value; x, y, z = Talairach coordinates. *The survived clusters were labelled according to Automated anatomical labelling atlas 3 (AAL3) (Rolls et al., 2020).

CER9), right side of dorsolateral superior frontal gyrus (rSFGdl) and left side of middle temporal gyrus (lMTG) (Table 3Figs. 5 and 6). The sensory attributes of one tastant include taste quality, taste palatability and taste intensity (Gutierrez and Simon, 2021) and these three distinct attributes are thought to be processed by different brain circuits (Breslin, 2013; Perez et al., 2013; Wallroth and Ohla, 2018). The intensities of three types of oral solutions in the present study were rated as iso-intense in the pilot experiment (please see supplementary materials – **table S1**); nevertheless, they were rated as different in the formal experiment in the MRI scanner with sucrose solutions perceived as more intense than tannin and capsaicin solutions. Subjective-rated intensity of a solution might be influenced by circumstance, and being in the MRI scanner is such a different circumstance (Kishi et al., 2017). To handle this situation, we introduced the ratings of pleasantness (palatability) and intensity of each individual in the MRI as covariates into the ANOVA. Hence, we suggest that the significant differences of BOLD signals among the stimuli according to the ANOVA in the present study were mainly related to the differences of the qualities of the three types of oral stimuli. Based on this, we speculate that these three regions located in CER9, rSFGdl and lMTG play a role in the recognition and discrimination of sucrose, tannin and capsaicin solutions. In the following we will discuss the significances of the three brain regions regarding taste processing.

Cerebellar lobule IX (CER9): There are previous studies already showing that the cerebellum was activated by taste stimulations (Barry et al., 2001; O'Doherty et al., 2001; Small et al., 2003). However, it is not clear whether the cerebellar response contributed to taste identification. In the present study, we found that all three types of oral stimulations activated CER6 compared with baseline (Table 1) and their BOLD signals were significantly different in CER9 (Table 3). Both CER6 and CER9 belong to the cerebellar **posterior** lobe, which have little or no connection with the cerebral cortical sensorimotor areas (Schmahmann, 2019). Instead, they are linked with areas of the cerebral cortices concerned with cognition, e.g., the prefrontal cortex. Damages to the posterior lobe involving CER4 through CER9 cause cerebellar cognitive affective syndrome (CCAS) but no motor deficit (Schmahmann, 2019). Small et al. conducted an event-related fMRI study to dissociate regions responding to taste intensity and taste affective valence (palatability) and they found that the cerebellum responded to taste intensity irrespective of affective valence (palatability) (Small et al., 2003). Unfortunately, in their study, they did not point out whether it is the posterior part or the anterior part of the cerebellum that was activated. For human olfaction, it has been found that odorants induced activation primarily in the **posterior** lateral regions and this activation was concentration-dependent. It was also observed that sniffing in the absence of an odorant induced activation primarily in the **anterior** part of cerebellum (Sobel et al., 1998). Hence, it was proposed that the cerebellum coordinates sniff volume in relation to odorant concentration. For gustation, a similar view was proposed that the cerebellum coordinates oral taste volume in relation to taste intensity (Small et al., 2003; Frost et al., 2015). Based on the present study, we extend the speculations concerning the roles of cerebellum in gustation such that the cerebellum (CER9) plays a role in identifying different oral stimulations by linking to the cerebral cortices concerned with cognition.

The right dorsolateral superior frontal gyrus (rSFGdl): The SFGdl corresponds to the dorsolateral part of Brodmann area 8 and 9 (BA 8 and BA 9) which are involved in cognitive control and **memory** processing (Petrides, 2000; Watanabe, 2017). ‘‘Gustatory imagery", thinking about taste in the absence of actual taste stimuli, activates the frontal gyri (Kobayashi et al., 2004). According to Kobayashi et al., the middle and superior frontal gyri participate in the generation of gustatory hallucinations by the retrieval of gustatory information from the storage of long-term memories and thus they are thought to mediate ‘‘top-down" control of gustatory processing. In the present study, sucrose, tannin and capsaicin activated rSFGdl at significantly different levels. This might correspond to the different memories evoked by the three different stimuli qualities in the frontal gyri, which contributes to the recognition of different taste qualities.

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The left middle temporal gyrus (MTG): The MTG has been suggested to be involved in various functions (Giraud et al., 2004; Sato et al., 2012), which do not seem to be very relevant to taste identification. However, deficiencies in taste and smell recognition abilities have been observed following temporal lobectomy (Henkin et al., 1977; Small et al., 1997). The anteromedial temporal lobe, which is close to amygdala, is thought to play a role in recognizing taste quality (Small et al., 2005). In addition, when estimating taste intensity, patients with excisions from either the left or the right anteromedial temporal lobe were also less accurate compared to a control group (Small et al., 2001).

In the traditional view of gustatory processing, the primary taste cortex – the insula – constitutes the first cortical representation of a taste quality. It is meant to process detection of a taste quality, whereas the recognition of the quality is a function ascribed to the secondary taste cortex – the orbitofrontal cortex (OFC) (Rolls, 2019). In the present study, we found three brain regions responding differently to different oral "taste" qualities, which might suggest that they play a role in identification of oral "taste" qualities. These regions, however, do not belong to either the primary or the secondary taste cortices. Instead, they are distributed in cerebellum, frontal cortex and temporal cortex, suggesting that identification of different oral qualities depends on a distributed network of brain areas.

Within the **insula**, we found overlapping regions co-activated by all three types of stimuli (Table 2). Here, one limitation of the current study has to be noted that the baseline used for analysis was a condition where participants did not receive any oral stimulation instead of a baseline corresponding to a condition where participants were presented with only solvents, that is water in our case. Therefore, the influence of solvent (water) cannot be subtracted during analysis. It has been shown that water also activates the insula in fMRI (de Araujo et al., 2003), as does the artificial saliva, used in other taste fMRI studies (Saker et al., 2014; Avery et al., 2020). Hence, the observation of overlapping brain regions (Table 2) co-activated by all three types of stimuli in the insula might be, at least to some degree, because they all had the same solvents – water. However, other human gustatory fMRI studies without the limitation mentioned above also have shown that responses in the insula occurred to all oral stimuli irrespective of their modality (somatosensory or gustatory) (Cerf-Ducastel et al., 2001; De Araujo and Rolls, 2004; Rudenga et al., 2010). This is consistent with the multi-sensitive nature of central gustatory neurons observed in primate studies, where the insula contains not only **taste-specific neurons** specifically tuned to the five basic tastes (sweet, salt, bitter, sour and umami), but also **somatosensory-specific neurons** encoding capsaicin, viscosity, fat texture and temperature, and **multimodal neurons** responding to both somatosensory and taste stimulations (Rolls, 2019). These findings agree with the view that the convergence of gustatory and oral somatosensory inputs could be present already at earlier stages of taste processing in the insula (Verhagen et al., 2004). Some authors also argue that the gustatory and oral somatosensory systems are widely overlapping within the central nervous system (Simon et al., 2008). In the *introduction*, we have mentioned that gustation is defined as the sensation that results from the direct stimulation of the "gustatory receptor" whereas somatosensations correspond to stimulations directly acting on somatosensory receptors (e.g., mechanoreceptors, thermoreceptors and nociceptors). If a stimulant has the potential to activate multiple receptors, the definition of a stimulant will be complicated. For example, salts and acids in moderate-to-high concentrations, are thought to evoke somatosensory sensations (Green and Gelhard, 1989; Green and Lawless, 1991). Consequently, a "taste" stimulus can have both gustatory and oral somatosensory components (Rudenga et al., 2010). In this perspective, whether the "oral astringency" is a taste or an oral sematosensation or both should depend on more studies regarding the peripheral receptors involved. In addition, it has to be noted that in the present study, the pungent stimulus was not a purely capsaicin stimulus but a capsaicin-ethanol mixture because capsaicin was needed to be dissolved with a small amount of ethanol first, although both ethanol and capsaicin activate transient receptor potential channels for vanilloid (TRPV1) and then produce similar pungent sensations (Simon and Gutierrez, 2017; Leijon et al., 2019; Gutierrez and Simon, 2021).

In studies on primates, the firing rate (spikes/s) of single neuron in the insula can be recorded by microelectrodes during oral taste stimulations. It has been found that an neuron of insular taste cortex (bo139c2, the name of the neuron) responded to different taste stimuli (including glucose – sweet, quinine – bitter, sodium chloride (NaCl) – salty, hydrochloric (HCl) – sour, monosodium glutamate – umami) with significantly different levels of firing rates (Verhagen et al., 2004), suggesting that the insula plays a role in the identification of taste qualities. Similar to the insula, neurons in the secondary taste cortex – the OFC – also respond to different taste qualities and other types of oral stimulations, e.g., capsaicin and oils, with significantly different firing rates (Rolls et al., 2003). However, this phenomenon at the level of single neuron cannot be directly detected at the macroscopic level, as in our case, with the fMRI-based BOLD signals. Most human fMRI studies including the present one failed to observe significantly different BOLD signals to different "taste" stimuli within the gustatory cortices – insula and OFC. One possibility was that most human fMRI studies relied on small sample size and relatively low spatial resolution (Avery et al., 2020). Another possibility is that taste representations may not be uniform from one subject to another and thus significant results cannot be observed at the group level (Schoenfeld et al., 2004).

In the *introduction*, we briefly introduced two **spatial** taste coding models, that is the **topographic model** and **population coding model**. However, gustatory processing in the central nervous system is viewed as interactive distributed neural networks of feedforward and feedback pathways, arranged in series, in parallel and recurrently (Katz et al., 2002; Jones et al., 2006;

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Lemon and Katz, 2007; Fonseca et al., 2018). Hence, the temporal feature, i.e., time, matters in taste coding. One fact is that rodents can demonstrate recognition of a taste within 200 ms (Halpern and Tapper, 1971; Boughter et al., 2002; Perez et al., 2013). Humans with normal taste function also can respond to a change in taste in about 400 ms (Halpern, 1986). Nevertheless, the temporal resolution of fMRI is poor. It typically lies between 5 and 8 s (Faurion et al., 2005). This limited temporal resolution could also be one of the reasons why human fMRI studies failed to verify the results from primate studies.

To see whether the brain responses to ‘‘taste” stimulations were stable over time within subjects in this fMRI experiment, we examined the brain activations of a subset of participants, who were scanned in two separate visits with an average interval of 18 days. The functional imaging data were compared between two visits at the group level (Table 4). Two clusters respectively in the left orbital part of inferior frontal gyrus and right lingual gyrus were activated stronger in the first visit than in the second. The orbital part of inferior frontal gyrus contains Brodmann area 47 which exerts a prominent function in language processing and comprehension (Ardila et al., 2017). The lingual gyrus belongs to the visual cortex which plays a role in reading words (Mechelli et al., 2000). In the fMRI scanner, the participants needed to see a screen with words guiding them whether to be still, move their mouths, or swallow the liquids. When being scanned in the second visit, they had already experienced how to behave in the scanner as guided by the screen in the first visit. This adaptation to the situation might be the cause of these differences observed in fMRI analysis. In any case, brain regions that we thought to respond to ‘‘taste” stimulants, such as insula, showed no significant difference between the two visits on a group level. Hence, we suggested that brain responses to ‘‘taste” stimulations were relatively stable over time within subjects when doing group analysis in this fMRI experiment.

In summary, we observed three brain regions distributed in the cerebellum, frontal cortex and temporal cortex which responded differently to the three types of oral stimulations, suggesting that brain regions outside the primary and the secondary taste cortices also contribute to the identification of different taste solutions in the mouth.

AUTHOR CONTRIBUTIONS

TH and YZ concepted the idea. YZ drafted the article. YZ and DT collected the data. YZ and PH analyzed the data. All authors revised it critically for intellectual content and approved the final version of the manuscript.

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DECLARATIONS OF INTEREST

None.

AVAILABILITY OF DATA AND MATERIAL

All data and material are available upon request.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroscience.2023.03.011.

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Discussion and Outlook

In our first study (Publication 1), we observed no significant changes on taste and smell function after one-year zinc therapy using paired t tests. However, the sample size (n = 14) was too small to evaluate the curative effect of oral zinc therapy, also, we were unable to receive a precise documentation of the dose of zinc treatment from each patient. However, zinc therapy was not the primary interest in the present study. We selected subjects with taste disorders treated with zinc because taste function is more likely to change under zinc therapy (Shatzman & Henkin, 1981; Henkin et al., 1999a). The focus of the present study was to explore the associations between changes of taste function and changes of salivary parameters instead of how zinc therapy would affect taste function or saliva parameters.

In our study, we found that Δ salivary flow rate was negatively correlated with Δ taste strip scores, indicating that when the salivary flow rate increased, the taste strips scores would decrease, and vice versa. This is in accordance with our previous cross-sectional research, that is, the taste strip score correlated negatively with the salivary flow rate, and patients with taste disorders exhibited a higher salivary flow rate compared to healthy controls (Walliczek-Dworschak et al., 2017a).

When we reviewed other studies investigating the relationship between taste function and salivary flow rate, we did not find a uniform picture. Gustatory loss can be accompanied by increased, decreased, or unchanged salivary flow rates (Walliczek-Dworschak et al., 2017a) and the correlations has been reported to vary for different taste qualities. For example, one study (Heinzerling et al., 2011) showed that there was a negative correlation between salt perception and salivary flow rate, whereas no correlation was found for bitterness or sweetness, and contradictory results were reported for sourness. Other studies showed that bitter taste sensitivity correlates positively with unstimulated saliva flow rate (Marquezin et al., 2016). In addition, also a negative correlation has been observed for sourness and salivary flow rate (Heinzerling et al., 2011).

One reason for this inconsistency of results may relate to the different methods used in the respective studies. For example, results may differ when taste function was evaluated by self-ratings or chemosensory tests, whether salivary flow rate was assessed as stimulated or unstimulated, or whether participants were healthy or patients with taste disorders. Hence, when considering the relationship between salivary flow rate and taste function, individual taste qualities (sweet, salt, sour, bitter) could be studied and discussed separately using comparable techniques in future studies (unfortunately, individual taste qualities could not be analysed in a meaningful way in the present study). Moreover, considering that the

salivary pH and the salivary buffer capacity are highly dependent on the salivary flow rate (they increase when the salivary flow rate increases and vice versa), (Gron & Messer, 1965; Dawes, 1969; Bardow et al., 2000; Bardow et al., 2001) there could be also an optimal range of salivary flow rate for the best taste sensitivity.

Salivary pH is maintained at a relatively constant level, i.e., 6.5-7.4, buffering acids and thereby diminishing the rate of dental demineralisation (Ericsson, 1959; Bardow et al., 2000). Several previous investigations showed that salivary pH interacts with the salivary flow rate and is important for sour (Norris et al., 1984; Christensen et al., 1987; Lugaz et al., 2005) and sweet taste perception (Matsuo & Yamamoto, 1992; Aoyama et al., 2017). In the present study, Δ salivary pH was found to be significantly different between two groups - salivary pH tended to increase in no-group while it decreased in the im-group (Table 4, Publication 1), indicating that the increased taste sensitivity might be accompanied by decreased salivary pH during the zinc therapy. However, more research is needed to confirm this tendency.

In some previous investigations, proteolytic activity of human saliva plays a role in the perception of bitter, fatty, and salty stimuli (Dsamou et al., 2012; Mounayar et al., 2013; Stolle et al., 2018) and enhanced in-mouth proteolysis is a key peri-receptor factor associated with higher gustatory sensitivity (Dsamou et al., 2012). One hypothesis is that the mucosal pellicle forms a barrier that controls the accessibility of tastants to the receptors (Dsamou et al., 2012). A thinner or looser pellicle due to higher proteolytic activity would then be associated with a facilitated tastant-taste receptor interaction (Dsamou et al., 2012). In the present study, for the no-group whose taste strip scores decreased, their salivary proteolytic activity also decreased combined with increased salivary total protein (Table 3, Publication 1), which support this hypothesis (Dsamou et al., 2012). However, we also observed an increased salivary total protein without concomitantly decreased salivary proteolysis in the im-group whose taste strip scores increased (Table 3, Publication 1). This difference might be explained by the small sample size in the im-group (n = 5) which did not reflect the significant changes in proteolysis. The overall contradictory results on the relation between taste function and salivary proteolysis might also be interpreted in light of the differences between correlations with individual taste qualities (sweet, salt, sour, bitter). In our study, the taste strip score represents the combined function of the four basic tastes, but the negative correlation may exist only between salivary proteolysis and a specific taste quality, such as bitter as shown in previous studies (Dsamou et al., 2012).

CaVI (gustin) in saliva has been associated with the growth and development of taste buds (Shatzman & Henkin, 1981) and a lower caVI concentration is associated with lower levels

of total parotid salivary zinc in subjects with reduced taste function. Our previous cross-sectional study also found there was a positive correlation between the caVI concentration and taste scores (Walliczek-Dworschak et al., 2017a). What we found in present study, patients in no-group showed a significant decreased caVI concentration, is also in accordance to these findings.

Although, for total patients, there was no significant change in taste function after one-year zinc therapy, the improvements of symptoms of total patients were significant. It is not unusual that the patients' subjective complaints about taste disorders symptoms are not paralleled by their objective taste capacities. Qualitative taste disorders which can only be diagnosed by self-report (Landis & Heckmann, 2004) do not have to coexist with quantitative taste disorders and for quantitative taste disorders, many individuals do not even notice their taste deficiency (Landis et al., 2005). A study on 48 patients with qualitative dysgeusia showed that two thirds experienced spontaneous resolution of the dysgeusia (evaluated by self-ratings), with an average duration of 10 months and mood state (evaluated by BDI scores) related to resolution rates (Deems et al., 1996). In the present study, we found that Δ BDI scores were positively correlated with Δ taste disorder symptom ratings, i.e., when taste disorder symptoms improved, patients' depressive symptoms also improved and vice versa. This suggests that, psychotherapy might help these patients to feel better, without necessarily improving their gustatory sensitivity.

One study investigating subjects with oral sensory complaints including burning mouth syndrome, idiopathic taste aberrations and xerostomia indicated that salivary and taste analyses were helpful in distinguishing healthy subjects from subjects with complaints (Nagler & Hershkovich, 2004). In the present study, we could observe both improvement of symptoms of taste disorders and changes of saliva-related parameters of total patients (Table 3, Publication 1). However, the sample size was small and a healthy control group was missing. Hence, we cannot conclude that the improvement of complaints could be reflected by saliva-related parameters. Still, the results suggest that salivary parameters may be useful in the distinction between healthy subjects and patients with qualitative taste disorders and thus call for more investigations on saliva testing as an objective measurement to evaluate either taste dysfunction or taste complaints.

Because of the high rate of dropouts, there are only 14 samples in the present study and the aetiologies and subtypes of their taste disorders are heterogeneous. As is shown in Publication1, Table 2, the first patient's hypogeusia is reported after a surgery while other patients' taste disorder (either dysgeusia or hypogeusia or both) are idiopathic. Theoretically, saliva parameters could change in order to compensate for the recovery of

taste function (secondary change). However, changes of salivary parameters could also be the primary cause of taste disorders (primary change). Samples in current study exhibit heterogeneity but they are too small to be divided into subgroups which could be analysed separately. Thus, based on the present work, several directions can be suggested for future studies to investigate the association of changes between saliva-related parameters and taste function. At first, larger sample sizes are needed. The presently observed changes in saliva-related parameters such as total protein, proteolysis, salivary flow rate, and pH, could be the priorities to be studied. More patients with taste disorders with different aetiologies and subtypes should be included. Healthy controls plus a group of untreated patients would also be desirable. As mentioned above, studies found that individual taste qualities could be influenced by saliva differently (Heinzerling et al., 2011; Marquezin et al., 2016). Hence, the suggestion would be to not only measure general function, but also measure sensitivity for specific taste qualities (sweet/sour/salty/bitter) using validated assessment tools. At last, when measuring taste function with psychophysical tests, it is also important to record taste complaints with symptom scales so that the association between taste complaints and salivary parameters could be studied in greater detail.

In our second study (Publication 2), on a group level, a weaker functional connection between right and left OFC was observed in patients with taste loss compared to healthy controls during taste stimulations. In primates, OFC receives direct inputs from the primary taste cortex - IC (Baylis et al., 1995) and neurons in the OFC can respond to the prototypical tastes (Rolls et al., 1990; Baylis & Rolls, 1991). Human neuroimaging studies also showed that the OFC can be activated by gustatory stimuli (O'Doherty et al., 2001; Zald et al., 2002; Rolls, 2008). Hence, the OFC is considered to contain the secondary taste cortex in humans (Rolls, 2019). It also plays an important role in integrating gustation with retronasal olfaction and oral somatosensation into a “flavor” (Small et al., 2007). The right and left OFCs are anatomically separated in two hemispheres. In the previous study (Hummel et al., 2007) of our laboratory, when participants receiving taste stimulations, activations of the right and left OFCs in the patient group tended to be stronger than that in control group. Interestingly, at the same situation, the functional connectivity between right and left OFCs was weaker in patient-group compared to control group based on the FCA in the present study.

To our knowledge, no other study has investigated how brain functional connectivity changes after a long-term taste loss. However, there was a study investigating the effects of chronic *peripheral* olfactory loss on brain functional connectivity where patients with long-

term peripheral olfactory loss and healthy controls were asked to do a sniffing task in the MRI scanner. The FCA revealed that compared to healthy controls, patients with olfactory loss showed a decrease in functional connectivity (Kollndorfer et al., 2015a) involving the anterior prefrontal cortex, the anterior cingulate cortex, the entorhinal cortex and the cerebellum (Kollndorfer et al., 2015a). In other words, long-term peripheral olfactory loss is associated with decreased functional connectivity among the brain regions relevant to olfactory processing. This is consistent with what we have found on our patients with long-term taste loss.

Generally, there were two types of task conditions in the present study that is taste condition and water condition. Taste condition is a condition that participants were receiving sweet or sour taste stimulations (0.1ml sweet/sour solutions). Water condition is a condition that participants were perceiving purely water (0.1ml water). Importantly, water has been mentioned as an independent taste modality (Rosen et al., 2010). The functional connectivity of ROIs has been compared between taste and water conditions but there were no significant differences between two conditions at a group level. This might be because the sample size was too small to show the significant difference; or the difference between cerebral processing of taste solutions and that of pure water does not manifest itself in the level of functional connectivity.

Interestingly, we observed, when participants perceiving purely water (in water condition), a weaker functional connectivity between the left frontal pole (lFP) and the left superior frontal gyrus (lSFG) in the patient group compared to the control group. FP contains areas associated with many higher cognitive functions such as drawing analogies and making plans (Fuster, 2002; Bludau et al., 2014). The SFG is generally thought as a core brain region in cognitive control systems (Niendam et al., 2012). Cognitive functions are expected to modulate taste-related activations in gustatory cortices (Jones et al., 2006). The decrease in functional connectivity between brain regions relevant to cognition may contribute to the perceived taste loss. Several studies (Steinbach et al., 2010; Sakai et al., 2017; Contri-Degiovanni et al., 2020) have demonstrated that people with cognitive impairments or with dementia in the early stage exhibited significant impairments of taste sensitivity in comparison to age-matched healthy controls. These findings suggest a close relation between taste function and cognition. Unfortunately, the cognitive abilities of our participants were not evaluated at that time. Nevertheless, the finding seems to emphasize the association between taste function and cognition. Cognitive functions should receive more attention when patients complain about taste loss, especially in idiopathic taste loss.

In this perspective, fMRI could be an available tool to follow up patients and evaluate brain changes at the level of functional connectivity.

At an individual level, each participant exhibited a unique pattern of ROI-to-ROI functional connectivity (RRC) matrix. This was true for patients and controls. This individual variation was expected because sensory systems are highly plastic (Goldstone, 1998; Kollndorfer et al., 2015a) at both cellular (Cadiou et al., 2014) and cognitive levels (Bende & Nordin, 1997), which relates to learning/training experiences (Gilbert & Sigman, 2007; Kollndorfer et al., 2015b). As mentioned above, at a group level, we found a weaker functional connectivity between the lFP and the lSFG in the patient group compared to the control group. In individual level, we found that z-scores corresponding to the lFP – lSFG connection were more than 0.25 in 9 out of 12 healthy controls but were less than or equal to 0.25 in all patients with taste loss (Table 4, Publication 2). This provides an impression that there might be a useful criterion regarding the z-scores that could differentiate patients with taste loss from healthy controls. This impression suggests the possibility that fMRI might help diagnosing taste loss as an additional tool in future by focusing on specific functional connections.

However, the shortcoming of the present study is not only the small sample size but also the uncertainty and inconsistency regarding the etiology of taste loss. Regarding the seven patients in our study, three patients claimed that their taste losses started after head traumas. However, their structural MR scans did not show any lesions that could be related to the taste loss in the brain. Three patients claimed that their taste loss began after infections of the upper respiratory tract (also with no lesions visible in structural brain imaging). The remaining patient had idiopathic taste loss. The common characteristic of these patients was the long-term taste loss and the impaired ability to identify taste qualities based on psychophysical taste testing. So far, we could only suggest that symptoms of taste loss as well as impaired taste identification ability are associated with decreased central functional connectivity between some brain regions. We cannot make certain whether the symptoms of taste loss cause the decreased functional connectivity of the brain regions or the other way around. In addition, the sample size of the current study was too small. Introducing age and gender as covariates further reduced the power of analysis. Hence, we framed the study as exploratory and hope these observations might provide some useful references for other researchers to design related studies. Future studies with a larger sample size, age/gender-matched controls and with subgroups, e.g., 1) a subgroup including patients with long-term peripheral taste loss and 2) a subgroup including patients with idiopathic taste loss (possibly related to earlier central cognitive damages), should

elucidate the possible causal relationship of the decreased functional connectivity of brain regions and the symptom of taste loss.

In our third study (Publication 3), the main aim was to investigate human brain responses to three types of oral stimuli, sweet (sucrose), astringent (tannin) and pungent (capsaicin) solutions within the same experimental framework. To achieve this, we measured brain activations using fMRI during sweet, astringent, and pungent oral stimulations. We found that activation levels (BOLD signals) for the three types of oral stimuli within the primary taste cortex, the insula, were similar to each other. In contrast, significant differences of BOLD signals to the three stimuli were found in three separated brain regions located respectively in cerebellum_9 (Lobule IX of cerebellar hemisphere, CER9), right side of dorsolateral superior frontal gyrus (rSFGdl) and left side of middle temporal gyrus (lMTG) (Table 3, Figure 5 and 6, Publication 3). The sensory attributes of one tastant include taste quality, taste palatability and taste intensity (Gutierrez & Simon, 2021) and these three distinct attributes are thought to be processed by different brain circuits (Breslin, 2013; Perez et al., 2013; Wallroth & Ohla, 2018). The intensities of three types of oral solutions in the present study were rated as iso-intense in the pilot experiment; nevertheless, they were rated as different in the formal experiment in the MRI scanner with sucrose solutions perceived as more intense than tannin and capsaicin solutions. Subjective-rated intensity of a solution might be influenced by circumstance, and being in the MRI scanner is such a different circumstance (Kishi et al., 2017). To handle this situation, we introduced the ratings of pleasantness (palatability) and intensity of each individual in the MRI as covariates into the ANOVA. Hence, we suggest that the significant differences of BOLD signals among the stimuli according to the ANOVA in the present study were mainly related to the differences of the qualities of the three types of oral stimuli. Based on this, we speculate that these three regions located in CER9, rSFGdl and lMTG play a role in the recognition and discrimination of sucrose, tannin and capsaicin solutions. In the following we will discuss the significances of the three brain regions regarding taste processing.

Cerebellar lobule IX (CER9): There are previous studies already showing that the cerebellum was activated by taste stimulations (Barry et al., 2001; O'Doherty et al., 2001; Small et al., 2003). However, it is not clear whether the cerebellar response contributed to taste identification. In the present study, we found that all three types of oral stimulations activated CER6 compared with baseline (Table 1, Publication 3) and their BOLD signals were significantly different in CER9 (Table 3, Publication 3). Both CER6 and CER9 belong to the cerebellar **posterior** lobe, which have little or no connection with the cerebral cortical

sensorimotor areas (Schmahmann, 2019). Instead, they are linked with areas of the cerebral cortices concerned with cognition, e.g., the prefrontal cortex. Damages to the posterior lobe involving CER4 through CER9 cause cerebellar cognitive affective syndrome (CCAS) but no motor deficit (Schmahmann, 2019). Small et.al. conducted an event-related fMRI study to dissociate regions responding to taste intensity and taste affective valence (palatability) and they found that the cerebellum responded to taste intensity irrespective of affective valence (palatability) (Small et al., 2003). Unfortunately, in their study, they did not point out whether it is the posterior part or the anterior part of the cerebellum that was activated. For human olfaction, it has been found that odorants induced activation primarily in the **posterior** lateral regions and this activation was concentration-dependent. It was also observed that sniffing in the absence of an odorant induced activation primarily in the **anterior** part of cerebellum (Sobel et al., 1998). Hence, it was proposed that the cerebellum coordinates sniff volume in relation to odorant concentration. For gustation, a similar view was proposed that the cerebellum coordinates oral taste volume in relation to taste intensity (Small et al., 2003; Frost et al., 2015). Based on the present study, we extend the speculations concerning the roles of cerebellum in gustation such that the cerebellum (CER9) plays a role in identifying different oral stimulations by linking to the cerebral cortices concerned with cognition.

The right dorsolateral superior frontal gyrus (rSFGdl): The SFGdl corresponds to the dorsolateral part of Brodmann area 8 and 9 (BA 8 and BA 9) which are involved in cognitive control and memory processing (Petrides, 2000; Watanabe, 2017). “Gustatory imagery”, thinking about taste in the absence of actual taste stimuli, activates the frontal gyri (Kobayashi et al., 2004). According to Kobayashi et al., the middle and superior frontal gyri participate in the generation of gustatory hallucinations by the retrieval of gustatory information from the storage of long-term memories and thus they are thought to mediate “top-down” control of gustatory processing. In the present study, sucrose, tannin and capsaicin activated rSFGdl at significantly different levels. This might correspond to the different memories evoked by the three different stimuli qualities in the frontal gyri, which contributes to the recognition of different taste qualities.

The left middle temporal gyrus (MTG): The MTG has been suggested to be involved in various functions (Giraud et al., 2004; Sato et al., 2012), which do not seem to be very relevant to taste identification. However, deficiencies in taste and smell recognition abilities have been observed following temporal lobectomy (Henkin et al., 1977; Small et al., 1997). The anteromedial temporal lobe, which is close to amygdala, is thought to play a role in recognizing taste quality (Small et al., 2005). In addition, when estimating taste intensity,

patients with excisions from either the left or the right anteromedial temporal lobe were also less accurate compared to a control group (Small et al., 2001).

In the traditional view of gustatory processing, the primary taste cortex – the insula – constitutes the first cortical representation of a taste quality. It is meant to process detection of a taste quality, whereas the recognition of the quality is a function ascribed to the secondary taste cortex – the orbitofrontal cortex (OFC) (Rolls, 2019). In the present study, we found three brain regions responding differently to different oral "taste" qualities, which might suggest that they play a role in identification of oral "taste" qualities. These regions, however, do not belong to either the primary or the secondary taste cortices. Instead, they are distributed in cerebellum, frontal cortex and temporal cortex, suggesting that identification of different oral qualities depends on a distributed network of brain areas.

Within the insula, we found overlapping regions co-activated by all three types of stimuli (Table 2, Publication 3). Here, one limitation of the current study has to be noted that the baseline used for analysis was a condition where participants did not receive any oral stimulation instead of a baseline corresponding to a condition where participants were presented with only solvents, that is water in our case. Therefore, the influence of solvent (water) cannot be subtracted during analysis. It has been shown that water also activates the insula in fMRI (de Araujo et al., 2003), as does the artificial saliva, used in other taste fMRI studies (Saker et al., 2014; Avery et al., 2020). Hence, the observation of overlapping brain regions (Table 2, Publication 3) co-activated by all three types of stimuli in the insula might be, at least to some degree, because they all had the same solvents – water. However, other human gustatory fMRI studies without the limitation mentioned above also have shown that responses in the insula occurred to all oral stimuli irrespective of their modality (somatosensory or gustatory) (Cerf-Ducastel et al., 2001; De Araujo & Rolls, 2004; Rudenga et al., 2010). This is consistent with the multi-sensitive nature of central gustatory neurons observed in primate studies, where the insula contains not only **taste-specific neurons** specifically tuned to the five basic tastes (sweet, salt, bitter, sour and umami), but also **somatosensory-specific neurons** encoding capsaicin, viscosity, fat texture and temperature, and **multimodal neurons** responding to both somatosensory and taste stimulations (Rolls, 2019). These findings agree with the view that the convergence of gustatory and oral somatosensory inputs could be present already at earlier stages of taste processing in the insula (Verhagen et al., 2004). Some authors also argue that the gustatory and oral somatosensory systems are widely overlapping within the central nervous system (Simon et al., 2008). In the *introduction*, we have mentioned that gustation is defined as the sensation that results from the direct stimulation of the "gustatory receptor" whereas

somatosensations correspond to stimulations directly acting on somatosensory receptors (e.g., mechanoreceptors, thermoreceptors and nociceptors). If a stimulant has the potential to activate multiple receptors, the definition of a stimulant will be complicated. For example, salts and acids in moderate-to-high concentrations, are thought to evoke somatosensory sensations (Green & Gelhard, 1989; Green & Lawless, 1991). Consequently, a “taste” stimulus can have both gustatory and oral somatosensory components (Rudenga et al., 2010). In this perspective, whether the “oral astringency” is a taste or an oral sematosensation or both should depend on more studies regarding the peripheral receptors involved.

To see whether the brain responses to “taste” stimulations were stable over time within subjects in this fMRI experiment, we examined the brain activations of a subset of participants, who were scanned in two separate visits with an average interval of 18 days. The functional imaging data were compared between two visits at the group level (Table 4, Publication 3). Two clusters respectively in the left orbital part of inferior frontal gyrus and right lingual gyrus were activated stronger in the first visit than in the second. The orbital part of inferior frontal gyrus contains Brodmann area 47 which exerts a prominent function in language processing and comprehension (Ardila et al., 2017). The lingual gyrus belongs to the visual cortex which plays a role in reading words (Mechelli et al., 2000). In the fMRI scanner, the participants needed to see a screen with words guiding them whether to be still, move their mouths, or swallow the liquids. When being scanned in the second visit, they had already experienced how to behave in the scanner as guided by the screen in the first visit. This adaptation to the situation might be the cause of these differences observed in fMRI analysis. In any case, brain regions that we thought to respond to “taste” stimulants, such as insula, showed no significant difference between the two visits on a group level. Hence, we suggested that brain responses to “taste” stimulations were relatively stable over time within subjects when doing group analysis in this fMRI experiment.

Summary in German

Hintergrund

„Gustation“ bezieht sich auf die Empfindung, die durch die direkte Stimulation der Schmeckrezeptoren in den Schmeckknospen entsteht. Ob ein Lebensmittel im Mund geschluckt werden sollte, wird hauptsächlich aufgrund der Schmeckempfindung entschieden. So sind zum Beispiel süße, salzige und umami-Geschmäcker appetitanregend, während bittere Geschmäcker aversiv sind und eher abgelehnt werden. Schmeckstörungen führen häufig zu negativen Auswirkungen auf die Essgewohnheiten und den Ernährungszustand, was sich wiederum auf die menschliche Gesundheit auswirken kann. Der Speichel spielt eine wesentliche Rolle bei der Schmeckwahrnehmung. Es gibt nach wie vor viele offene Fragen in Bezug auf das Schmecksystem, z. B. die Beziehung zwischen Speichelparametern und Schmeckfunktion und die zerebrale Verarbeitung von oralen Schmeckreizen.

Hypothese/Fragestellung

In Publikation 1 untersuchten wir die Hypothese, dass Veränderungen der Schmeckfunktion von Veränderungen der Speichelparameter begleitet werden. Die Frage war, ob sich die Verbesserung oder Verschlechterung von Schmeckstörungen in Veränderungen der Speichelparameter widerspiegeln könnte. Wir gingen davon aus, dass sich mit der Verbesserung oder Verschlechterung der Schmeckfunktion von Patienten mit Schmeckstörungen nach einer einjährigen Behandlung mit einer oralen Zinktherapie auch einige Speichelparameter der Patienten verändern werden.

In Publikation 2 stellten wir die Hypothese auf, dass sich die funktionellen Verbindungen zwischen einigen Hirnregionen bei Patienten mit Schmeckverlust von denen gesunder Kontrollpersonen mit normaler Schmeckfunktion während oraler Schmeckstimulationen unterscheiden. Ziel dieser Studie ist es, die Unterschiede der funktionellen Verbindungen zwischen anatomisch getrennten Hirnregionen von Patienten mit Schmeckverlust und gesunden Menschen mit normaler Schmeckfunktion zu untersuchen.

In Publikation 3 stellten wir die Hypothese auf, dass bei gesunden Probanden mit normaler Schmeckfunktion die Reaktionen in bestimmten Hirnregionen unterschiedlich ausfallen würden, wenn sie verschiedene orale Reize erhalten, nämlich süße (Saccharose), adstringierende (Tannin) und scharfe (Capsaicin) Lösungen. Eine weitere Hypothese war, dass die drei verschiedenen oralen Reize eine Unterregion in der Insula ko-aktivieren kwürden. Mit dieser Studie sollte untersucht werden, welche Hirnregionen durch orale süße (Saccharose), adstringierende (Tannin) und scharfe (Capsaicin) Lösungen in gesunden menschlichen Gehirnen aktiviert werden und ob die jeweiligen Aktivierungsniveaus unterschiedlich sind.

Material und Methoden

In Publikation 1 nahmen vierzehn Patienten mit Schmeckstörungen (6 Männer, 8 Frauen) sowohl an der ersten Sitzung (Ausgangswerte) als auch an der Nachuntersuchung teil. In der ersten Sitzung wurden die Speichelparameter (Flussrate, Gesamtproteine, Proteolyse, Katalase, gesamte antioxidative Kapazität [TAC], Kohlensäureanhydrase VI [caVI] und Speichel-pH) und die Schmeckfunktion mit "Schmeckstreifen" bestimmt. Außerdem erfassten wir die Intensität der Symptome mit Hilfe visueller Analogskalen und des Beck-Depressions-Inventars (BDI). Anschließend nahmen die Patienten ein Jahr lang eine orale Zinktherapie zur Behandlung ihrer Schmeckstörungen ein. Nach einem Jahr wurden die Speichelparameter und die Schmeckfunktion erneut mit denselben Methoden untersucht. Die Ergebnisse der Schmeckuntersuchungen und der Speichelparameter wurden zwischen den beiden Besuchen verglichen.

In Publikation 2 wählten wir 26 Paare von Hirnregionen, die mit der Schmeckverarbeitung in Verbindung stehen, als Interessengebiete (ROIs) aus. Mit Hilfe der funktionellen Magnetresonanztomographie (fMRT) wurden die Gehirnreaktionen von 7 Patienten (5 Frauen, 2 Männer) mit Schmeckverlust und 12 gesunden Kontrollpersonen (6 Frauen, 6 Männer) gemessen, während sie Schmeckreize (Schmeckbedingung) und Wasser (Wasserbedingung) erhielten. Die Daten wurden mittels ROI-zu-ROI-Analyse der funktionellen Konnektivität (FCA) mit der CONN-Toolbox analysiert. Die funktionellen Verbindungen wurden zwischen der Patientengruppe und der gesunden Kontrollgruppe in beiden Bedingungen verglichen.

In Publikation 3 wurden vierundzwanzig gesunde Teilnehmer (10 Männer, 14 Frauen) einbezogen. Alle unterzogen sich drei fMRT-Messungen, um ihre Gehirnaktivierungen aufzuzeichnen. Bei jeder fMRT-Messung wurde den Teilnehmern eine der drei Arten von oralen Reizen (Saccharose-, Tannin- und Capsaicin-Lösungen) in den Mund gegeben. Die durch die drei Arten von oralen Stimuli ausgelösten Hirnaktivierungen wurden in einem Inner-Subjekt-Design verglichen.

Ergebnisse

Bei Publikation 1 wiesen Patienten mit eingeschränkter Schmeckfunktion einen Rückgang der Speichelproteolyse und des caVI sowie einen Anstieg des Gesamtproteins im Speichel auf. Patienten mit erhöhter Schmeckfunktion wiesen ebenfalls einen Anstieg des Speichelproteins auf. Die Δ Speichelflussrate war negativ mit den Δ-Schmeckstreifenwerten korreliert. Der Δ Speichel-pH-Wert war bei Patienten mit erhöhter Schmeckfunktion signifikant niedriger als bei Patienten mit verminderter Schmeckfunktion. Der Δ BDI korrelierte positiv mit beiden Δ-Symptomwerten. Bei allen Patienten nahmen die Symptombewertungen ab, während das Gesamteiweiß im Speichel anstieg; Speichelflussrate, Proteolyse und caVI nahmen im Vergleich zum Ausgangswert signifikant ab.

Für Publikation 2 beobachteten wir auf Gruppenebene in der Patientengruppe eine schwächere funktionelle Konnektivität zwischen dem linken und rechten orbitofrontalen Kortex in der Schmeckbedingung ($T(17) = 6,79$, Verbindungsschwelle: $p < 0,05$, p-FWE korrigiert) und zwischen dem linken frontalen Pol und dem linken superioren frontalen Gyrus in der Wasserbedingung ($T(17) = 5,16$, Verbindungsschwelle: $p < 0,05$, p-FWE korrigiert).

Bei Publikation 3 beobachteten wir drei verteilte Hirnunterregionen, die sich jeweils im Lobulus IX der Kleinhirnhemisphäre, auf der rechten Seite des dorsolateralen Gyrus frontalis superior und auf der linken Seite des Gyrus temporalis middle befanden und die signifikant unterschiedlich auf die drei Arten von oralen Stimulationen reagierten ($p < 0,001$, p-unkorrigiert, Clustergröße > 50).

Schlußfolgerungen

Veränderungen der gemessenen Schmeckfunktion sowie der von den Patienten berichteten Schmeckbeschwerden gingen mit Veränderungen der Speichelparameter einher. Es zeiget sich also, dass Speichelparameter bei der Diagnose von Patienten mit Schmeckstörungen nützlich sein können, und dass die Speicheluntersuchung bei der Untersuchung von Schmeckstörungen bedeutsam ist. Die Analyse der funktionellen Konnektivität auf der Grundlage von fMRT-Daten ergab Unterschiede auf Gruppenebene zwischen Patienten mit Schmeckstörungen und Gesunden. Mit der fMRT konnte die unterschiedlichen Gehirnreaktionen auf verschiedene orale Stimulationen bei gesunden Probanden untersucht werden. Die Ergebnisse deuten darauf hin, dass fMRT ein nützliches Instrument zur Untersuchung der zerebralen Verarbeitung von Schmeckreizen ist und bei der Bewertung von Schmeckstörungen hilfreich sein könnte.

Summary in English

Background

Gustation or taste sensation refers to the sensation that results from the direct stimulation of the gustatory receptors residing in taste buds. Whether a food in the mouth should be swallowed is determined mostly based on gustation (taste sensation). For example, sweet, salty and umami tastes are appetizing, while bitter tastes are aversive and likely to be rejected. Taste disorders often lead to negative effects on eating habits and nutritional status which consequently may affect human health conditions. Saliva plays an essential role in taste perception. There remain lots of mysteries regarding gustatory system, e.g., the relation between salivary parameters and taste function and the cerebral processing of oral taste stimulations.

Hypothesis/Question

In Publication 1, we hypothesized that changes of taste function are accompanied by changes of salivary parameters. The question was that could the improvement or deterioration of taste disorders be reflected by changes on salivary parameters? We assumed that as the taste function of patients with taste disorders improve or decline after one-year-time treated with oral zinc therapy, some salivary parameters of patients will concomitantly alter.

In Publication 2, we hypothesized that the functional connections between some brain regions in patients with taste loss are different from that in healthy controls with normal taste function during oral taste stimulations. This research aims to explore the differences of functional connections of anatomically separated brain regions between patients with taste loss and healthy people with normal taste function.

In Publication 3, we hypothesized that for healthy subjects with normal taste function, brain responses in some brain regions would be different when receiving different oral stimulations, that is sweet (sucrose), astringent (tannin) and pungent (capsaicin) solutions. We also hypothesized that three different taste solutions could co-activate a sub-region in insula. This research aims to investigate which brain regions are activated by oral sweet

(sucrose), astringent (tannin) and pungent (capsaicin) solutions in the healthy human brains and whether the respective activation-levels are different.

Materials and methods

In Publication 1, fourteen patients with taste disorders (6 males, 8 females) participated in both the first session (baseline) and the return visit session. At their first session, we assessed their salivary parameters (flow rate, total proteins, proteolysis, catalase, total anti-oxidative capacity [TAC], carbonic anhydrase VI [caVI], and salivary pH) and taste function with “taste strips”. We also recorded their symptoms’ intensities using visual analogue scales and the Beck Depression Inventory (BDI) scores. Then, patients were taking oral zinc therapy to treat their taste disorders for one year. After one year, their salivary parameters and taste function were evaluated again with the identical methods. Comparisons regarding taste scores and salivary parameters were made between two visits.

In Publication 2, we selected 26 pairs of brain regions related to taste processing as our regions of interests (ROIs). Functional magnetic resonance imaging (fMRI) was used to measure brain responses in 7 patients (5 females, 2 males) with taste loss and 12 healthy controls (6 females, 6 males) as they received taste stimulations (taste condition) and water (water condition). The data was analyzed using ROI-to-ROI functional connectivity analysis (FCA) with CONN toolbox. The functional connections were compared between patient group and healthy control group in both conditions.

In Publication 3, twenty-four healthy participants (10 males, 14 females) were included. All of them underwent three fMRI measurements to record their brain activations. In each fMRI measurement, one of the three types of oral stimuli (sucrose, tannin and capsaicin solutions) were presented to the mouth of participant. Brain activations stimulated by three types of oral stimuli were compared in a within-subject design.

Results

For Publication 1, patients with decreased taste function exhibited a decrease in salivary proteolysis and caVI, and an increase in salivary total protein. Patients with increased taste

function also showed an increase in salivary total protein. Δ Salivary flow rate was negatively correlated with Δ taste strip scores. Δ Salivary pH was significantly lower in patients with increased taste function compared to patients with decreased taste function. Δ BDI was positively correlated with both Δ symptoms ratings. Across all patients, symptom ratings decreased while salivary total protein increased; salivary flow rate, proteolysis and caVI decreased significantly compared with baseline.

For Publication 2, on a group level, we observed weaker functional connectivity in the patient group between the left and right orbitofrontal cortex in the taste condition ($T(17) = 6.79$, connection threshold: $p < 0.05$, p-FWE corrected) and between the left frontal pole and the left superior frontal gyrus in the water condition ($T(17) = 5.16$, connection threshold: $p < 0.05$, p-FWE corrected) relative to control group.

For Publication 3, we observed three distributed brain sub-regions respectively located in Lobule IX of cerebellar hemisphere, right side of dorsolateral superior frontal gyrus and left side of middle temporal gyrus, which responded significantly different to the three types of oral stimulations ($p < 0.001$, p-uncorrected, cluster size > 50).

Conclusions

Changes of both taste function evaluated by psychophysical tests and taste complaints reported by patients were accompanied by changes in salivary parameters, indicating that salivary parameters have the potential to be useful in the diagnosis of patients with taste disorders and that assessment of saliva is of importance in research on taste dysfunction. Functional connectivity analysis based on fMRI data revealed differences on a group level between patients with taste disorders and healthy controls. FMRI could detect the difference of brain responses to different oral stimulations in healthy subjects. These results suggested that fMRI is a useful tool to investigate the cerebral processing of gustatory stimulations and might be helpful in evaluating taste disorders.

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