Comprehensive recordings of taste function in patients with dysgeusia – A Pilot Study

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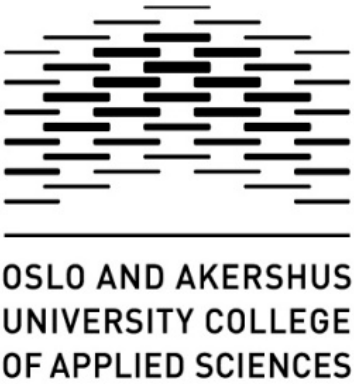

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ABBREVIATIONS

AVI	alanine-valine-isoleucine (recessive haplotype of TAS2R38 gene)
BDI-FS	Beck Depression Inventory Fast Screen
CMS	Common Mode Sense electrode
DRL	Driven Right Leg electrode
EEG	Electroencephalogram
EGM	Electrogustometry
ERP	Event-related potentials
FDP	Fungiform papillae density
fMRI	Functional Magnetic Resonance Imaging
gERP	Gustatory event-related potentials
ISI	Inter-stimulus interval
LPA	Left pre-auricular point
PAV	proline-alanine-valine (dominant haplotype of TAS2R38 gene)
PROP	N-Propylthiouracil
RPA	Right pre-auricular point
TAS2R38	Gene coding for the protein Taste receptor 2 member 38 (bitter taste receptor)
TDI	Threshold Discrimination Identification (olfactory function score)
UWS	Unstimulated Whole Saliva
2AFC	Two Alternative Forced Choice

NORWEGIAN ABSTRACT

Bakgrunn og formål:

Til sammenlikning med andre sanser, som syn og hørsel virker kanskje ikke smakssansen så viktig, men den kan ha en drastisk innvirkning på livskvalitet, matinntak og generell allmenntilstand. Forskning på og kunnskap om kjemosensoriske lidelser, spesielt smaksforstyrrelser, er mangelfullt. Å studere lukt og smak er utfordrende, og innhentet data er gjerne subjektive og kvalitative heller enn objektive og kvantitative.

Dysgeusia er en type smaksforstyrrelse hvor pasienter opplever forvrengt smaksoppfattelse. Formålet med dette prosjektet var i) å belyse perifer og sentral prosessering av smak hos pasienter med smaksforstyrrelser gjennom omfattende tester, og ii) beskrive mulige forhold mellom psykofysiske, fysiologiske og elektrofysiologiske funn.

Metoder og materialer:

Pasienter med smaksforstyrrelse ble rekruttert fra Lukt- og smaksklinikken ved Universitätsklinikum, Technische Universität Dresden. Kontrollgruppen ble rekruttert fra normalbefolkningen for å matche pasientene i alder og kjønn. Deltakerne fylte ut spørreskjemaer om medisinsk historie. De gjennomgikk en depresjonsscreeningtest, og evaluerte sitt eget funksjonsnivå på lukt og smak. Spyttsekresjonsrate (mL/min) ble beregnet ut i fra 5 minutter lang oppsamling av ustimulert spytt. Lukt- og smaks funksjon ble testet med validerte metoder; Sniffin' sticks, og smakssprayer og smaks-strimler. Deteksjonsterskel for smak ble testet med elektogustometri (EGM). Deltakernes bitter-smaksreseptorer fenotype ble bestemt ved hjelp av PTC/PROP smaksstrips. Tettheten av fungiforme papillae på tungen ble evaluert ved å følge Denver Papillae Protocol. Sentral prosessering av smak ble målt som fremprovoserte elektriske potensialer (event related potentials, ERP). Hver økt varte ca. 2,5 timer pr. deltaker. Alle deltakerne ble bedt om å ikke røyke, spise eller drikke noe annet enn vann 60 min før undersøkelsene.

Resultater:

Resultater viser at (i) pasientene hadde dårligere score på lukt- og smakstestene, (ii) pasientene var mer deprimerte, (iii) papillatetthet var lavere i pasientgruppen, (iv) pasientene krevde sterke stimulus for å oppfatte smak, og (v) pasientene viste mindre respons for target-

stimuli på ERP. Assosiasjoner ble funnet mellom alder og lukt- og smaksfunksjon, og mellom smaksfunksjon og papillatetthet.

Konklusjon:

Både perifere, og sentrale faktorer ble funnet hos pasienter med dysgeusia, som kan være årsak til smaksforstyrrelser. Videre studier er nødvendige for å finne ut om disse faktorene bidrar til smaksforstyrrelser.

ABSTRACT

Background and aim:

Dysgeusia is a type of taste disorder. In comparison with other senses like vision and hearing the sense of taste may not seem that important, but it can have drastic impact on the quality of life, nutritional intake, and general state of health for the patient. Knowledge and research on chemosensory disorders, and especially taste disorders, is lacking. The study of taste and smell disorders challenging, and obtained data tends to be subjective, and qualitative rather than quantitative.

The aim of this project was to (i) comprehensively elucidate the peripheral and central gustatory processing in patients with dysgeusia, and (ii) describe possible relations between psychophysical, physiological, and electrophysiological findings.

Methods and materials:

Patients with dysgeusia was recruited from the Smell and Taste Clinic, University Hospital, TU Dresden. A control group was recruited to match the patients' age and sex. All participants filled out a questionnaire about medical history, went through a depression screening test and self-evaluated their smell and taste function. Saliva secreation rate (mL/min) was calculated from unstimulated saliva collected for 5 minutes. Smell and taste function was tested using validated techniques; Sniffin' sticks, and taste sprays and strips. Taste detection threshold was tested with elctrogustometry (EGM). The participants were phenotyped for bitter-taste receptors. The density of fungiform papillae on the tongue was evaluated using The Denver Papillae Protocol. Central processing of taste was evaluated with gustatory event-related potentials (gERP). Each session lasted about 2,5 hours pr. participant. All participants were asked not to smoke, eat, or drink anything but water 60 min before their session.

Results:

The major findings are (i) the patients have lower smell and taste scores compared to the controls, (ii) patients scores higher on the depression questionnaire, (iii) the fungiform papillary density is lower in patients than controls, (iii) patients require stronger electrical stimuli to detect taste, and (iv) patients showed less activation for target stimulus in gERP. Furthermore, taste function in the patient group was associated with fungiform papillae

density. In both groups, there was correlation between taste function, and PROP phenotype and PROP-intensity rating.

Conclusion:

This study found differences between dysgeusia patients and controls in both peripheral taste tests and gERPs. Further study is needed to determine these factors as contributory to taste disturbance.

1 INTRODUCTION

The sense of taste may not seem that important in comparison to the other senses we humans possess, but it can have drastic impact on the quality of life, nutritional intake, and general state of health for the patient (1, 2). The study of taste and smell disorders is not so straight forward due to the challenge of obtaining objective data. Instead, data on smell and taste tends to be subjective, and qualitative rather than quantitative (3). Although the field of chemosensory disturbances is not well known, the prevalence and the impact on quality of life is better known for olfactory disorders than gustatory disorders (4). It is therefore interesting to shed some light on gustatory chemosensory processing and the mechanisms behind gustatory disorders. In this study, subjective and objective taste function of patients suffering from dysgeusia – a type of taste disorder - was evaluated using a variety of techniques.

1.1 Taste disorders

1.1.1 Types of taste disturbance/classification

Taste disturbances can be divided into three groups; ageusia, hypogeusia, and dysgeusia. They can be classified as either quantitative or qualitative taste disorders (Table 1.1). Ageusia *(greek "no taste")* is absence of one or more of the five basic tastes: salt, sweet, sour, bitter and umami. Hypogeusia *("under taste")* is decreased taste sensitivity. Dysgeusia *("bad taste")* is a distortion or misinterpretation of a taste, with the gustatory stimuli often being perceived as bitter, sour, or metallic by the patient. Dysgeusia can be further divided into parageusia and phantogeusia. Parageusia *("beside taste")* is a triggered taste distortion, for instance while eating. Phantogeusia ("taste illusion") is a gustatory hallucination (3, 5).

Table 1.1: Classification of taste disorders

Quantitative taste disorders	Ageusia Hypogeusia Hypergeusia
Qualitative taste disorders	Dysgeusia └> Parageusia └> Phantogeusia

1.1.2 Prevalence

Little is known about the prevalence of taste disorders in the general population. One study found that 5% of the general population were hypogeusic (6). A study of patients suffering from smell and taste disorders found that 57,7% of the patients had a combined smell- and taste dysfunction, while only 8,7% had a taste disorder alone (7).

1.1.3 Causes

Taste disorders can be caused by a multitude of things (5):

i) Bad tasting materials in the mouth (e.g. dentures or gingivitis)

ii) Problems with transporting the tastants to the taste buds (e.g. dry mouth, alterations of the saliva, or candida in the mouth).

iii) Damaged taste pores (e.g. due to trauma or tumour).

iv) Damage to peripheral nerves innervating the taste buds (e.g. surgery, dental procedures, trauma, or Bell's palsy).

v) Damage to central neural structures (e.g. tumour, stroke, trauma causing cranial nerve damage).

vi) Systemic diseases (e.g. diabetes, immunological connective tissue diseases).

vii) Neurological disorders (e.g. Parkinson's Disease, Epilepsy)

viii) Taste disorder can be a side effect of cancer and cancer therapy.

ix) Several medications can have taste disorder as a side effect.

1.1.4 Measuring and treating taste dysfunction

There is lack of diagnostic tools and treatments for patents with taste disorders. As compared to taste disorders, evaluation and treatment of olfactory disorders are more standardized and better established (1, 5, 8). There have been some studies suggesting that a zinc supplement may be beneficial, but the effect of other treatment suggestions have not been confirmed (1).

Flavour perception is largely mediated by retronasal stimulation of olfactory receptors during mastication and deglutition. Patients with olfactory disorders (hyposmia/anosmia) may therefor sometimes complain of losses in taste only. This confusion of gustatory and olfactory mediated sensations makes it necessary to test the olfactory function in addition to gustatory function in patients complaining of taste disorders (9). Tests available for measuring gustatory function are often based on psychophysical techniques, such as taste strips or taste solutions (1). Psychophysical techniques rely on the patient's perception of taste and smell, and are thus subjective. There are techniques which offer an objective measure of the patient's gustatory and olfactory functional level, like EEG-derived event related potentials (ERP) (10, 11), magnetoencephalography (MEG) (12), and functional brain imaging (fMRI) (13). Both EEG-ERP and fMRI has been pointed out as possible useful diagnostic tools (10, 13, 14), however, none of them are routinely used in diagnostics today. EEG is a non-invasive technique that provides a detailed and precise insight of patients' objective cortical and peripheral gustatory perception. To evaluate activation at the central level functional Magnetic Resonance Imaging (fMRI) must be used, however, it is a costly and time consuming analysis, and not suitable for larger study groups (15).

1.1.5 Relevance for Biomedical Laboratory Science

Biomedical laboratory science involves analysing biological materials to aid in diagnosing a patient. This study is a small contribution for better understanding a patient group which receives little attention, but nevertheless deserves good diagnostic and treatment options. As a biomedical laboratory scientist, one is also bound by the ethical guidelines for the profession, to contribute to biomedical scientific knowledge (16). As with any other field of study for a biomedical laboratory scientist, the work done in this study requires understanding about factors which may affect the results, and ways to minimize possible errors.

The study of taste is relevant for biomedical laboratory scientists because the mouth can be considered the "gate keeper" for voluntary ingestion of food. It gives important information about the nutritional value of food being eaten, and initiates metabolism in the oral cavity.
Knowledge about processes and conditions in the oral cavity is necessary for the understanding of several other functions in the body, e.g. digestion. Not to mention its usefulness when it comes to analysing spit samples (both biochemically and molecularly), and working with microbiological swab samples from the mouth.

1.3 The sense of taste

1.3.1 Anatomy and physiology of the peripheral gustatory system

The tongue, papillae, and taste buds:

The upper surface of the tongue is covered in small bumps called lingual papillae. There are three types of papillae with gustatory function: fungiform, foliate and circumvallate papillae. In addition, there are two types of supporting papillae: filiform and conical papillae. The actual taste organ are the taste buds. The taste buds can be found on the gustatory papillae, and consists of an opening where tastants can enter (gustatory pore), supporting cells, and sensory cells with nerves leading to the central nervous system (5).

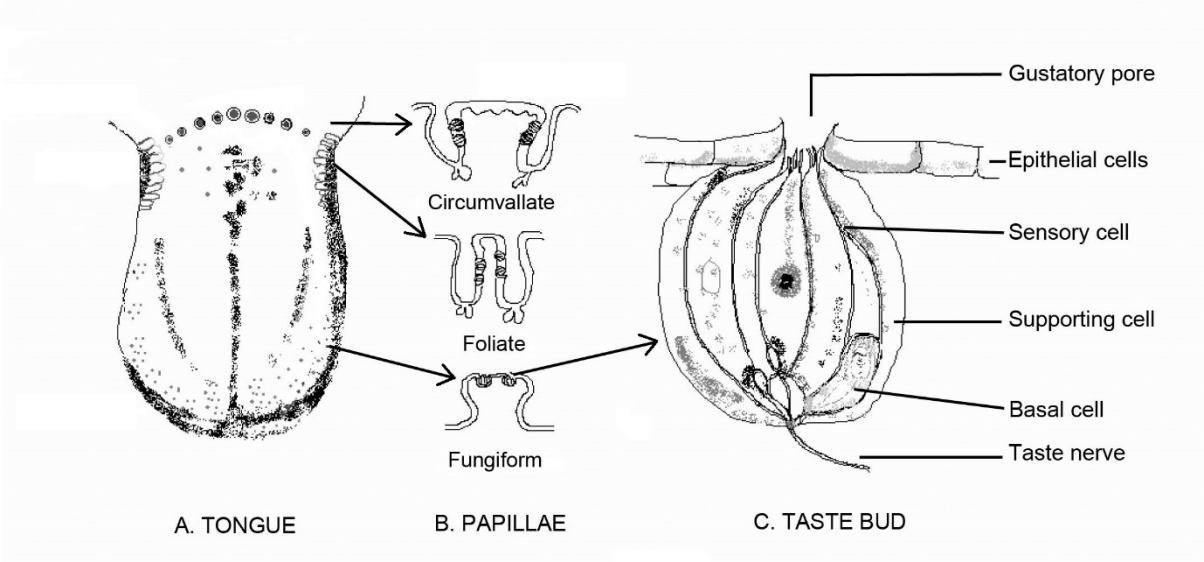


Fig. 1.1: Illustration of the peripheral gustatory structures. Figure modified from source image (17).

Innervation:

The nerve fibres innervating taste buds belong to three cranial nerves; the facial nerve (CN VII), the glossopharyngeal nerve (CN IX), and the vagus nerve (CN X). Taste buds on the anterior two-thirds of the tongue are innervated by the facial nerve. Taste buds on the posterior one-thirds of the tongue, and in the pharynx, are innervated by the glossopharyngeal nerve. Taste buds in the larynx are innervated by the vagus nerve (5) (Fig. 1.2).

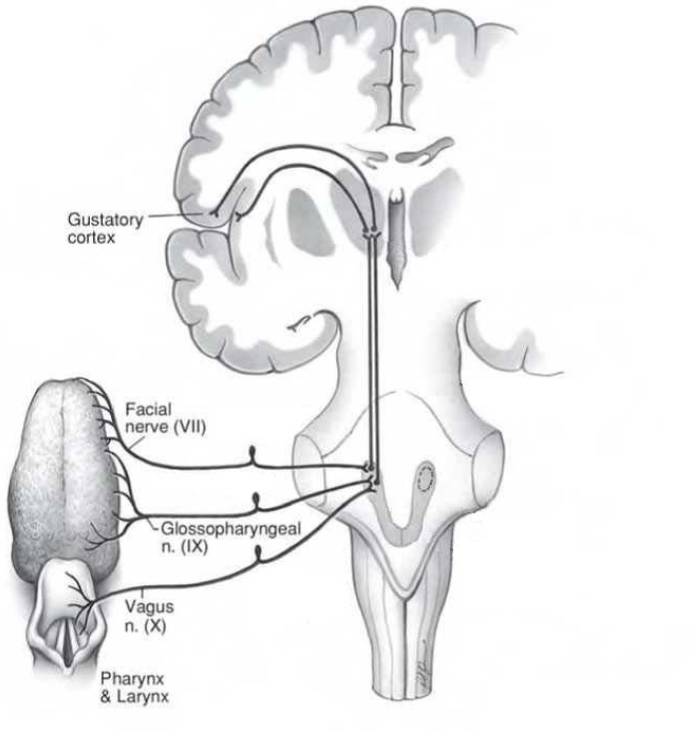


Fig. 1.2: Innervation of chemosensory receptors and gustatory neuron pathway through the central nervous system. Figure modified from source image (18).

Central processing:

Gustatory signals are processed in the gustatory cortex in the brain. The primary gustatory cortex consists of two substructures: the anterior insula on the insular lobe and the frontal operculum on the inferior frontal gyrus of the frontal lobe (19).

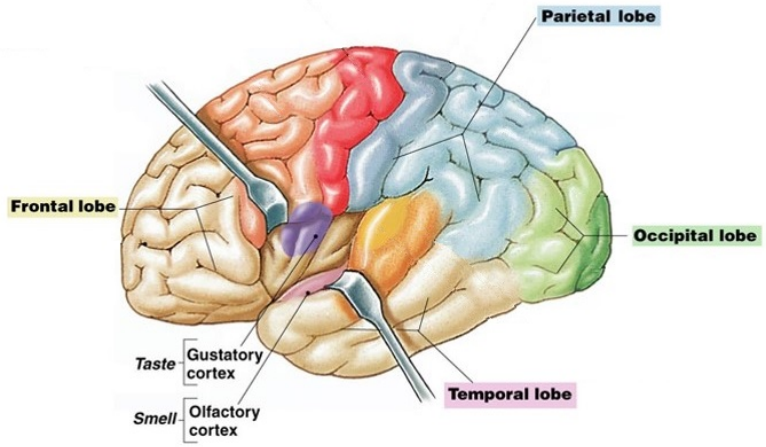


Fig. 1.3: Gustatory cortex shown in purple. Figure modified from source image (20).

Molecular physiology:

Taste receptors are, as the name indicates, receptors which facilitates taste sensation. When food or other substances enter the mouth, the molecules bind to taste receptors on the sensory cells in the taste buds. There are taste receptor for all the main tastes, sweet, sour, salty, bitter and umami. The sweet, bitter and umami taste is mediated through G protein-coupled receptors, while salty and sour taste is mediated through ion channels (5). It is proposed that sour taste may also be mediated through a transient receptor potential (TRP) channel (21).

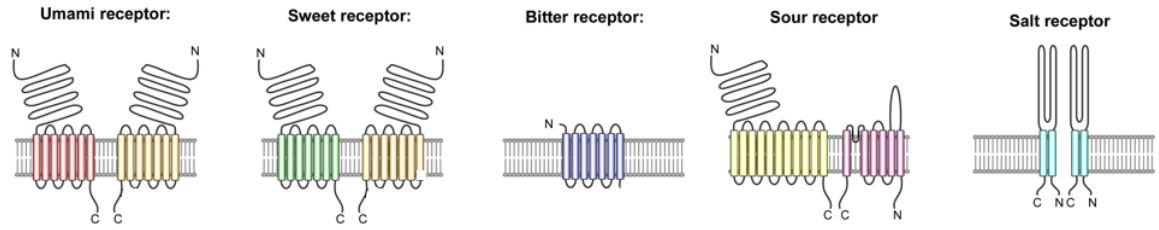


Fig. 1.4: The different taste receptors. Figure modified from source image (21).

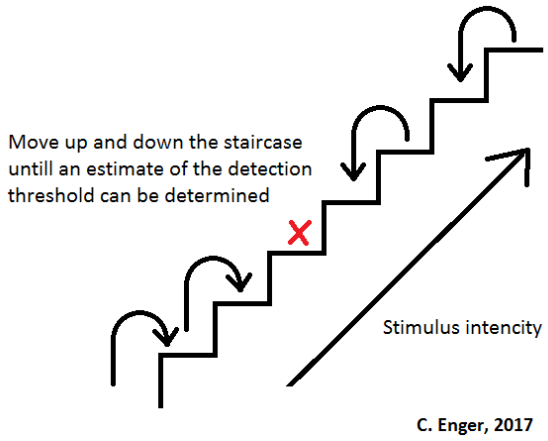
The ability to taste 6-n-propyltiouracil (PROP), a bitter compound, is controlled by three polymorphisms in the TAS2R38 PROP-taste receptor gene, which give rise to two common haplotypes: PAV, the dominant (taster) variant and AVI, the recessive (non-taster) one. PROP-tasters are PAV homozygote or PAV/AVI heterozygote, while PROP-non-tasters are AVI homozygote (22, 23). The existence of bitter-tasting genes may be linked to the advantage of being able to detect poisonous and spoiled food (24). The ability to taste PTC and PROP are correlated and reflect the same polymorphism (25).

1.4 Psychophysics

Psychophysics can be defined as "the scientific study of the relation between stimulus and sensation" (26). Some common principles implemented in psychophysical techniques are staircase designs and forced choice methods.

The staircase design

When determining the detection threshold for a certain stimulus, psychophysics often uses adaptive staircases. This means estimating when the subject can detect the stimulus and not by presenting the stimulus many times over with varying intensity, moving up and down in stimulus-strength like on a staircase.



The most commonly used staircase design is the 2-down-1-up staircase with fixed-step size. If the participant makes the correct response two times in a row, the stimulus intensity is reduced by one step size. If the participant makes an incorrect response the stimulus intensity is increased by one step size. A movement up or down on the intensity scale is referred to as a reversal. After a certain number of reversals, the test is concluded, and a threshold is estimated from the average value of the reversal-point of the last few runs. Usually the last four reversal-points are used to calculate the threshold, but this number and the number of reversal that are run through depends on the experimental set-up (27).

2 Alternative forced-choice method

A Two-alternative forced choice method (2AFC) is often utilized, meaning the subject is forced to choose one of two alternatives given to them as the correct one (first observation versus second observation). By using a 2AFC experimental paradigm the response bias is minimized (28). Compared to using a simple yes-no model, performance levels in 2AFC are higher, and permits measurement of sensitivity to smaller stimulus differences (29). A forced choice procedure does not have to involve only two alternatives. In psychophysics, 3 and 4 alternative forced choice is also often used.

1.5 Electroencephalography and gustatory event related potentials

1.5.1 EEG

Electroencephalography (EEG), is a technique that records the electrical impulses created by the neurons in the cerebral cortex. There is a constant move of ions in and out of, and between neurons within the brain. These ion movements cause voltage fluctuations. By placing electrodes on the scalp, the voltage differences created by these fluctuations can be measured, and a recording of the electrical activity happening in the cerebral cortex can be made. This recording, called electroencephalogram, shows the voltage plotted against time with stronger voltage creating higher amplitudes. EEG measurement consists of active electrodes placed on the scalp, reference electrodes placed in relatively electrically inactive locations, and one ground electrode. The voltage differences recorded are the potential differences between active electrodes and reference electrodes. The ground electrode is mainly for preventing power line noise interference. It is worth noting that EEG is a measure of the sum of electrical activity on the brain surface underneath the electrode. Electrical signals from single neurons, or from deeper within the brain are not strong enough to be recorded by EEG (30).

1.5.2 ERP

EEG-derived event related potentials (ERP) or evoked potentials are the electrophysiological responses exhibited by the brain when sensory nerves are stimulated (10). When a subject is exposed to a certain type of stimulus during an EEG recording, we can find changes in the voltage within a section of the EEG that are specifically related to the brain's response to this kind of stimulus (31). The response can be visualised as a waveform with electric current shown as a function of time passed since stimulus-onset. The peaks, or waves, are given names based on their direction (negative or positive), and the timing of their appearance. For instance, P300, or simply P3, is a positive peak appearing 250-400 msec after the stimulus (Fig 1.5). The first peaks, which appear within the first 100 msec after stimulus onset, are "sensory" or "exogenous", and reflect the physical response to the stimulus. Later peaks, appearing after 200-300 msec, are "cognitive" or "endogenous" peaks, and reflect the mental processing of the stimulus (32).

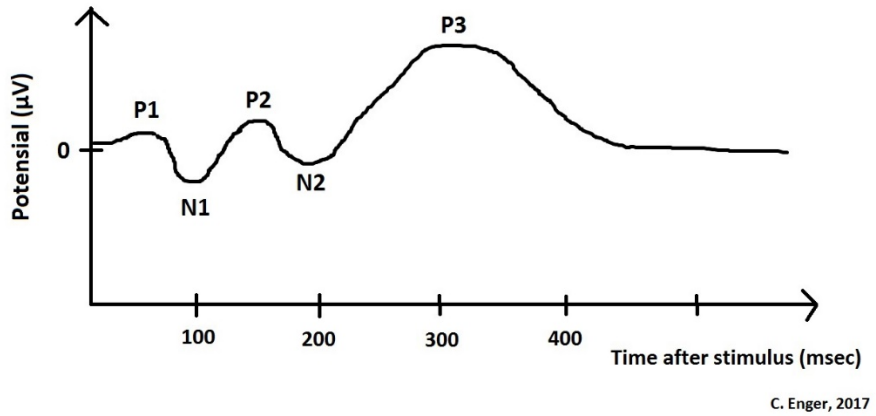


Fig. 1.5: The components of an ERP.

Recording gustatory ERPs using a high-resolution system

One way to record ERPs is using a multi-channel high-resolution 128-channel system. This system uses 128 active electrodes plus two grounding electrodes – Common Mode Sense (CMS) active electrode, and Driven Right Leg (DRL) passive electrode. An EEG amplifier measures voltage differences between a point on the scalp (an active electrode), and a reference electrode. The reference electrodes are placed in areas with low electrical activity: earlobes, mastoid process, bony part of nose (dorsum). External electrodes are also placed over and under left eye, and under right eye for tracking eye blinking. The electrodes are placed in a specific pattern using the 10-5 system. Electrode placement systems use defined landmarks on the scull: above the bridge of the nose, (Nasion, Nz), the occipital protuberance (Inion, Iz), and just in front of the ears (left and right pre-auricular point, LPA and RPA). These four points make up two axes; Nz-Iz line and LPA-RPA line. The crossing point of these to axes are called the vertex (Cz). Following contours made by these landmarks, electrodes are spaced apart with a distance of 5% or 10% of the total Nz-Iz or LPA-RPA distance of the skull (33).

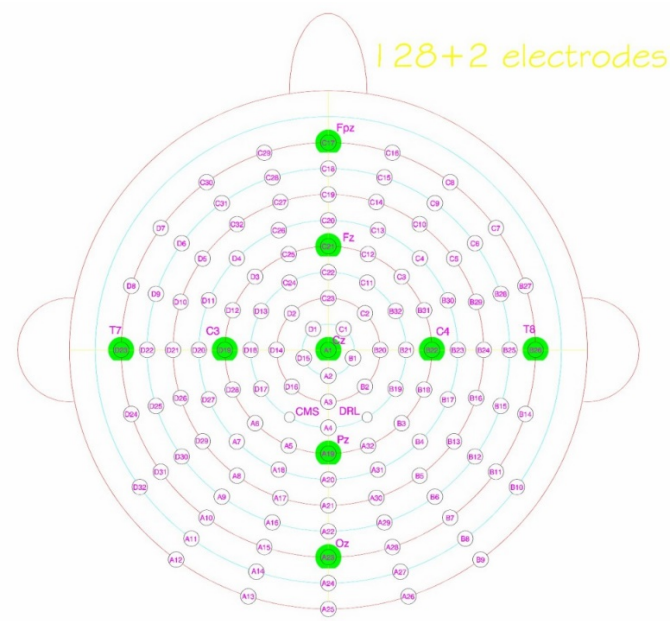


Fig. 1.6: BioSemi 128 channel high-resolution system for recording EEG (34).

Averaging the ERP signal

EEG signals can be noisy, and they contain the electrical activity of a lot of cortical neurons together. Especially in a high-resolution system, it is difficult to distinguish the gustatory response from all the other signals in the recording. The ERPs need to be extracted from this background activity. This can be done by averaging the signals. In theory, the random activation will cancel each other out, and the non-random activation (the gustatory response) will be left (35).

The data from the EEG recording containing the stimulus reaction is separated out from the rest of the recording. We can define a section of EEG (an epoch) that begins a certain amount of time before stimulus onset (pre-stimulation time) and ends a certain amount of time later (post-stimulation time). This epoch will then include the brain's response to the stimulus. We can examine this defined bit of the recording to find changes specifically related to the stimulus event. Chemsosensation elicits a slower reaction in addition to the deliverance of the stimulus being a little slower than auditory and visual stimuli. This should be taken into consideration when choosing pre-stimulation and post-stimulation time. Epochs with noisy signals or eye blinking are rejected. All accepted epochs are then filtered, baseline corrected and layered on top of each other. This layering creates a single average wave representing the stimulus reaction.

1.5.3 Gustometer

A gustometer is a device for administering gustatory stimuli. The gustometer can be controlled through a computer to deliver a pre-programmed, precise sequence of stimuli. The sequence defines which stimulus should be delivered when, and with what concentration, flow rate, duration, and inter-stimulus interval (ISI, time passed between stimuli).

Fig. 1.7: Computer controlled gustometer. The different taste-solutions are filled into separate pipettes. The tastants are delivered to the subject with exact timing and portioning, as determined by the sequence pre-programmed in the computer. Photo: C. Enger (2017).

2 AIM

The aim of this project was to (i) comprehensively elucidate the peripheral and central gustatory processing in patients with dysgeusia, and (ii) highlight possible associations between psychophysical, physiological, and electrophysiological findings.

3 MATERIALS AND METHODS

3.1 Participants

The study was conducted according to the declaration of Helsinki and approved by the Ethics Committee of Medical Faculty Carl Gustav Carus, Technical University Dresden. Informed written consent was obtained from the participants prior to the study. A total of 9 patients with taste dysfunction and 9 healthy controls were included in this study. Patients with complaints of distorted taste sensation were be recruited from the Smell and Taste Dysfunction Clinic, Department of Otorhinolaryngology, University Medical School, Technische Universität (TU), Dresden. The control group was recruited from the city of Dresden, mainly from the University Clinic and the TU campus, Dresden. Inclusion criteria for the patients: Suffering from dysgeusia, non-smoker. Exclusion criteria for the controls: Age <40 years, smoking, diabetes.

3.2 Methods

In this study, participants' health status was ascertained with a detailed medical history. Salivary rate was measured (sialometry). The participants' olfactory and gustatory function was tested using psychophysical techniques. PROP tasting ability was established. Taste threshold was measured using electrogustometry (EGM). Fungiform papillary density was assessed using Denver staining protocol. Finally, EEG derived gustatory event-related potentials were recorded. A complete session including all the tests took about 2,5 hours for each participant. The participants were asked not to eat, smoke, or drink anything but water 60 minutes before the session.

3.2.1 Questionnaires

Prior to testing all participants were asked about their medical history. All participants were screened for depression using the Beck Depression Inventory Fast Screen (BDI-FS). The questionnaire consists of 7 items. The items concern tiredness, pessimism, feeling of failure, loss of joy, self-rejection, self-criticism, and thoughts of suicide. Each item response was rated on a 4-point Likert-type scale ranging from 0 to 3, based on the severity of each item, giving a possible score 0-21. Scores of 10–21 indicate severe depression; 7–9 moderate; 4–6 mild and 0–3 minimal depression (36). The participants were also asked to score their own

general subjective smell and taste perception on a scale from 0 to 8, where 0 = no smell perception, and 8 = very good smell perception.

3.2.2 Measurement of salivary flow rate: Sialometry

Unstimulated whole saliva (UWS) was collected from all participants to determine salivary secretory rates. The saliva was collected with a spitting method in plastic containers and weighed, and then secretion rate was calculated (g/ml = ml/min). Saliva collection was performed before the other tests so that the olfactory and gustatory stimulation done in the next test wouldn't affect the salivation.

3.2.3 Assessment of olfactory function

Sniffin' sticks

The olfactory function of all the participants was assessed using a validated method – the extended Sniffin' Sticks test kit (Burghart, Wedel, Germany).

Sniffin' sticks are odour-containing felt-tip pens which are used to assess orthonasal olfactory function. The test is divided into three sub-tests to assess odour detection threshold (olfactory threshold), odour discrimination and odour identification. Each sub-test utilizes its own set of pens. The sets contain 3x16, 3x16 and 12 pens for threshold test, discrimination test and identification test, respectively.

The odour was presented prenasally and bilaterally to the subject by holding the pen ~2 cm in front of both nostrils and slightly moving it back and forth for a few seconds. As odour detection requires the most concentration from the subject and is the lengthiest test, this was performed first. The pens belonging to the first two sub-tests (threshold and detection tests) are colour coded to be recognizable to the test administrator. The subject was therefore blindfolded during the first two-thirds of the experiment to prevent them seeing the colour code on the pen that is presented to them. The whole experiment followed a forced-choice procedure. The subject had to choose an answer every time a pen/triplet of pens were presented to them. The subject was scored on each sub-test, ultimately resulting in a cumulative TDI score (Threshold Discrimination Identification) of maximum 44 points (16+16+12 points).

Olfactory threshold was established by presenting the subject with a triplet of sniffin' sticks pens in randomized order – one of which contained odorant in a certain concentration, and two which did not contain any odorant. The subjects had to identify the pen they believed to contain the odour. Triplets with varying concentration in the odour-containing pen was presented until the patient's threshold for olfaction was established through a staircase method. The staircase design utilised in this test was the 2-up-1-down staircase with fixed-step size. The odour-containing pens are arranged in a 1:2 dilution series with 16 stages, or steps, starting at 4 %. Starting with the lowest concentration/step, each triplet was presented to the subject twice. If the participant made the correct response two times in a row, the stimulus intensity was reduced by one step size – meaning the pen one step below in odour concentration was presented next. If the participant made an incorrect response the stimulus intensity was increased by the one size. A movement up or down on the intensity scale is referred to as a reversal. The test was concluded after seven reversals. A threshold was estimated from the reversal-point of the last four runs.

Odour discrimination ability was assessed by presenting the patient with three pens; two containing same odorant and one containing a different odorant. The patient had to identify the pen which differed from the others.

Finally, odour identification ability was assessed by presenting the subject with one odorant at the time. The subject had to identify the smell out of four options given to them (1, 37).

3.2.4 Assessment of gustatory function

Gustatory function was assessed using two whole-mouth taste tests; taste strips and taste sprays.

Taste strips

The subject was given filter-paper impregnated with four of the basic taste qualities sweet, sour, bitter, or salty flavouring. The test did not include the umami taste, as it is not well known to Westerners. The strips contain the four tastes in four different concentration levels (sixteen strips altogether). The concentrations are listed in table. 3.1. The taste strips were presented with increasing concentration in a randomized order. The participant could taste the strip with their mouth closed, using the whole tongue. The participant was asked to identify the type of taste the strip elicited, thus giving a measure of recognition threshold for bitter,

sweet, sour, and salty taste. One point was awarded for every right answer, giving a final taste score of maximum 16 points (9).

Table 3.1: Concentrations of solutions for taste strips.

Solute	*Conc. 1*	*Conc. 2*	*Conc. 3*	*Conc. 4*
Sweet: Sucrose (g/mL)	0,4	0,2	0,1	0,05
Sour: Citric acid (g/mL)	0,3	0,165	0,09	0,05
Salty: NaCl (g/mL)	0,25	0,1	0,04	0,016
Bitter: Quinine hydrochloride (g/mL)	0,006	0,0024	0,0009	0,0004

Taste sprays

Taste sprays are a rapid screening test for the four basic tastes presented at supra-threshold concentrations. The test kit consisted of four spray bottles containing taste solutions (sweet, sour, salty, and bitter). The following concentrations were used: sweet: 0,1 g/mL sucrose; sour: 0,05 g/mL citric acid; salty: 0,075 g/mL NaCl; bitter: 0,0005 g/mL quinine hydrochloride. One pump of the spray bottle was administered onto the tongue of the participant. The participant had to identify the taste as sweet, sour, salty, or bitter. One point was given for each correct answer, giving a score of maximum 4 points (38).

3.2.5 Phenotyping of bitter taster/non-taster ability: PROP sensitivity

Bitter-taster status was determined using a simple supra-threshold method. A filter-paper impregnated with propylthiouracil (PTU/PROP) was given to the participant (N-Propylthiouracil Test Paper, Precision Laboratories, USA). PROP together with phenylthiocarbamide (PTC) belong to the class “thioureas”, containing a chemical group responsible for bitter taste. Whether the subject tasted a bitter taste or not determined their bitter-taster status (39). In addition, the subjects were asked to rate the intensity of the bitter taste on a visual analogue scale (VAS) from 0 to 10, where 0 = no taste at all, and 10 = extremely strong taste.

3.2.6 Measurement of taste threshold: Electrogustometry (EGM)

Detection threshold for electrical stimuli of the tongue was determined using an electrogustometer (RION TR-06, Rion, Kokubun, Japan). The experimental setup consisted of a current-delivering electrode (5 mm stainless steel) placed intermittently in contact with the subject's tongue and a grounding electrode placed on the subject's neck. The current-delivering electrode was held in contact with the surface of the subject's tongue for 2 seconds at the time, twice in a row. One time the electrode delivered an electrical stimulus (stimulus time: 0,5 sec), the other it did not. The operator controlled during which of the two contacts current was delivered. The test person was blinded with regards to the stimulation timing and levels. A Two-alternative forced choice method (2AFC) was utilized, meaning the subject is forced to choose one of two temporal intervals (first observation versus second observation). The test person indicated which contact, number one or two, elicited sensation by hand signalling to the operator. Duplets of stimulus/non-stimulus with varying current strength is delivered until the patient's threshold for stimulus detection was established using a 2-up/1-down staircase method with fixed step size. Stimulus strength range: -6 dB to 24 dB (3-400μA), step size: 2 dB. The test was concluded after seven reversals. A threshold was estimated from the reversal-point of the last four runs. The whole procedure was performed four times at different locations on the tongue, giving four threshold values. The four locations can be imagined as quadrants of the tongue; left and right side on the front of the tongue (innervated by chorda tympani), and the left and right side at the back of the tongue (innervated by the glossopharyngeal nerve) (Fig. 3.1). Thresholds above 50 mA, in the absence of chorda tympani degeneration, may be contaminated by trigeminal responses, but thresholds within the normal range do not overlap with responses attributable to activation of nerve fibres carrying tactile information (28).

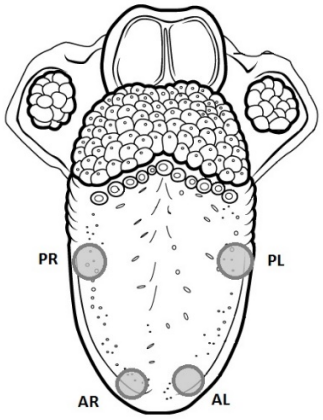


Fig. 3.1: Locations for application of electrical stimuli for EGM. Figure modified from source image (40).

3.2.7 Calculating fungiform papillary density

The Denver Papillae Protocol (DPP) was followed (41). The subject was directed to dry their tongue with a paper hand towel and leave tongue protruding from their mouths. Approximately 3 mL of blue food dye was applied to the apex of the tongue using a cotton swab. The subject then swallowed once to remove excess dye. The subject was asked to position themselves with their elbows on the table, holding their chin in their hands, tongue extended comfortably. The subjects were encouraged to secure the tongue gently between their teeth. A piece of filter paper with a 9,5mm diameter circular cut-out was placed on the tongue on the left side of the tongue anteriorly. Images of the tongue were then taken using a Nikon Coolpix S520 digital camera (16 megapixels) mounted on a tripod for stability. The image was uploaded in the software ImageJ. Each papilla observed in the picture was scored using the Denver Papillae Protocol Dichotomous Key to determine it as fungiform or not (Fig. 3.2), and then marked using the "Multi-point tool" function in the software. The number of papillae marked as fungiform papillae gave a FP raw score. Each image was assed twice blindly. The two scores were then compared. If the higher FP raw score was within 10% of the lower FP raw score, the final FP score was set as the average of the two raw scores. If the two FP raw scores differed by more than 10%, the image was reassessed. The final FP score divided by the area encircled by filter paper gives the papillary density:

$$Density = \frac{FP\ score}{Area}$$

$$Density = \frac{FP\ score}{\pi r^2} \quad (r = 0{,}45\ cm)$$

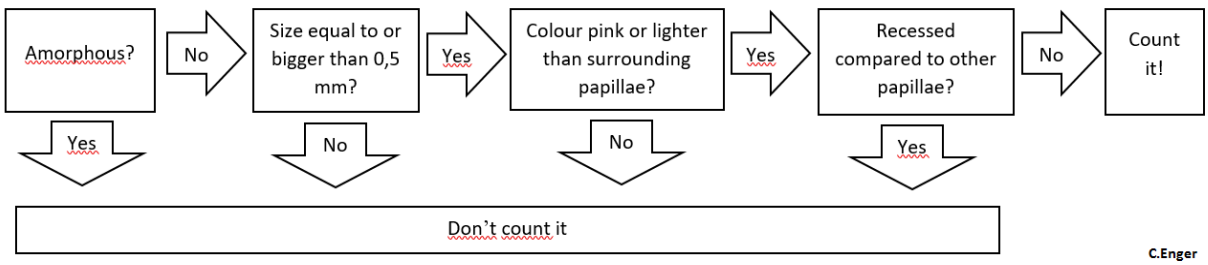


Fig. 3.2: Schematic representation of the Denver Papillae Protocol Dichotomous Key.

3.2.8 Recording EEG-related gustatory event-related potentials (gERP)

Experimental setup

Gustatory event-related potentials (gERP) were compiled using EEG-signals recorded while the subject is gustatory stimulated. The gustatory stimuli were delivered through a computer controlled gustometer (Burghart gustometer GU001, Wedel, Germany), and consisted of two different dilutions of a salty solution. The EEG signals evoked by the gustatory stimuli were amplified and recorded using a 128-channel system from Biosemi (Lowpass: 30 Hz, Highpass: 0,16 Hz, Decimation: ¼, Sample rate: 512 Hz) (Hardware: AD-box and USB2 receiver, Active Two, Biosemi, Amsterdam, Netherlands. Software: Biosemi ActiView606, Biosemi, Amsterdam, Netherlands). The signals were referenced against electrodes placed on the earlobes, mastoid processes, and nose. Eye blinking was monitored with electrodes placed over and under the eyes. During recordings of the gustatory ERPs, participants received brown noise through headphones to mask the switching clicks from the gustometer (42). Each session of recording evoked potentials lasted about 35 minutes. The session was divided into three equal blocks with two short breaks in between. The participants were asked to count the number of light salty stimuli they felt in each block to keep the focused.

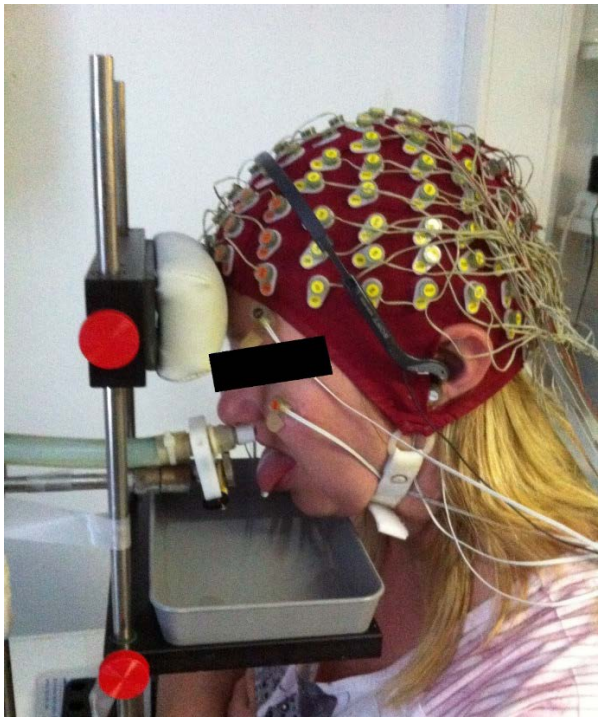


Fig. 3.1: Experimental setup: Taste stimuli are delivered from gustometer and sprayed on to the tongue. The electrical activity in the brain is recorded using a 128-channel EEG system. Sounds from the gustometer are masked with brown noise. Photo: C. Enger (2017).

Stimulus

The stimuli the participants were exposed to were two salty stimuli with equal duration and amount, but different concentration. One strong and one weak stimulus was created by diluting a 150 g/L NaCl-solution by 50 % and 15 % respectively[1]. 15 % = target stimulus, 50 % = non-target.

Sequence design

The sequence consisted of a total of 120 stimuli. During the recording, the subject was given a break every 40th stimulus. Each stimulus had a 500 msec duration with 12 sec interstimulus interval (ISI). Pulse volume 100 µL. Average volume flow 200 µL/sec. The sequence was built as an oddball paradigm with weak stimulus being delivered in randomized order in between every 2-5 strong salty stimuli, i.e. the weaker stimulus being the target stimulus. To avoid tactile and thermal stimuli, the taste stimuli were delivered intermittently in a continuous spray of background solution at a constant temperature of 36ºC. The background solution was a tasteless watery solution composed of the main ionic components of saliva (25 mM KCl and 2,5 mM $NaHCO_3$).

Data analysis

ERP averaging:

The data was processed using Cartool 3.55 (Denis Brunet, Functional Brain Mapping Laboratory, University of Geneva, Switzerland). One epoch was defined as beginning 1000 msec before stimulus and ending 2000 msec after stimulus. Epochs with noisy signals or eye blinking were rejected. All accepted epochs were filtered through a Butterworth filter: High

[1] 50%= 1283 mM 15%=171 mM

pass 0,5 Low pass 30. In addition, a 50-Hz Notch filter was applied to attenuate power-line effects. Finally, the epochs were baseline corrected (inferior limit: -512 (-1000 ms), superior limit: 0), and averaged. Signals from the Fz (C21), Cz (A1) and Pz (A19) electrodes were exported to MATLAB for further analysis.

Measurement:

The responses were investigated in a time-amplitude space, and peak to peak maximum amplitude measurements were performed using MATLAB R2013a (MathWorks, Massachusetts, USA).

3.3 Statistical analysis

Results were analysed using SPSS 24.0 for Windows. Normality test were employed to check whether the data were normally distributed. Independent and paired t-tests were used to compare normally distributed continuous variables in the patient and control group. For data not normally distributed, non-parametric tests were utilized. Chi-square test was utilised to compare dichotomous variables and Pearson's correlation was used to measure the strength and direction of linear relationships between pairs of continuous variables. All differences were considered significant at $p < 0.05$.

4 RESULTS

4.1 Group characteristics

Participant characteristics and demographic variables are presented in Table 4.1. The participants in the dysgeusia patient group and the control group had a comparable mean age, with the patient's age ranging from 41 to 83, and the controls age ranging from 40 to 67 years. The age distribution of the two groups is shown in figure 4.1. The two groups did not differ significantly in their age, gender make-up, smoking habits, or number of chronic diseases. The two groups did not differ significantly in their age, gender make-up, smoking habits, or number of chronic diseases. Chronic disease was reported by three participants in the control group (all three suffered from hypertension) and six participants in the patient group (suffering from hypertension, hypothyroidism, irritable bowel syndrome, and diabetes). Both controls and patients with chronic diseases were on medication. There were no significant differences in the use of medication between groups. The patients scored significantly higher than the controls on the depression screening test (BDI-FS).

Table 4.1: Characteristics of patients and controls.

	Patients with dysgeusia	*Controls*
Gender, n (%)		
Male	1 (11,1 %)	3 (33,3 %)
Female	8 (88,9 %)	6 (66,7 %)
Age in years (mean±SD)	60,7±13,9	56,3±8,4
Use of alcohol, n (%)		
Yes	5 (55,6 %)	7 (77,8 %)
No	4 (44,4 %)	2 (22,2 %)
Smoker, n (%)		
Yes	0 (0 %)	0 (0 %)
No	9 (100%)	9 (100%)
Use of medication, n (%)		
Yes	8 (88,9 %)	3 (33,3 %)
No	1 (11,1 %)	6 (66,7 %)
Chronic disease, n (%)		
Yes	6 (66,7 %)	3 (33,3 %)
No	3 (33,3 %)	6 (66,7 %)

Number of diagnoses (mean±SD)	1,3±1,9	0,3±0,5
BDI-FS score, 0-21 (mean±SD)	2,4±2,6*	0,2±0,7*
Minimal depression, n (%)	5 (55,6 %)	9 (100 %)
Mild depression, n (%)	4 (44,4 %)	0 (0 %)

*p=0,032

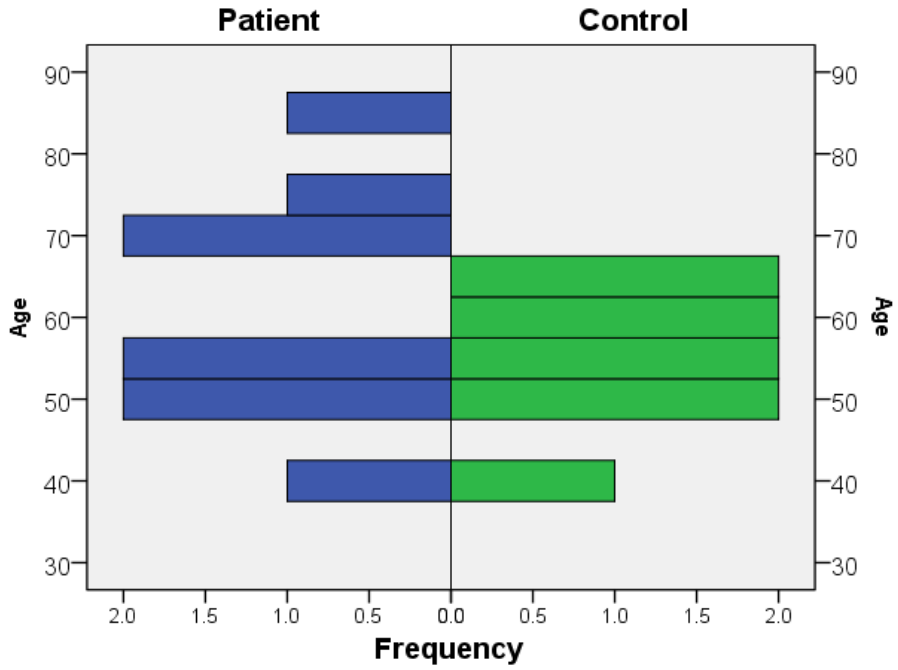


Fig. 4.1: Age distribution of patients and controls included in the study.

4.2 Patients' taste dysgeusia characteristics

Bitter taste dysgeusia was reported by 55% (n=5), salty taste dysgeusia was reported by 22% (n= 2), metallic/bitter taste dysgeusia was reported by 11% (n=1) and sweet/salty dysgeusia was reported by 11% (n=1) of the patients in this study. The patients' diagnoses regarding are shown in table 4.2, and the distribution of types of dysgeusia is shown in figure 4.2. None of the controls had dysgeusia.

Table 4.2: Patients' self-reported dysgeusia

Patient	*Type of dysgeusia*
1	Bitter dysgeusia
2	Metallic and bitter dysgeusia
3	Salty dysgeusia
4	Bitter dysgeusia
5	Bitter dysgeusia
6	Salty dysgeusia
7	Sweet and salty dysgeusia
8	Bitter dysgeusia
9	Bitter dysgeusia

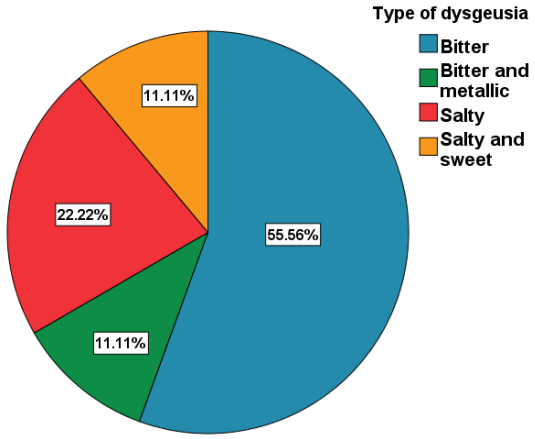


Fig. 4.2: Distribution of type of dysgeusia in patient group.

4.2 Sialometry

No significant differences were found in saliva flow rate between the two groups (Table 4.3). The mean secretion rate was about the same, but there were much greater differences in spread in the patient group than in the control group (Fig. 4.3).

Table 4.3: Sialometry results of patients and controls.

	Patients with dysgeusia	*Controls*
Sialometry (ml/min) (mean±SD)	0,44±0,30	0,39±0,18

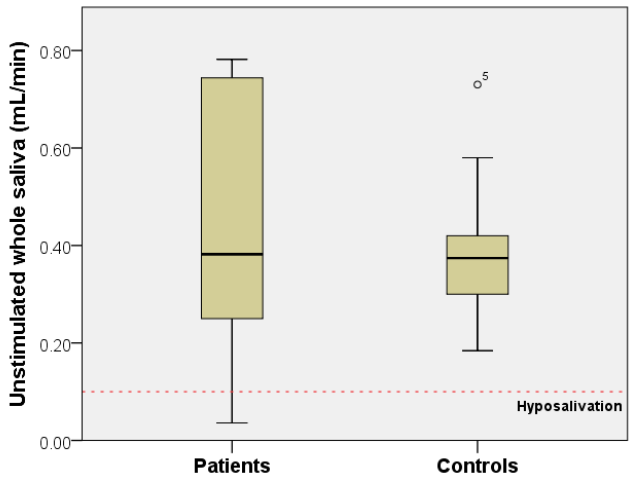


Fig 4.3: Comparison of sialometry results in patients with dysgeusia and controls

4.3 Olfactory results

The patient group had lower mean olfactory score (TDI score) than the control group, and rated their ability to smell lower than the controls did (Table 4.4). Some of the patients had TDI scores indicating anosmia (Table 4.6). No significant differences were found in measured and self-reported olfactory mean score in the two groups. Normative data for smell are shown with red dotted lines (Fig. 4.4). There was a strong negative correlation with age and smell scores in the patient group (r=-0,88, p=0,002).

Table 4.4: Olfactory function results (TDI score) of patients and controls.

	Patients with dysgeusia	*Controls*
TDI score (mean±SD)	26,5±10,8	32,4±4,1
Self-reported smell function, 0-8 (mean±SD)	3,7±2,1	5,2±1,7

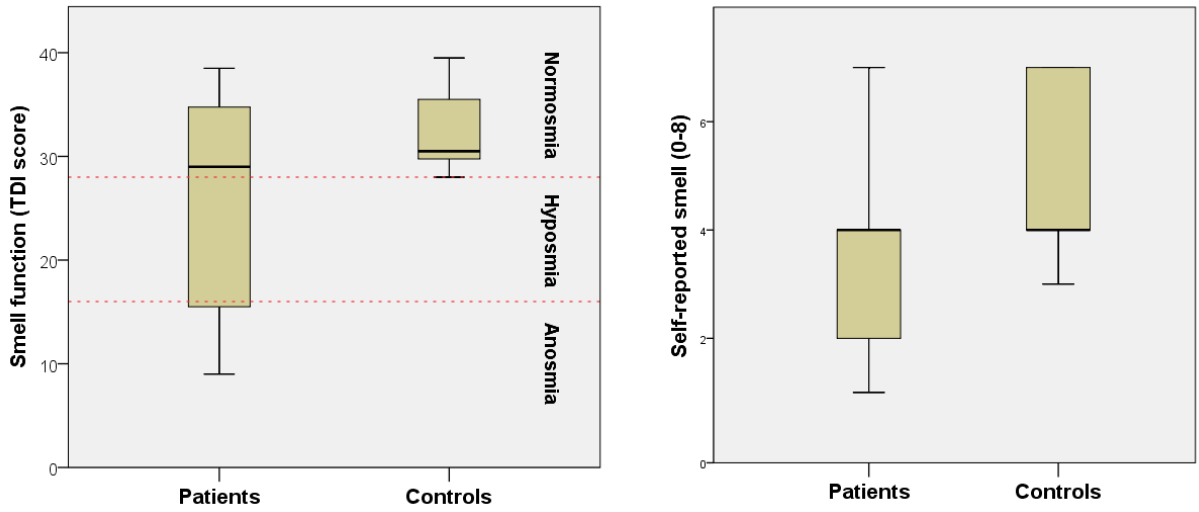


Fig. 4.4: Comparison of self-reported and tested smell function in patients with dysgeusia and controls.

4.4 Gustatory results

The patient group had lower mean gustatory scores than the control group, and rated their ability to smell and taste lower than the controls did (Table 4.5). Both self-reported taste and tested taste are significantly poorer in the patient group. For both smell and taste the patient tend to rate their chemosensory function below average, while the controls rated themselves above average. Normative data for smell and taste are shown with red dotted lines (Fig. 4.5). There was a strong negative correlation with age and taste scores in the patient group (r=-0,70, p=0,035). The control group showed a moderate negative correlation with age and taste (r=-0,62, p=0,077).

Table 4.5: Gustatory function results of patients and controls.

	Patients with dysgeusia	*Controls*
Taste strips *(mean±SD)*	9,8±1,9*	12,7±2,0*
Taste sprays *(mean±SD)*	3,4±0,9	4,0±0,0
Self-reported taste function, 0-8 (mean±SD)	2,8±1,2**	4,9±1,6**

*p=0,006
**p=0,007

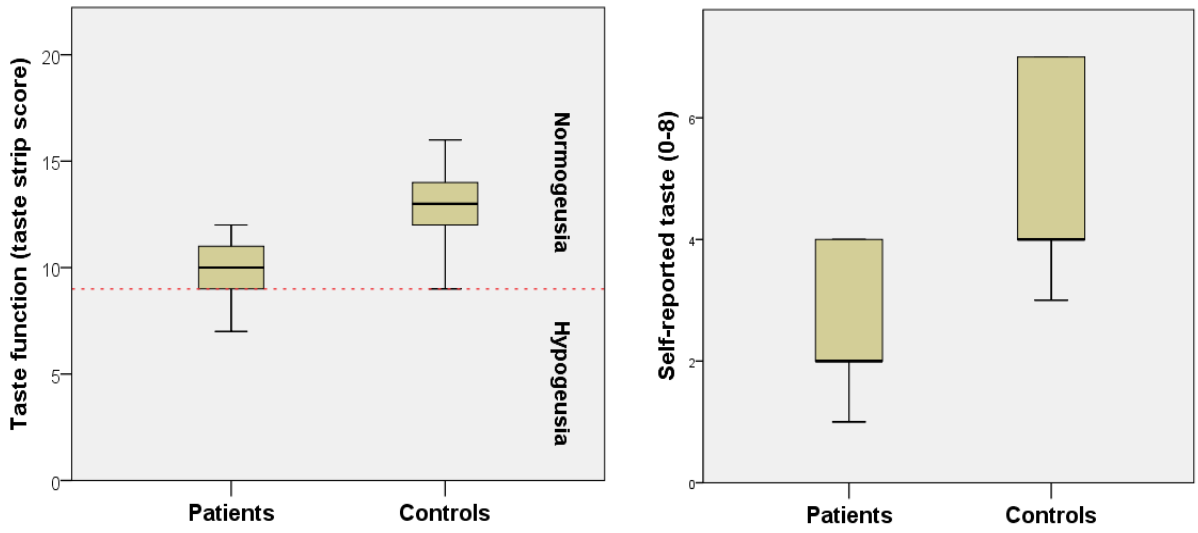


Fig 4.5: Comparison of self-reported and tested taste function in patients with dysgeusia and controls.

Table 4.6: Patient diagnosis (qualitative smell and taste disorders).

Patient	*Quantitative smell disorder*	*Quantitative taste disorder*
1	-	Hypogeusia
2	-	-
3	-	Hypogeusia
4	-	-
5	Anosmia	Hypogeusia
6	Anosmia	-
7	-	Hypogeusia
8	Anosmia	-
9	-	-

4.5 PROP-sensitivity results

There were more bitter-taster in the patient group, but they had a lower mean rating of the PROP-taste intensity than the controls (Table 4.6). Taste scores were significantly positively correlated with PROP phenotype in controls (r=0,76, p=0,018). There was also a correlation

in the patient group, but not significant (r=0,61, p=0,078). Taste scores were also correlated with PROP intensity rating both in patients (r=0,76, p=0,019) and controls (r=0,67, p=0,047). There were also positive correlations between PROP phenotype and PROP intensity rating in both groups in patients (r=0,65, p=0,058) and controls (r=0,95, p=0,000).

Table 4.6: PROP phenotype results of patients and controls.

	Patients with dysgeusia	*Controls*
PROP phenotype		
Taster, n (%)	8 (88,9 %)	7 (77,8 %)
Non-taster, n (%)	1 (11,1 %)	2 (22,2 %)
PROP-intensity rating, 0-10 (mean±SD)	4,7±2,7	6,4±3,8

4.4 Electrogustometry results

The patients had significantly higher electrogustometry scores than controls in all areas of the tongue (Table 4.7). In the control group, strong negative correlations between EGM results and PROP phenotype at anterior left site (r=-0,74, p=0,024), anterior right side (r=-0,81, p=0,008), posterior left side (r=-0,75, p=0,020) and posterior right side (r=-0,79, p=0,011). The controls also had correlating EGM and PROP intensity results at anterior left site (r=-0,66, p=0,050), anterior right side (r=-0,78, p=0,013), posterior left side (r=-0,65, p=0,052) and posterior right side (r=-0,70, p=0,034).

Table 4.7: Electrogustometry results of patients and controls

Location on tongue (mean±SD)	*Patients with dysgeusia*	*Controls*	*p-value*
Anterior left side	5,1±9,1	-4,1±2,8	S (p=0,017)
Anterior right side	7,7±11,7	-4,3±2,9	S (p=0,015)
Posterior left side	9,2±10,6	-3,4±2,6	S (p=0,007)
Posterior right side	9,0±11,0	-3,5±2,7	S (p=0,009)

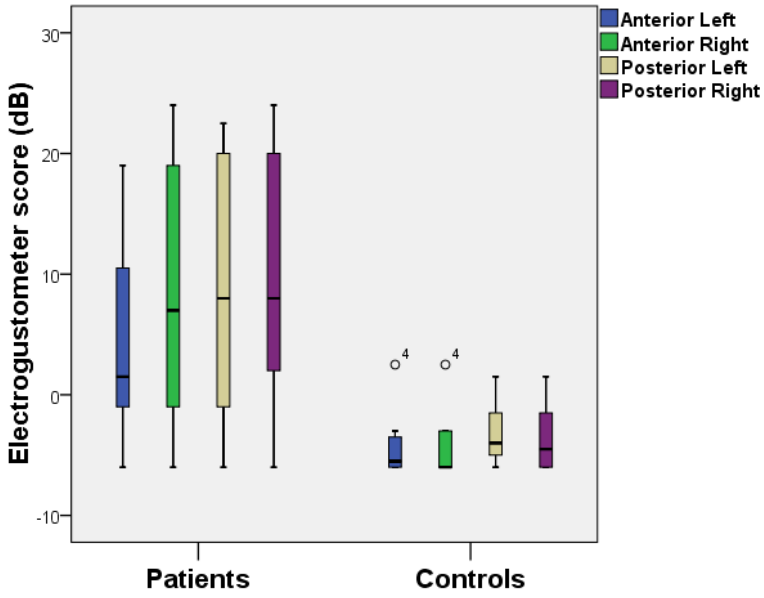


Fig 4.6: Comparison of electrogustometry results in patients with dysgeusia and controls.

4.5 Papillae density results

There was no significant difference in fungiform papillae density, but the patients had lower density than the controls. In the patient group, there was a significant positive correlation between fungiform papillae density and taste strip score (r=0,79, p=0,011). No correlation was found with PROP phenotype.

Table 4.8: Fungiform papillae density results of patients and controls.

	Patients with dysgeusia	*Controls*
Fungiform papillae density (/cm^2) (mean±SD)	22,0±8,4	25,1±6,2

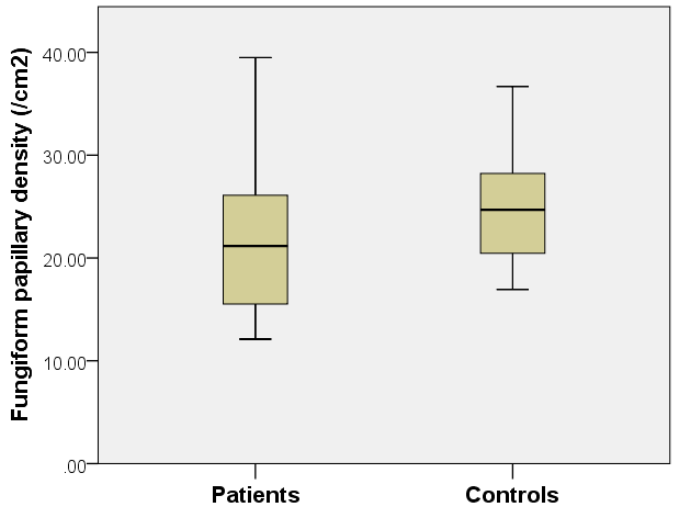


Fig 4.7: Comparison of fungiform papillae density in patients with dysgeusia and controls

4.6 ERP results

Peak latency and amplitude were measured for N1, P2, N2 and P3 peaks. Mean values from all latencies and amplitudes are given in as well as their p-values are given in Table 1, 2 and 3 in the appendix.

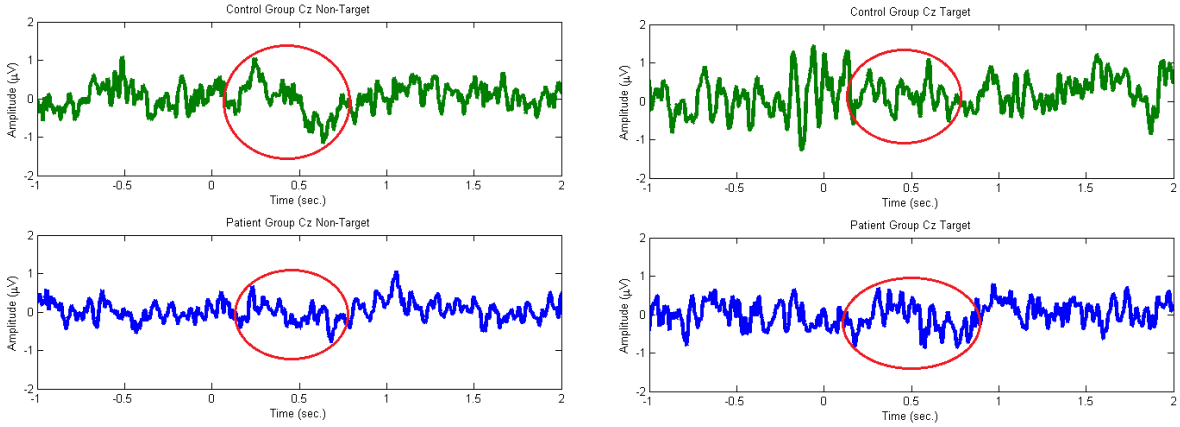


Fig. 4.7: Demonstration of averaged ERP signals from CZ electrode for one subject from each group to both stimulants. Non-target ERP is show to the left, and target ERP is shown to the right. Green waveform on top represents a control subject, while blue on bottom represents a patient. Red circles denote the area of response (ERP).

Using independent t-test, a significant difference between the groups' recordings was found in P2 latency for target stimulus in the PZ electrode (p=0,022). Mean P2 latency was longer for the controls than the patient. Using repeated measures ANOVA, a significant interaction between type of stimulus (target/non-target) and group (patient/control) was found ($F_{1,15}$=4,6, p=0,049, ηp^2=0,24). However, in the t-test corrected with Bonferroni, the results were not significant.

The p-values for the within-group comparison of target and non-target responses are given in Table 4 in the appendix. The patients had significant within-group differences between target and non-target signal in P2 amplitude for FZ electrode (p=0.014), and in N1 and N2 amplitude for PZ electrode (p=0,015 and 0,018 respectively). The target amplitudes were bigger than the non-target amplitudes.
The controls had significant within-group differences between target and non-target signal in N1 amplitude and P3 latency for FZ electrode (p=0,04/p=0,032), in N2 amplitude and P3 latency for CZ electrode (p=0,038/p=0,037), and in P2 latency for PZ electrode (p=0,018). The amplitudes and latencies were larger for target than non-target.

There were significant differences in target and non-target amplitude complexes in the control group (Table 4.9).

Table 4.9: Comparison of N1P2 and N2P3 amplitude complexes in patients and controls.

Amplitude in µV (mean±SD)	*Patients with dysgeusia*	*Controls*
Non-target N1P2complex	2,27±1,03	2,62±1,31*
Target N1P2complex	3,24±1,65	2,88±1,24*
Non-target N2P3complex	2,57±0,91	2,24±0,86**
Target N2P3complex	3,25±1,82	4,44±2,05**

*p=0,048

**p=0,005

5 DISCUSSION

The aim of this study was to comprehensively elucidate the peripheral and central gustatory processing in patients with dysgeusia, and describe possible relations between psychophysical, physiological, and electrophysiological findings. The major findings are (i) patients scores higher on the depression questionnaire, (ii) the patients have lower smell and taste scores compared to the controls, (iii) patients require stronger electrical stimuli to detect taste, (iv) the fungiform papillary density is lower in patients than controls and (v) patients had more difficulty distinguishing target and non-target stimuli. Furthermore, age was associated with smell and taste function in patients. The taste function in the patient group was associated with fungiform papillae density. In both groups, there was correlation between taste function, and PROP phenotype and PROP-intensity rating.

Almost no literature describes prevalence of dysgeusia. However, patients with taste dysfunctions accounts for around 8% of patients complaining of smell and taste disorders. This includes patients with both qualitative and quantitative taste disorders. The proportion of dysgeusia patients among this very small number of taste disorder patients will therefore be even smaller. Recruiting these patients naturally pose a certain challenge, and getting a large sample size was difficult. Since the control group was matched to the patient group, the total sample size for the study is low. This is the major reason behind the extremely low sample power in this study. Due to the high age of the patients, the matched controls had to be older as well. Recruiting controls without any chronic diseases or medicinal use in this age group was challenging. Therefore, participants in both groups suffer from chronic diseases. However, only controls with normal smell and taste function were included, and the none of the medications taken by the control individuals include taste disturbance as a side effect. Some of the participants had to be excluded from the study due to unusable EEG recordings (e.g. because of artefacts, excessive eye blinking or noisy signals). This also contributed to the small sample size.

This study is ongoing. Hence are the results presented here preliminary results. No certain conclusions can be drawn based upon data from such a small sample size, but speculations about trends seen in the data can be made.

Other studies have found that patients suffering from smell or taste disorder have a reduced quality of life, and may report depression due to their problem (43, 44). The patients in the current study scored significantly higher on the depression screening test than the controls. However, none of the participants scored so high as to be labelled “moderately depressed”. Half of the patients scored high enough to be labelled “mildly depressed”, while the other half of the patients were labelled “minimally depressed. All the controls were labelled “minimally depressed”.

The participants’ self-rating of their smell and taste seems to coincide with their test scores. For the mean smell score of the patients there are a few anosmic individuals who pull down the average, and lowers it in comparison to the controls. For the mean taste score however, there is an actual difference for the whole group compared to the controls. In addition, the patients had a lower mean intensity-rating of the PROP-taste strips than the controls, even though the patients had a higher percentage of taster-phenotypes. As the mean intensity rating for the control group included more “0”-answers, one might expect the mean values of both groups to be the same, or the patients’ mean rating to be higher, if the groups had to difference in taste perception. Several studies have found that smell and taste function deteriorates with age (44-46). In this study, there were no significant differences in age between in patient and control group. Any differences found in smell and taste would therefore presumably be due to the patients’ condition, not their age.

When it comes to salivary rate, the patient results span a greater spectrum than the control results. On average, normal unstimulated flow rate is 0,3-0.4 mL/min (47, 48). Two of the patients had salivary rate below 0,1 mL/min, which is considered hyposalivation (49). Dry mouth (xerostomia) can be a reason for dysgeusia occurring (5), so it is not so surprising to see some patients with low salivary rate, however the non-hyposalivaric patients seem to produce more saliva than the controls. The patients with the highest salivary rates are also the ones who report their taste disturbance as being constant. It is possible that the increased salivary rate in the non-xerostomic dysgeusia patients is correlated with their dysfunction – that the internal taste stimulus is making them salivate more. In the current study only

unstimulated saliva was collected. Both because unstimulated whole saliva flow rate correlates more with dry mouth than stimulated whole saliva rate (50), and because the equipment for collecting stimulated saliva was unavailable at the time of data collection. It might be of interest to also collect stimulated saliva.

Fungiform papillae density is slightly lower in the patient group. It was found that taste function and papillae density was positively correlated. This makes sense, since a greater number of fungiform papillae would presumably mean a greater number of taste buds. Some studies find that PROP sensitivity is associated with the density of fungiform papillae on the anterior surface of the tongue (51-53), while others do not (54). This study found no correlation with papillae density and PROP phenotype.

There were clear significant differences in the detection threshold for electrical stimuli in patients and controls. The patients have much higher thresholds, indicating they require a stronger stimulus to taste something. There was also a moderate correlation with EGM and taste scores in both groups, but no correlation with papillae density. The reason for the increased electrical detection threshold might be the conduction of the signal, rather than a decreased number of papillae, and hence fewer taste buds.

The controls presented some differences in non-target and target stimulus N2 amplitudes, and N2 and P3 latencies. Although not all differences came out as significant, where there were significant differences, a tendency can be seen for the same amplitude/latency in the other electrodes as well. The N2 and P3 peaks are representative of the cognitive processing of the stimulus. As the participants were given the task of distinguishing one stimulus from the other, and count them mentally, it is expected to see the greatest difference between target and non-target here. The amplitudes and latencies for the target stimulus were increased compared to non-target amplitudes and latencies. This is the expected pattern in this kind of oddball paradigm (32), which means the controls were able to discriminate the target and non-target stimuli successfully.

The patients' showed some significant differences between the stimulus types, but these differences seem to be random, and not consistent in the measurement zones (e.g. a significant difference between target and non-target was found for N2 amplitude in the PZ electrode, but the difference in the FZ and CZ electrodes were far from significant). This indicates that the patients had more difficulty distinguishing the stimuli. This inconsistency may be due to their condition, based on the nature of their distorted taste processing. The given task of discriminating the stimuli, seem to be harder for the patients because they are struggling to process both the target and non-target stimuli. Also, they are putting a lot of sensorial and cognitive efforts to process the stimuli and completing the task in comparison with the controls. The within-group differences can therefore not be expected to be that big. One gERP study utilizing one-sided stimulation which included preliminary data from a hemi-augeusic patient saw an absence of response in the ageusic side, while the non-geusic side showed a response (14). This indicates that a lack of gERP response could be expected in ageusic patients. The patients in the current study however, were not agausic. As the patients to varying degree have a more or less constant taste sensation in their mouth, it may make it more difficult for them to perform the task given to them, namely differentiating the two gustatory stimuli and count them mentally. One patient had trouble distinguishing the salty stimuli coming from the gustometer from the underlying constant salty taste in her mouth caused by the dysgeusia. It is possible that other types of dysgeusia also distorts or masks the gustatory stimuli. This assumption is supported by the fact that the N2P3 amplitude complexes were significantly different in the control group, but not in the patient group.

The repeated measures ANOVA would be the best statistical method to analyse this type of data, and for this reason repeated measures ANOVA was applied to this data even though the sample size was small – a characteristic which would speak against using the use of ANOVA. The only significant result found was stimulus-group interaction for P3 amplitude in PZ electrode. If the sample size had been bigger, the t-test would likely have shown significance as well. Since this is only an explorative study, and the sample size is so small, it was decided to use t-tests instead.

One study of gERP found that the female participants had larger gERP responses than men (14). As this study group selection is so heavily female, this could not be explored in this study.

Even though there are more men in the control group as compared to the patient group, this should not lead to different conclusions of the results. This study generally found the patient group to have poorer function than the controls. Several studies have found women to outperform men when it comes to smell and taste, and even gERPs. Therefore, having less men in the control group should theoretically not reduce the differences between the groups, but rather increase them.

It can be challenging to obtain good EEG recordings without any noise. If the participant breathes to heavily, moves their head or blinks a lot, it creates artefacts and distortions in the EEG signals. Especially the eldest participant struggled more to hold still during the recording. Noise in the signals makes it more difficult to distinguish the peaks when measuring. Even though participants with excessively noisy EEG recordings were excluded from the dataset, the possibility of some small errors being made in the peak-to-peak measurements cannot be excluded completely. However, these will not be large errors, and will not likely affect the result in a consequential manner.

6 CONCLUSION

The preliminary findings in this study were that patients were more depressed than controls. The patients had a poorer sense of smell and taste compared to controls. Peripheral evaluation found that the patients required higher thresholds of stimuli before they could detect taste. Differences were also found in cortical processing of taste between patients and controls. The patients had a harder time distinguishing taste stimuli. This may be due to the nature of their disorder, with the constant or intermittently underlying taste disturbance masking or distorting external taste stimuli. As this study is on-going, no certain conclusions can be drawn at this point, and further research is necessary to determine these factors' contribution to taste disturbance.

REFERENCES

1. Welge-Luessen A, Hummel T. Management of Smell and Taste Disorders : A Practical Guide for Clinicians. Stuttgart: Georg Thieme Verlag; 2014.
2. Sakai M, Ikeda M, Kazui H, Shigenobu K, Nishikawa T. Decline of gustatory sensitivity with the progression of Alzheimer's disease. International Psychogeriatrics. 2016;28(03):511-7.
3. Ackerman BH, Kasbekar N. Disturbances of taste and smell induced by drugs. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 1997;17(3):482-96.
4. Hummel T, Nordin S. Olfactory disorders and their consequences for quality of life. Acta Oto-Laryngologica. 2005;125(2):116-21.
5. Doty RL. Handbook of Olfaction and Gustation. 2nd ed. New York: Marcel Dekker; 2003.
6. Welge-Lüssen A, Dörig P, Wolfensberger M, Krone F, Hummel T. A study about the frequency of taste disorders. Journal of Neurology. 2011;258(3):386-92.
7. Deems DA, Doty RL, Settle RG, Moore-Gillon V, Shaman P, Mester AF, et al. Smell and taste disorders, a study of 750 patients from the University of Pennsylvania Smell and Taste Center. Archives of otolaryngology–head & neck surgery. 1991;117(5):519-28.
8. Cowart BJ, Young IM, Feldman RS, Lowry LD. Clinical disorders of smell and taste. Occupational medicine (Philadelphia, Pa). 1997;12(3):465-83.
9. Mueller C, Kallert S, Renner B, Stiassny K, Temmel A, Hummel T, et al. Quantitative assessment of gustatory function in a clinical context using impregnated" taste strips". Rhinology. 2003;41(1):2-6.
10. Ikui A. A review of objective measures of gustatory function. Acta Oto-Laryngologica. 2002;122(4):60-8.
11. Kobal G. Gustatory evoked potentials in man. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section. 1985;62(6):449-54.
12. Kobayakawa T, Ogawa H, Kaneda H, Ayabe-Kanamura S, Saito S. Spatio-temporal Analysis of Cortical Activity Evoked by Gustatory Stimulation in Humans. Chemical Senses. 1999;24(2):201-9.
13. Hummel C, Frasnelli J, Gerber J, Hummel T. Cerebral processing of gustatory stimuli in patients with taste loss. Behavioural brain research. 2007;185(1):59-64.
14. Hummel T, Genow A, Landis BN. Clinical assessment of human gustatory function using event related potentials. Journal of Neurology, Neurosurgery & Psychiatry. 2010;81(4):459-64.
15. Sollai G, Melis M, Pani D, Cosseddu P, Usai I, Crnjar R, et al. First objective evaluation of taste sensitivity to 6-n-propylthiouracil (PROP), a paradigm gustatory stimulus in humans. Scientific Reports. 2017;7:40353.
16. BioingeniørfagligInstitutt. Etikk for bioingeniører. 2 ed. Grønland, Oslo: NITO; 2013.
17. Jacob T. Papillae and taste buds. 2007.
18. Pathway of the gustatory system. http://aibolita.com/nervous-diseases/46980-gustation-taste.html2015.
19. Kobayakawa T, Endo H, Ayabe-Kanamura S, Kumagai T, Yamaguchi Y, Kikuchi Y, et al. The primary gustatory area in human cerebral cortex studied by magnetoencephalography. Neuroscience letters. 1996;212(3):155-8.
20. Functional Areas of Cerebral Cortex. Pearson Education Inc., publishing as Benjamin Cummings; 2007.

21. Kobayashi Y, Habara M, Ikezazki H, Chen R, Naito Y, Toko K. Advanced Taste Sensors Based on Artificial Lipids with Global Selectivity to Basic Taste Qualities and High Correlation to Sensory Scores. Sensors (Basel, Switzerland). 2010;10(4):3411-43.
22. Bufe B, Breslin PA, Kuhn C, Reed DR, Tharp CD, Slack JP, et al. The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. Current Biology. 2005;15(4):322-7.
23. Kim U-k, Jorgenson E, Coon H, Leppert M, Risch N, Drayna D. Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. Science. 2003;299(5610):1221-5.
24. Drewnowski A, Rock CL. The influence of genetic taste markers on food acceptance. The American journal of clinical nutrition. 1995;62(3):506-11.
25. Guo S-W, Reed DR. The genetics of phenylthiocarbamide perception. Annals of Human Biology. 2001;28(2):111-42.
26. Gescheider GA. Psychophysics : The Fundamentals. 3rd ed. ed. Hoboken: Taylor and Francis; 2013.
27. García-Pérez MA. Forced-choice staircases with fixed step sizes: asymptotic and small-sample properties. Vision research. 1998;38(12):1861-81.
28. Stillman J, Morton R, Hay K, Ahmad Z, Goldsmith D. Electrogustometry: strengths, weaknesses, and clinical evidence of stimulus boundaries. Clinical Otolaryngology & Allied Sciences. 2003;28(5):406-10.
29. Macmillan NA, Creelman CD. Detection theory: A user's guide: Psychology press; 2 004.
30. Niedermeyer E, Da Silva FL, Lopes Da Silva FH. Electroencephalography: Basic Principles, Clinical Applications, and Related Fields. Philadelphia: Philadelphia : Wolters Kluwer; 2004.
31. Light GA, Williams LE, Minow F, Sprock J, Rissling A, Sharp R, et al. Electroencephalography (EEG) and Event-Related Potentials (ERP's) with Human Participants. Current protocols in neuroscience / editorial board, Jacqueline N Crawley [et al]. 2010;CHAPTER:Unit-6.2524.
32. Sur S, Sinha VK. Event-related potential: An overview. Industrial Psychiatry Journal. 2009;18(1):70-3.
33. Oostenveld R, Praamstra P. The five percent electrode system for high-resolution EEG and ERP measurements. Clinical Neurophysiology. 2001;112(4):713-9.
34. BioSemi. 128 Channel ABC Layout.
35. Landis B, Hummel T, Lacroix J-S. Basic and clinical aspects of olfaction. Advances and technical standards in neurosurgery: Springer; 2005. p. 69-105.
36. Beck AT, Steer RA, Brown GK. BDI-FastScreen for medical patients. 1 ed: Pearson; 2013.
37. Hummel T, Sekinger B, Wolf SR, Pauli E, Kobal G. 'Sniffin'sticks': olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. Chemical senses. 1997;22(1):39-52.
38. Cecchini MP, Osculati F, Ottaviani S, Boschi F, Fasano A, Tinazzi M. Taste performance in Parkinson's disease. Journal of Neural Transmission. 2014;121(2):119-22.
39. Harris H, Kalmus H. The measurement of taste sensitivity to phenylthiourea (PTC). Annals of Human Genetics. 1949;15(1):24-31.
40. OpenStax. Anatomy & Physiology: OpenStax CNX; 2016. Available from: http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@8.24.

41. Nuessle TM, Garneau NL, Sloan MM, Santorico SA. Denver Papillae Protocol for Objective Analysis of Fungiform Papillae. Journal of Visualized Experiments : JoVE. 2015(100):52860.
42. Singh PB, Iannilli E, Hummel T. Segregation of gustatory cortex in response to salt and umami taste studied through event-related potentials. Neuroreport. 2011;22(6):299-303.
43. Temmel AP, Quint C, Schickinger-Fischer B, Klimek L, Stoller E, Hummel T. Characteristics of olfactory disorders in relation to major causes of olfactory loss. Archives of Otolaryngology–Head & Neck Surgery. 2002;128(6):635-41.
44. Boyce JM, Shone GR. Effects of ageing on smell and taste. Postgraduate Medical Journal. 2006;82(966):239.
45. Pavlidis P, Gouveris H, Anogeianaki A, Koutsonikolas D, Anogianakis G, Kekes G. Age-related changes in electrogustometry thresholds, tongue tip vascularization, density, and form of the fungiform papillae in humans. Chemical senses. 2013;38(1):35-43.
46. Glanville EV, Kaplan AR, Fischer R. Age, sex and taste sensitivity. Journal of Gerontology. 1964;19(4):474-8.
47. Dawes C. Physiological factors affecting salivary flow rate, oral sugar clearance, and the sensation of dry mouth in man. Journal of Dental Research. 1987;66(2 suppl):648-53.
48. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. The Journal of prosthetic dentistry. 2001;85(2):162-9.
49. Sreebny LM, Valdini A. Xerostomia. A neglected symptom. Archives of internal medicine. 1987;147(7):1333-7.
50. Suh KI, Lee JY, Chung JW, Kim YK, Kho HS. Relationship between salivary flow rate and clinical symptoms and behaviours in patients with dry mouth. Journal of oral rehabilitation. 2007;34(10):739-44.
51. Melis M, Atzori E, Cabras S, Zonza A, Calò C, Muroni P, et al. The gustin (CA6) gene polymorphism, rs2274333 (A/G), as a mechanistic link between PROP tasting and fungiform taste papilla density and maintenance. PloS one. 2013;8(9):e74151.
52. Miller IJ, Reedy FE. Variations in human taste bud density and taste intensity perception. Physiology & behavior. 1990;47(6):1213-9.
53. Bajec MR, Pickering GJ. Thermal taste, PROP responsiveness, and perception of oral sensations. Physiology & behavior. 2008;95(4):581-90.
54. Garneau NL, Nuessle TM, Sloan MM, Santorico SA, Coughlin BC, Hayes JE. Crowdsourcing taste research: genetic and phenotypic predictors of bitter taste perception as a model. Frontiers in Integrative Neuroscience. 2014;8:33.

APPENDIX

Table 1: Mean latencies and amplitudes for FZ electrode in patients and controls.

	FZ electrode (mean±SD)	*Patients with dysgeusia*	*Controls*
Non-target	N1 time (msec)	178±44	166±32
	N1 amplitude (μV)	-1,51±2,03	-0,38±1,58
	P2 time (msec)	265±73	244±42
	P2 amplitude (μV)	2,70±0,85	4,00±2,64
	N2 time (msec)	355±104	334±72
	N2 amplitude (μV)	-0,70±2,95	-0,54±1,34
	P3 time (msec)	436±142	412±95
	P3 amplitude (μV)	2,77±2,55	2,60±2,35
Target	N1 time (msec)	197±23	204±49
	N1 amplitude (μV)	-2,52±2,20	-2,39±1,97
	P2 time (msec)	305±83	266±38
	P2 amplitude (μV)	4,12±1,09	3,24±2,21
	N2 time (msec)	379±122	400±74
	N2 amplitude (μV)	-1,44±2,23	-1,59±2,63
	P3 time (msec)	469±119	520±86
	P3 amplitude (μV)	4,68±2,29	4,33±2,10

Table 2: Mean latencies and amplitudes for CZ electrode in patients and controls.

	CZ electrode (mean±SD)	*Patients with dysgeusia*	*Controls*
Non-target	N1 time (msec)	169±44	172±41
	N1 amplitude (μV)	-1,17±0,94	-1,02±0,92
	P2 time (msec)	241±52	273±56
	P2 amplitude (μV)	1,04±0,53	1,52±1,23
	N2 time (msec)	328±97	332±69
	N2 amplitude (μV)	-0,84±1,06	-0,74±0,57
	P3 time (msec)	423±140	450±118
	P3 amplitude (μV)	1,36±1,31	1,46±0,98
Target	N1 time (msec)	188±59	177±18
	N1 amplitude (μV)	-1,69±0,98	-1,54±0,74
	P2 time (msec)	273±68	288±66
	P2 amplitude (μV)	1,56±1,01	1,33±1,14
	N2 time (msec)	342±105	402±92
	N2 amplitude (μV)	-1,10±0,92	-1,84±1,34
	P3 time (msec)	497±141	577±119
	P3 amplitude (μV)	2,14±1,30	2,59±1,74

Table 3: Mean latencies and amplitudes for PZ electrode in patients and controls.

PZ electrode (mean±SD)		*Patients with dysgeusia*	*Controls*
Non-target	N1 time (msec)	166±49	197±52
	N1 amplitude (µV)	-0,96±0,63	-0,82±0,42
	P2 time (msec)	233±97	287±47
	P2 amplitude (µV)	0,99±0,83	1,02±0,95
	N2 time (msec)	359±111	380±119
	N2 amplitude (µV)	-0,92±0,42	-0,98±0,74
	P3 time (msec)	495±135	553±215
	P3 amplitude (µV)	1,26±0,45	2,06±1,57
Target	N1 time (msec)	220±57	208±38
	N1 amplitude (µV)	-1,73±0,57	-1,47±0,83
	P2 time (msec)	279±65*	345±43*
	P2 amplitude (µV)	1,30±1,03	1,11±0,62
	N2 time (msec)	396±100	447±89
	N2 amplitude (µV)	-2,29±1,50	-1,77±0,94
	P3 time (msec)	574±114	618±186
	P3 amplitude (µV)	1,77±0,96	1,51±1,46

*p=0,022

Table 4: P-values of within group comparison of mean non-target and target responses for FZ, CZ and PZ electrodes in patients and controls.

	Patients with dysgeusia			*Controls*		
	FZ	**CZ**	**PZ**	**FZ**	**CZ**	**PZ**
	p-value	*p-value*	*p-value*	p-value	p-value	p-value
N1 time	0.311	0.457	0.047	0.088	0.751	0.606
N1 amplitude	0.373	0.271	0.015	0.041	0.204	0.066
P2 time	0.342	0.269	0.252	0.288	0.618	0.018
P2 amplitude	0.014	0.190	0.485	0.543	0.744	0.816
N2 time	0.679	0.780	0.470	0.093	0.085	0.204
N2 amplitude	0.597	0.591	0.018	0.331	0.038	0.075
P3 time	0.622	0.277	0.198	0.032	0.037	0.510
P3 amplitude	0.139	0.224	0.166	0.144	0.109	0.461