Making Sense of Taste

Psychophysical, molecular biological and neurophysiological studies of umami taste processing in humans

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“Clouds come floating into my life, no longer to carry rain or usher storm, but to add color to my sunset sky.”

Rabindranath Tagore

To Noah, Simon and Goldy

To my mother

Contents

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Oslo, 2011

Preet Bano Singh

List of papers

The following papers (I-IV) are submitted in partial fulfilment of the requirements for the degree Philosophiae Doctor (Ph.D.) at the Faculty of Dentistry, University of Oslo, Oslo, Norway. The thesis is based on experimental work carried out at The Smell and Taste Clinic, Department of Otorhinolaryngology, University Medical School, Dresden, Germany and Laboratory of Sensory Neurobiology, INRA, Jouy-en-Josas, France.

Paper I Singh PB**,** Schuster B, Seo H-S. **Variation in umami taste perception in the German and Norwegian population.** Eur J Clin Nutr. 64; 1248-50. **2010.**

Paper II Raliou M, Grauso M, Hoffmann B, Schlegel-Le-Poupon C, Nespoulous C, Débat H, Belloir C, Wiencis A, Maud Sigoillot, Singh PB, Trotier D, Pernollet J-C, Montmayeur J-P, Faurion A, Briand L. **Human genetic polymorphisms in T1R1 and T1R3 taste receptor subunits affect their function.** Chem Senses. [Epub ahead of print]. **2011**.

Paper III Singh PB, Iannilli E, Hummel T. **Segregation of gustatory cortex in response to salt and umami taste studied through event-related potentials**. NeuroReport. 22; 299-303. **2011.**

Paper IV Iannilli E , Singh PB , Schuster B, Gerber J, Hummel T. **Taste laterality studied by means of umami and salt stimuli: a fMRI study.** J Neurosci. Submitted.

Abbreviations

AC	Adenylate Cyclase
ASIC	Acid-Sensing Channel
BNaCl	Brain Type Sodium Channel
BSA	Bovine Serum Albumin
cNMP	Cyclic Nucleotide Monophosphate
cAMP	Cyclic Adenosine Monophosphate
DAG	Diacylglycerol
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal Bovine Serum
EEG	Electroencephalogram
ENaC	Epithelial Sodium Ion Channel
ERP	Event Related Potentials
fMRI	Functional Magnetic Resonance Imaging
GMP	Guanylate Monophosphates
GPCR	G-protein Coupled Receptors
HEK293	Human Embryonic Kidney Cell Line
IMP	Inosinate Monophosphates

IP_3	Ionositol Trisphosphate
MDEG1	Mammalian Degenerin-1-Channel
mGLuRs	Metabotropic Glutamate Receptors
MSG	Monosodium Glutamate
NCAM	Neural Cell Adhesion Molecule
nsSNPs	Non-synonomous Single Nucleotide Polymorphisms
NTS	Nucleus Tractus Solitarius
OFC	Orbitofrontal Cortex
PLC-β2	Phospholipase C- β2
PDE	Phosphodiesterase E
SNAP25	Synaptosomal-Associated Protein 25
SNPs	Single Nucleotide Polymorphisms
SPM	Statistical Parametric Mapping
TRCs	Taste Receptor Cells
TRPM5	Transient Receptor Potential Melastanin 5
TRPV1t	Transient Receptor Potential Vanilloid 1 Variant
VPM	Ventroposterior Medial Nucleus
MDEG1	Mammalian Degenerin-1 Channel

1. Introduction

1.1 Historical perspective of taste

Vision, hearing, touch, smell and taste are the five classical sensory modalities that allow animals to establish an internal representation of the outer world. Among these five senses, the sense of smell and taste are known as chemical senses as they play a crucial role in detection of chemical substances in the environment. All environmental components required for survival enter our bodies through the nose and mouth. Olfaction is considered to be instrumental in locating potential food in the environment, while the sense of taste plays an important role in making ingestive decisions. As the role of diet is of great importance in human health, it has become increasingly important to study the cellular underpinnings of taste that contribute to the difference between lifelong health and chronic diseases. Gustatory clues ensure the maintenance of the energy supply through sweet tasting carbohydrates and umami tasting amino acids, whereas salty and sour tasting minerals maintain electrolyte balance. Bitter taste perception indicates that the food stuff may be toxic or poisonous. By providing important information regarding the nutritional value and toxicity of food substances, the sense of taste indicates whether it should be ingested or rejected (Scott and Mark 1987; Bartoshuk, 1991). Thus, the sense of taste serves as primary gatekeeper controlling voluntary ingestion of substances.

Although, the scientific knowledge about the chemical senses is relatively recent, these senses have played a significant role in the everyday life of humans since prehistoric times. This fact is evident from the examples in history of how important spices and perfumes have been, to knit ancient civilizations together. The *Silk*

Route, an extensive network of trade routes across the Asian continent connecting Asia, Northeast Africa and Europe, is such an example.

Already in 1566, work solely devoted to the sense of taste was published by Laurentius Gryllus and in 1581, nine types of different taste qualities were listed by Jean François Fernel (Witt et al., 2003). These nine taste qualities included sweet, bitter, sour, salty, astringent, pungent, harsh, fatty and insipid. Casserius in 1609 described the detailed structure of the tongue, and the lingual papillae were associated with the taste sensation by Marcello Malpighi in 1664 and Lorenzo Bellini in 1665 (Witt et al., 2003).

Scientific advancement leading to our present understanding of gustation has progressed considerably since the mid-seventeenth century. However, in the modern scientific world, the sensory systems such as vision and hearing have received much attention compared to chemical senses and they played a crucial role to explain the mechanisms of perception at the cerebral level. The dark period for chemosensory senses seems to have passed as chemical senses have received tremendous attention especially after Richard Axel and Linda B. Buck were awarded the Nobel Prize in medicine in the field of olfaction in 2004.

1.2 Morphology of peripheral gustatory system

The peripheral functional organisation of the taste system includes the taste papillae, taste buds, taste cells and their innervation. Different types of taste cells are found within each taste bud and taste buds are assembled within specific taste papillae (Fig 1). Three different cranial nerves are responsible for communication between the peripheral taste organs and the central nervous system and hence, taste perception.

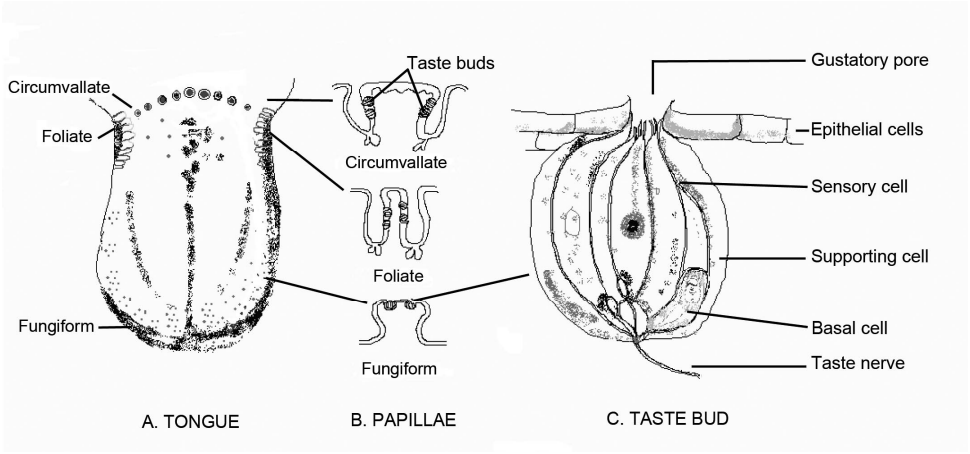

Figure 1. Schematic drawing by Prof. Tim Jacob showing (A) surface of tongue and localisation of different papillae on the tongue (B) fungiform papillae with apically situated taste buds, foliate papillae and cirumvallate papillae with laterally placed taste buds (C) structure of a taste bud with different types of cells and the apical taste pore. (Reproduced with permission from the author).

1.2.1 Taste papillae

"Many papillae are evident, I might say, innumerable, and the appearance is so elegant that they catch the view and the thoughts of the observer, and control him for a long time and not without enjoyment.....". This is the English translation of how Bellini in 1665 described the papillae on the human tongue (Witt et al., 2003).

On the dorsal surface of the tongue two categories of papillae are found:

A. Non-gustatory papillae

Filiform papillae and conical papillae are non-gustatory papillae, as they do not participate in the taste transduction. These papillae do not contain taste buds and probably have a purely mechanical function. Filiform papillae are quite abundant in number while the prevalence of conical papillae varies. (Petrén and Carlsöö,1976)

B. Gustatory papillae

Gustatory papillae bear taste buds and are present on the tongue, the epithelium of the palate, oropharynx, larynx and the upper esophagus. We distinguish between three types of gustatory papillae - fungiform papillae, foliate papillae and circumvallate papillae. The fungiform papillae are mushroom-shaped papillae intermingled between the long grass-shaped filiform papillae (Fig 2) and found predominantly in the anterior two third part of the tongue (Witt et al., 2003). Foliate papillae are oval shaped papillae located bilaterally along the lateral ridges of the tongue. Circumvallate papillae, described by Soemmering, 1806, are situated in a V-shaped line directly anterior to the Sulcus terminalis. They are round in structure and measure from 2 to 8 mm in diameter. The number of circumvallate papillae varies from 4 and 18 in humans, with an average of 9 papillae (Witt et al., 2003).

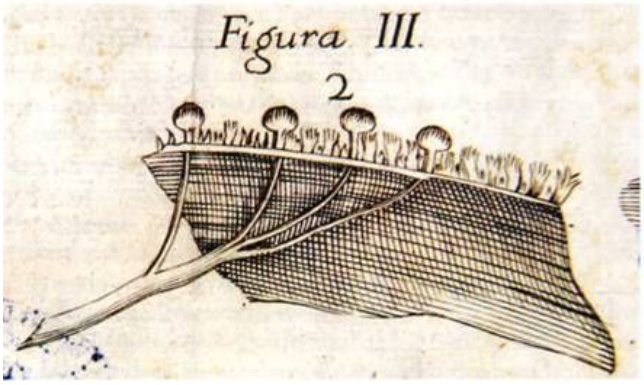

Figure 2. Drawing by Malpighi (1664) of fungiform papillae intermingled between grass-like filliform papillae when he discovered mucosal elevations on the tongue associated with nerve fibres.
(http://www.scienzagiovane.unibo.it/english/scientists/malpighi-3.html)

1.2.2 Taste buds

Taste buds are the principal organs responsible for the detection of gustatory stimuli. They are found in distinct papillae and are about 5 to 7 μm in diameter in humans (Arvidson, 1976). Taste buds are bulb-shaped structures, composed of taste cells (Fig 1). Each taste bud is characterised by a single gustatory pore, which is in direct contact with saliva. The taste cells within the bud extend their microvilli through the apical pore and provide a surface for binding of taste stimuli to the receptors and facilitation of taste transduction.

1.2.3 Taste cells

Each taste bud is composed of 50-100 taste receptor cells (TRCs). These cells comprise of a small number of proliferative basal cells and numerous elongated cells. Elongated cells are further divided into three categories- type I, type II and type III cells depending on their morphological characteristics, first defined by Murray (Murray, 1973). In some classifications the basal cells are referred to as type IV cells. These different types of TRCs have different roles in taste transduction.

Type I cells also known as “dark cells” (Delay et al., 1986; Nelson and Finger, 1993) seem to have a glial-like function. This fact is evident from the characteristic feature of these cells, in that they extend long and dense microvilli around other types of taste cells and that they express glial glutamate transporter (Lawton et al., 2000),facilitating functional isolation of different TRCs and transmitter clearance (Finger, 2005).

Type II cells or “light cells” contain a large, round nucleus and short microvilli. These cells express all the necessary elements for taste transduction of sweet, bitter and umami taste, namely, the T1R and T2R taste receptor families (Hoon et al., 1999; Miyoshi et al., 2001) and the components necessary for intercellular taste cascade, phospholipase C-β2, PLC-β2, transient receptor potential melastanin 5, TRPM5 (Miyoshi et al., 2001; Clapp et al., 2001) and gustducin (Boughter et al., 1997). These cells do not form conventional synapses and represent about 35% of the population of TRCs.

Type III cells or “ intermediate cells” form conventional synapses with the afferent fibres of the gustatory taste nerves (Murray, 1986) and are consequently rich in the synaptic membrane protein SNAP25 (Yang et al., 2007), the neural cell adhesion molecule NCAM (Nelson and Finger, 1993) and the neurotransmitter serotonin (Yee et al., 2001). The presence of a prominent synaptic contact confirms that these cells play a vital role in transmission of signal to the central nervous system (Finger, 2005).

Type IV cells also called “basal cells” are located at the bottom of the taste buds and might be progenitor cells of elongated cells. These are small, undifferentiated cells, and do not have microvilli that reach the gustatory pore (Murray, 1973).

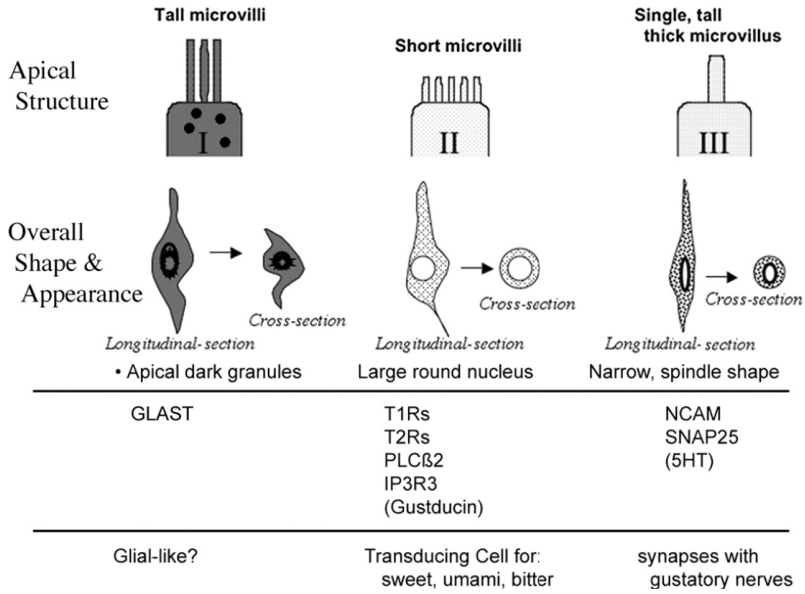

Figure 3. Schematic drawing by Thomas E. Finger, showing morphological (row 1) and biochemical (row 2) features of different types of taste cells. The bottom row suggests possible functions of each type of taste cells. (Reproduced with permission from the author).

Type V cells also called “marginal cells”. This cell type was not classified by Murray but is an extension of Murray’s nomenclature (Beidler and Smallman, 1965; Reutter and Witt, 1993). The role of these cells is not clarified but they might be taste bud stem cells (Beidler and Smallman, 1965).

1.2.4 Taste nerves

The taste buds are innervated by branches of three cranial nerves. The fungiform papillae on the anterior tongue are innervated by the chorda tympani branch of the **facial nerve (VII)**, while the greater petrosal branch of the same nerve goes to the palate. Both the foliate papillae and circumvallate papillae on the posterior tongue are innervated by the lingual branch of the **glossopharyngeal nerve (IX)**. The

superior laryngeal branch of the **vagus nerve (X)** carries out chemical responses in the larynx.

The role of the **trigeminal nerve (V)** in taste perception has been a matter of discussion for several years and is still not clarified. The somatosensory information is conveyed from the tongue to the trigeminal ganglion by this nerve, via the lingual fibres (Witt et al., 2003). The anatomical proximity of the gustatory and somatosensory nerve fibres indicates that there might as well be interactions between the gustatory and somatosensory sensations (Katz et al., 2000). The sense of taste is not just the perception of aroma of the food when introduced in the mouth, it is actually a combination of different sensations like smell, temperature, and texture of the food. However, little is known about how these different sensations interact with each other to give a particular taste sensation.

1.3 The gustatory pathway in humans

Through the gustatory part of cranial nerves the taste information terminates in the nucleus tractus solitarii (NTS) (Torvik, 1955). The second-order gustatory fibres ascend from the NTS towards the pons and project directly to the ventroposterior medial nucleus (VPM) (Beckstead et al., 1980) as there is no evidence for pontine taste relay at the current stage. From the thalamus the fibers terminate in the primary gustatory cortex, the anterior insula/frontal operculum (Small et al., 1999). There are projections from the primary gustatory cortex or “area G” to the caudal orbitofrontal cortex, (Baylis et al., 1995) which has been proposed as the secondary taste cortex.

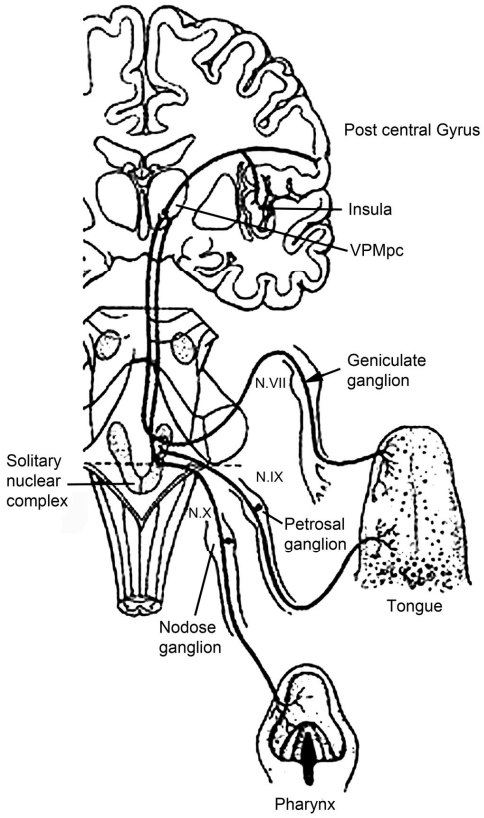

Figure 4. A schematic presentation depicting the gustatory pathway from the peripheral organs to the central nervous system. Note that the three gustatory nerves are following three different pathways from the TRCs to the solitary nucleus. (derived from Dodd and Castellucci,1991)

The laterality of the human gustatory pathway is yet not fully determined, however it is assumed to be similar to the nonhuman primate's gustatory system. From the TRCs the primary gustatory afferents project ipsilaterally to the nucleus of the solitary tract (Goto et al., 1983; Jyoichi et al., 1985; Nakajima et al., 1983). Further, there is supporting evidence on ipsilateral ascension of secondary taste fibres from the solitary nucleus of the medulla oblongata to the pons, as lesions caudal to the pons have shown taste disorders on the ipsilateral side of the tongue (Nakajima et

al., 1983; Uesaka et al., 1998). Although taste disorders following lesions to the pons are predominantly ipsilateral, contralateral and bilateral disturbances have also been reported (Onada and Ikeda, 1999). Similarly, both contralateral and ipsilateral taste deficits have been reported as a result of lesions in the higher order gustatory areas in the primary and secondary taste cortex (Bornstein, 1940; Pritchard et al., 1999). Nevertheless, there is substantial evidence in the literature that suggests bilateral representation of taste at the cortical level (Small, 2006).

Another related, still unresolved issue, concerns the hemispheric dominance of human gustation. Both right hemispheric (Small et al., 1999; Barry et al., 2001) and left hemispheric dominance (Faurion et al., 1998) of human gustation has been proposed. However, many studies report bilateral activation of the insular taste regions (O'Doherty et al., 2001; Small et al., 2003).

Further studies are needed to shed light on the laterality of the gustatory pathways from NTS to the cortex and to elucidate whether there exists a hemisphere dominance of gustatory processing in humans.

1.4 The umami taste

Human taste perception is divided into five categories: sweet, salt, sour, bitter and umami. The umami taste - also known as the "fifth taste"- was first discovered by Kikunae Ikeda (Ikeda, 1909) and is described as a meaty, mouth-filling, characteristic taste, naturally abundant in seaweed, fish, meat, mushrooms, tomatoes and cheese. This taste is now widely accepted as a unique taste quality, different from the other four tastes (Yamaguchi and Ninomiya, 2000; Beauchamp, 2009; Kurihara, 2009). The specific taste of umami is elicited by L-glutamate and is even more pronounced with monosodium glutamate (MSG), which is naturally

present in different palatable foods such as meat, seafood, vegetables, fruits, soy sauce, fermented beans, and dairy products (Yamaguchi and Ninomiya, 2000). MSG is also used as a flavor enhancer in the food industry, based on the fact that MSG enhances the palatability of foods (Yamaguchi 1991; Okiyama and Beauchamp 1998; Yamaguchi and Ninomiya 2000; Prescott 2004; Bellisle 2008), although MSG is not pleasant tasting when dissolved in water (Yamaguchi 1991). Another characteristic feature of MSG is that its effect is synergistically enhanced by the presence of ribonucleoties such as 5´-inosinate monophosphates (IMP) and 5´-guanylate monophosphates (GMP) (Yamaguchi 1967; Rifkin and Bartoshuk 1980; de Araujo et al., 2003). Glutamate is an amino acid, which, in addition to being an umami taste stimulant, also plays a key role in cellular metabolism (Newsholme et al., 2003) and is an important neurotransmitter in the central nervous system (Fonnum 1984).

MSG is more commonly consumed in Asian countries such as Japan, Korea, and Thailand, as compared to the USA and European countries (Löliger, 2000). Although the western world has been exposed to glutamate in their traditional as well as modern meals through meat, fish, and dairy products (Curtis 2009), it seems to be difficult for the general population to describe and discriminate the umami taste from other basic tastes. Although a large number of studies of umami taste have been conducted since the introduction of umami taste, little is known about the familiarity degree of umami taste in the western population.

The taste preference for amino acids has been suggested as a basic nutritional signal that reflects the amount of dietary protein in the body (Mori et al., 1991). Mori and colleagues showed that under severe protein deficiency, rats preferred NaCl

rather than MSG to maintain electrolyte and body fluid balance. In the absence of adequate umami taste perception, healthy individuals might show increased preference to saltiness, yet hypertension is often a complication of excess sodium intake. The hunger for salt might be decreased under adequate protein intake (Mori et al., 1991), which is further regulated by umami taste perception and knowledge of umami taste.

Several of the major health problems challenging the human population today such as obesity, heart diseases, hypertension, type-2 diabetes, and dental caries are all diet related. Variation in taste perception in healthy individuals might play an important role for the dietary choices made by them and, thus, resulting into diet-related health problems.

1.5 Molecular mechanisms of gustation

The taste sensation is initiated by the binding of tastants to the ion channels and receptors located apically on TRC. This coupling results in intracellular signal transduction through downstream components, depolarisation of the cell membrane and the subsequent release of neurotransmitter. The neurotransmitter released from the TRCs binds to the innervating nerves and, hence, taste is perceived in the gustatory cortex. TRCs use different receptor systems to mediate the five different taste qualities. Sour and salty taste is transduced through channel type receptors, whereas sweet, bitter and umami taste is mediated through serpentine transmembrane receptors coupled to trimeric G proteins (GPCR). In other words, the chemosensory transduction in TRCs employs many pathways and a single taste quality involves multiple cellular pathways.

1.5.1 Salt taste

Salt taste transduction plays an important role in electrolyte homeostasis in mammals. This taste is mediated through different mechanisms:

A. Amiloride-sensitive pathway

An amiloride-sensitive epithelial sodium ion channel (ENaC) was proposed as a salt receptor when it was demonstrated that amiloride, an inhibitor of Na^+ ion transport channel, substantially reduced the inward transport of sodium chloride in rat lingual epithelium (Heck et al., 1984). Furthermore, both neural (Brand et al., 1985) and behavioural (Schiffman et al., 1983) responses to NaCl were shown to be sensitive to the diuretic amiloride. This mechanism involves direct depolarization of the TRC induced by influx of Na^+ ions through the apically located amiloride-sensitive sodium channels. This further leads to neurotransmitter release (Gilbertson and Margolskee, 2003).

B. Amiloride-insensitive pathway

Amiloride could not completely inhibit the salt uptake, suggesting an additional pathway for salt taste transduction (Formaker and Hill, 1999). TRPV1t, a variant of transient receptor potential vanilloid 1 has been proposed as a candidate amiloride-insensitive taste receptor for salt transduction (Lyall et al., 2004). Another mechanism proposed is the “paracellular pathway” that involves the movement of Na^+ through the tight junctions on the basolateral membrane of TRCs and subsequently opening of sodium channel on the basal membrane rather than the apical membrane. (Elliot and Simon, 1990; Ye et al., 1991).

The molecular mechanisms underlying the salt taste including taste receptors are not yet fully elucidated. Future studies will be required to establish the role of the proposed receptors for salt taste transduction.

1.5.2 Sour taste

The perception of sour taste has two functions. Firstly, maintaining the electrolyte uptake as in salt taste and secondly, it serves as a signal that warns animals against ingestion of spoiled food, as it often tastes sour. The sour taste perception is directly related to protons (H^+) in humans and several transduction mechanisms have been proposed.

One of the mechanisms, applicable primarily to the lower vertebrates, is the direct proton-mediated inhibition of an apical K^+ channel (Kinnamon et al., 1988) which depolarises the TRC and subsequently induces the release of transmitter substance. Some new members of the ENaC family of ion channels have been cloned, and they seem to be involved in the acid sensing in TRCs (Gilbertson and Margolskee, 2003). Expression of multiple combinations of ENaC subunits is suggested to induce different acid responses in TRCs. The acid-sensing channel, ASIC is another member of the ENaC family, which might be responsible for sour taste transduction (Waldmann et al., 1997). Mammalian degenerin-1channel (MDEG1), also known as brain type Na^+ Channel or BNaCl has been identified in TRCs by in-situ hybridization and it also seems to play a role in acid sensing (Gilbertson and Margolskee, 2003). Finally, the paracellular pathway with high proton permeability through the tight junctions between TRCs, has been suggested to play a role in sour taste mediation (DeSimone et al., 1995).

Several members of the ENaC family have been identified in TRCs, but it is yet not clear what role these elements hold in the sour taste. With the recent advancements in molecular biology combined with electrophysiology, taste genetics and transgenic animal models, a complete understanding of taste transduction might be possible in the near future.

1.5.3 Sweet, bitter and umami taste

Three different families of GPCRs are responsible for taste transduction of the sweet, bitter and umami taste: T1R family, T2R family, and metabotrophic glutamate receptor family (mGluRs).

T1R family

The T1R receptor family belongs to class C of G protein coupled receptors. This family has three subunits T1R1, T1R2 and T1R3, which are taste bud specific receptors (Nelson et al., 2001). T1R1 is expressed commonly in fungiform papillae in taste buds, but not so often in circumvallate papillae. On the contrary, T1R2 is expressed in circumvallate papillae, but rarely expressed in fungiform papillae (Hoon et al., 1999). T1R3 is expressed in both fungiform and circumvallate papillae (Nelson et al., 2001; Kitagawa et al., 2001). These GPCRs assemble into heterodimers to form either an umami receptor (T1R1 +T1R3) or a sweet receptor (T1R2+T1R3) (Nelson et al., 2001; Li et al., 2002).

T2R family

T2R is the second family of GPCRs, which has been identified both in mice and humans (Adler et al., 2000; Matsunami et al., 2000). In humans, the T2R receptor family has at least 25 potentially functional genes and about 11 pseudogenes (Go et al., 2005). This subfamily of GPCRs is characterized by a short extracellular N-terminal, a seven transmembrane domain and an intracellular carboxy terminal (Chandrashekar et al., 2000). Functional expression studies have shown that T2R receptors are responsible for mediating the bitter taste quality (Chandrashekar et al., 2000).

mGluR family

The metabotropic glutamate receptors, or mGluRs belong to the class C of GPCRs. The expression of genes encoding glutamate metabotropic receptor, particularly mGluR4, taste-mGluR4 (a truncated form of mGluR4), mGluR1 and taste-mGluR1 (a truncated form of mGluR1) have been identified in taste buds of rats using RT-PCR, in situ hybridization and immunohistochemical studies (Chaudhari et al., 1996; Chaudhari et al., 2000; Toyono et al., 2002; Toyono et al., 2003). However, the role of these receptors in detection of umami taste is yet not clarified.

The bitter taste transduction

Numerous bitter substances with chemically diverse composition can interact with the T2R receptors located on the cell membrane of TRCs. The stimulation of these receptors cause the activation of G proteins coupled to them, particularly α-gustducin and G protein β/γ subunits (complex of β –gustducin3 and γ-gustducin

13) (Meyerhoff, 2005). Although the literature clearly shows the central role of α-gustducin (McLaughlin et al., 1992) and G protein β/γ subunits (Huang et al., 1999) in bitter taste transduction, the coupling of G protein to the intracellular downstream components is less understood. Two pathways are proposed for signal transduction for bitter taste stimuli. Bitter stimuli activate T2R receptors and activated receptors couple to G proteins, α-gustducin being the most likely candidate. Dissociation of the G protein complex, splits the signal into two different pathways (Fig 6). In one pathway, it is proposed that α -gustducin changes cyclic nucleotide monophosphate (cNMP) to nucleotide monophosphate through phosphodiesterase E (PDE) (McLaughlin et al.,1992). However, the role of decreased levels of cyclic nucleotides is yet not well understood (Meyerhoff, 2005). The other pathway activates β3/γ13 subunit, which further activates PLC- β2, which leads to increased levels of IP_3, release of calcium ions from intracellular storages and rise in cytosolic calcium concentration. Increased calcium levels in the TRCs stimulate the TRPM5 channels and leading to action potential formation and neurotransmitter release (Perez et al., 2003; Zhang et al., 2003; Damak et al., 2006).

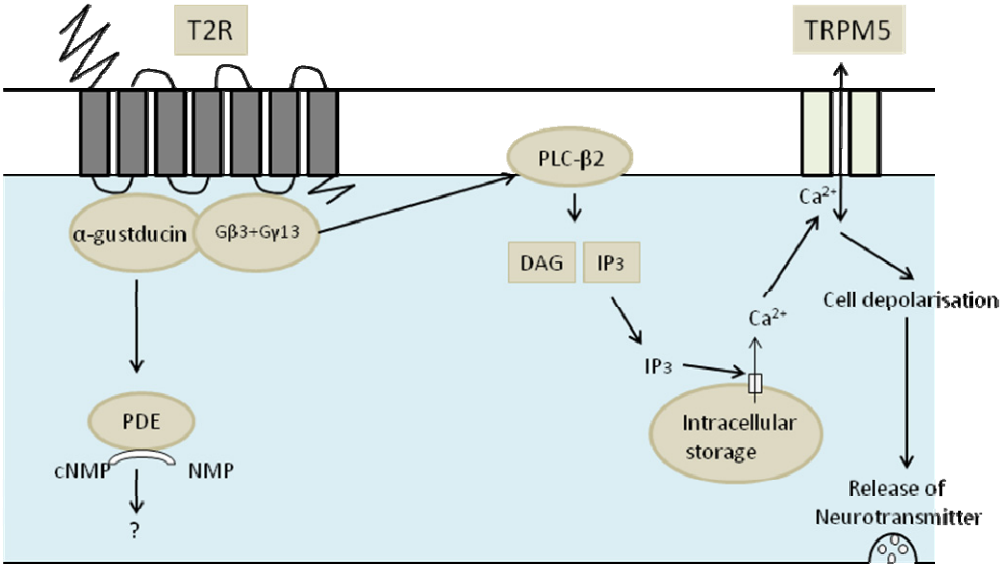

Figure 5. Schematic drawing showing proposed pathways for bitter taste transduction. NMP: nucleocide monophosphate, cNMP: cyclic nucleocide monophosphate, PLC-β2: Phospholipase C-β2, PDE: Phosphodiesterase E, IP_3: Ionositol trisphosphate. (Singh PB, 2011)

The sweet taste transduction

Two subunits of the T1R family, T1R2 and T1R3 form a heterodimer to mediate the sweet taste (Bachmanov et al., 2001; Kitagawa et al., 2001; Li et al., 2002, Montmayeur et al., 2001; Max et al., 2001; Nelson et al., 2001; Sainz et al., 2001).Two pathways have been proposed for sweet taste transduction (Fig 6).

1. **G_s-cNMP pathway:** When sweet stimuli bind to the G protein G_s coupled T1R2/T1R3 heterodimer, the $G\alpha_s$ subunit gets activated, which starts a cascade of reactions. Adenylate cyclase (AC) is stimulated to generate cyclic adenosine monophosphate (cAMP). cAMP activates protein kinase A which further phosphorylates a K^+ channel and the channel closes. Closing of the channel depolarizes the taste cell and results in increased levels of Ca^{2+} ions

and a subsequent release of trasmitter substance (Gilbertson and Margolskee, 2003).

2. **G_q/Gβγ-IP3 pathway**. This pathway is opted by artificial sweeteners where they activate the G proteins coupled to phospholipase C (PLCβ2) by either the α subunit of G_q or by Gβγ subunits. Activated PLCβ2 generates diacylglycerol (DAG) and inositol triphosphate (IP_3), which further release Ca^{2+} ions from the internal storage, resulting in release of neurotransmitter (Gilbertson et al., 2000; Margolskee, 2002).

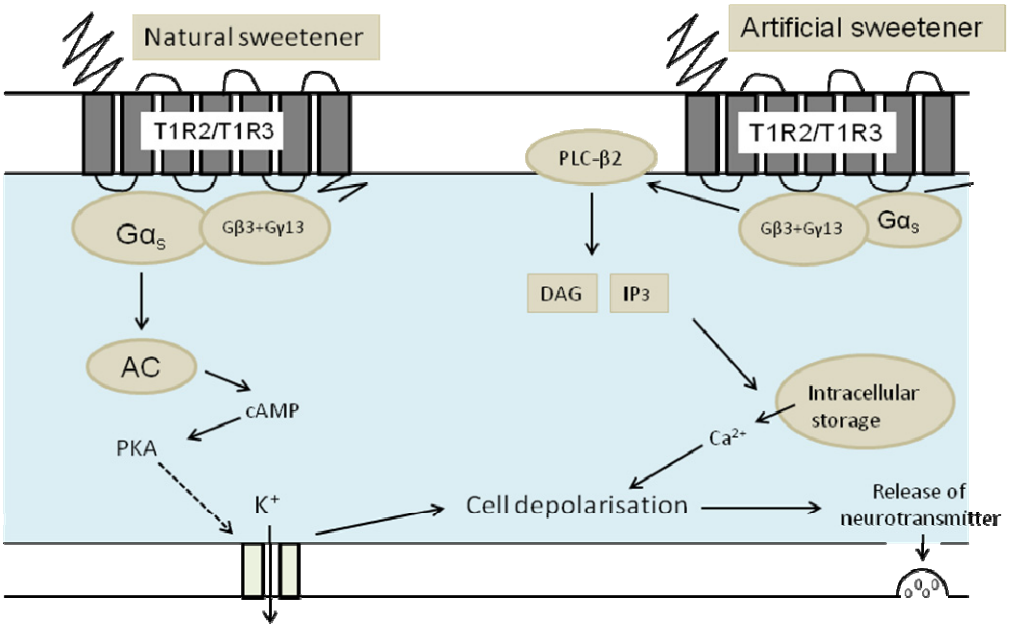

Figure 6. Heterodimer T1R2/T1R3 generates two different intracellular pathways depending on whether it is activated by natural or artificial sweetners. Both the pathways eventually lead to depolarization of the cell and release of transmitter substance. (Singh PB, 2011)

It is presently not clarified how sugar molecules mediate cAMP activation while artificial sweeteners mediate IP_3 responses. In addition, sweet taste is also suggested to be mediated through amiloride sensitive Na^+ channels, which leads to cation influx when stimulated by sweet stimuli (Gilbertson and Margolskee, 2003).

The umami taste transduction

Several GPCRs have been proposed as taste receptors for glutamate. Both ionotropic and metabotropic receptors in TRCs can be activated by monosodium glutamate, however it is the metabotropic receptor family that is believed to be responsible for umami taste transduction (Chaudhari and Roper, 1998). The first candidate molecule discovered, the taste specific variant of metabotropic glutamate receptor 4 (taste-mGLuR4), is expressed in rat circumvallate papillae on the posterior tongue (Chaudhari et al., 1996; Yang et al., 1999; Chaudhari et al., 2000). The second candidate is a heterodimer of two taste-specific GPCRs, theT1R subunits T1R1 and T1R3 (Li et al., 2002; Nelson et al., 2001), which is also the best understood receptor for umami. Finally, the third receptor proposed for umami is a truncated mGLuR1, which, like mGluR4, is found in rat circumvallate papillae on the posterior tongue (San Gabriel et al., 2005). Furthermore, it has been proposed that ionotropic glutamate receptors expressed in taste cells may play a role in glutamate transduction or signalling between taste cells and/or nerve fibers (Kinnamon and Vandenbeuch, 2009).

As in the case of sweet taste transduction, the Gα subunit coupled to the heterodimer T1R1/T1R3 modulates cAMP levels, and the βγ subunit stimulates PLC pathway. The βγ subunit of the G protein is suggested as a dominant part of the

umami taste transduction (Zhang et al., 2003). When glutamate binds to the receptor, Gβ3γ13 stimulates PLC-β2, which in turn produces DAG and IP_3. Second messenger IP_3 causes release of Ca^{2+} ions from intracellular stores. Increased levels of intracellular Ca^{2+} activates the monovalent cation channel TRPM5, which allows influx of Na^{+} ions. This leads to membrane depolarization and release of transmitter substance. The neurotransmitter in this case is believed to be ATP (Finger et al., 2005). The Gα subunit leads to the activation of PDE, which subsequently decreases the level of intracellular cAMP. The final target following the decrease in cAMP concentration is not yet confirmed, although disinhibition of a cAMP-suppressible channel has been suggested (Chaudhari et al., 1996).

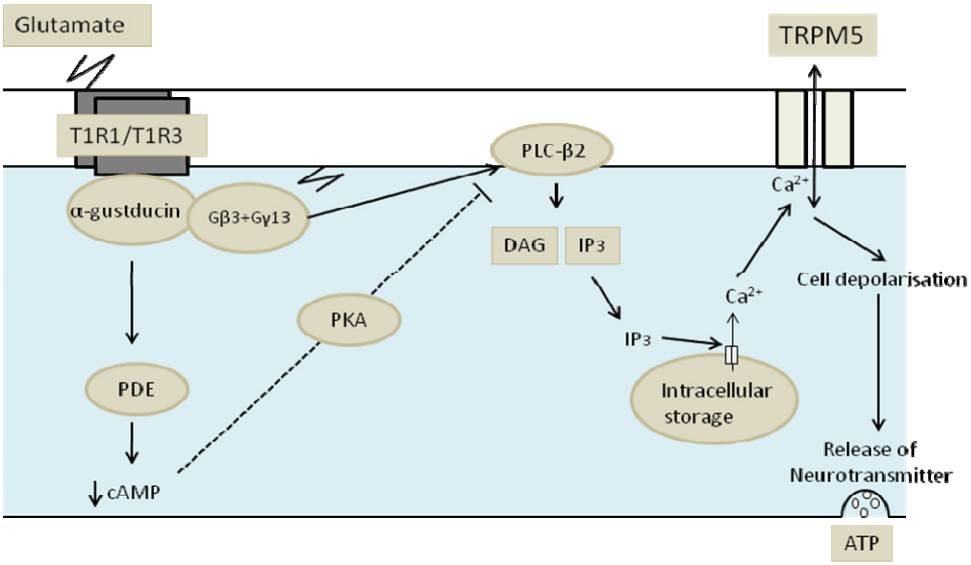

Figure 7. Schematic drawing showing proposed pathways for umami taste transduction. PLC-β2: phospholipase C-β2, PDE: phosphodiesterase E, cNMP: cyclic nucleocide monophosphate, IP_3: ionositol trisphosphate, PKA: protein kinase A. (Singh PB, 2011)

Considerable advancement has been made in the recent years elucidating the umami taste transduction. Umami taste receptors have been proposed and it has been shown that the heterodimer T1R1/T1R3 plays a pivotal role in detecting both glutamates and nucleotides. Yet, there are features in umami taste transduction that do not correspond well to the umami taste receptors reported to date as T1R1/T1R3 knockout mice retain considerable amount of glutamate taste response suggesting the presence of additional receptors and/or unknown interactions among the receptors (Zhao et al., 2003; Damak et al., 2003).

2. Taste disorders

Most of the patients complaining of taste related disorders usually are suffering from a smell disorder rather than an isolated gustatory problem (Deems et al., 1991; Fujii et al., 2004). A study from the Pennsylvania Smell and Taste Centre demonstrated that the prevalence of complaints from patients concerning loss of olfactory function alone was 20.4%, a combination of olfactory and gustatory function was 57.7% and gustatory function alone was 8.7% (Deems et al., 1991). Although, taste dysfunction occurs less frequently than smell disorders, but when taste disruption does occur, it has a much bigger impact on patient's life concerning their nutritional status, weight loss and quality of life as compared to loss of olfactory function (Mattes and Cowart, 1994).

The differences in prevalence of olfactory and gustatory disorders can be explained in terms of the central and peripheral anatomy of these two closely related chemosensory systems. The olfactory information is carried by a single cranial nerve (I), while gustation is mediated through three cranial nerves (VII, IX, and X). The olfactory nerve has a very vulnerable position as its axons pass through the cribriform plate of the ethmoid bone and the axons are easily damaged/stretched/teared if a person is subjected to head injury (Cowart, 2011). The gustatory nerves on the other hand, have a profound position and are not so easily damaged during head injury. Moreover, all the three nerves have to be damaged bilaterally to induce a complete loss of sense of taste, which happens in very rare cases.

Considering the peripheral anatomy, the olfactory receptors are located in a small area in the nasal cavity, easily subjected to damage by physiological changes in the

nose. Taste receptors, on the other hand, are located in whole of the oral cavity; on the tongue, palate, larynx, pharynx and epiglottis (Cowart, 2011).

2.1 Classification of taste disorders

The classification of gustatory disorders follows the same scheme as olfactory disorders. It is divided into two categories: quantitative taste disturbance or qualitative taste disturbance. Ageusia and hypogeusia are quantitative taste disorders, while dysgeusia, phantogeusia and gustatory agnosia are some of the qualitative taste disorders.

Ageusia: complete loss of ability to perceive taste.

Hypogeusia: decreased taste perception.

Dysgeusia/Parageusia: distortion of taste perception related to nutritional input (perception of unpleasant taste instead of normally pleasant taste).

Phantogeusia/Pseudogeusia: presence of permanent bad taste sensation that is not produced by external stimuli.

Gustatory agnosia: loss of ability to recognize taste sensation, while the different components of gustatory processing, and cognitive functions are intact.

2.2 Measurement of taste dysfunction

While altered taste function is a common complaint in the general population, medical care lacks appropriate diagnostic tools and treatment regimes (Deems et al., 1991; Hoffman et al., 1998). In the clinical context, evaluating, diagnosing and treating olfactory disorders is well established as compared to the assessment of

taste function is still less standardized (Hummel et al., 2009). Contemporary tests available to measure the gustatory function very often measure the sense of taste based on the subjective judgments, either by using taste strips (Mueller et al., 2003; Landis et al., 2009) or taste solutions (Halpern, 1997). Lately, there has been focus on non-invasive gustatory assessment techniques like event related potentials (Kobal, 1985; Hummel et al., 2009), magnetoencephalography (Kobayakawa et al., 1999) and functional brain imaging (Small et al., 1999; Faurion et al., 2005) to avoid bias of the investigated subject. However, none of these techniques are yet clinically used as standard tool for assessment of the gustatory function.

3. Study aims

Monosodium glutamate elicits a specific umami taste and is thought to increase palatability of food. In order to comprehensively study the mechanisms of the taste perception of L-glutamate, this work compiles results from several research fields namely, psychophysics, molecular biology and neurophysiology.

At the **perception level**, the aim was to explore individual variation in the perception of glutamate in the healthy population. At the **cellular level**, the question referred to the role of nsSNPs present in the umami taste genes on taste transduction of glutamate. At the **neurophysiological level**, the aim was to determine the topographical differences in cortical processing of umami and salt taste by using gustatory event related potentials (ERP). Finally, the laterality of the gustatory pathway was elucidated by using **functional brain imaging** in humans.

The specific aims were:

- To explore the umami taste genetics and elucidate the prevalence of non-tasters of umami in a healthy European population. (Paper I)
- To establish whether the SNPs identified in the umami taste receptors impair the receptor's response to MSG. (Paper II)
- To investigate the neurophysiological basis of gustatory processing of salt and umami taste in the human brain by means of EEG derived ERPs. (Paper III and Paper IV)
- To determine the laterality of the human gustatory cortex using fMRI. (Paper IV)

4. Methodological strategies

Several techniques were used in this thesis to better understand the processing of umami taste in humans. This section will provide a background for **1)** psychophysics, **2)** functional expression and calcium imaging. **3)** event related potentials, and **4)** functional magnetic resonance imaging.

4.1 Psychophysics

" To explain the mind, we have to show how minds are built from mindless stuff, from parts that are much smaller and simpler than anything we'd consider smart."

Marvin Minsky-The Society of Mind, 1985

Psychophysics is defined as the quantitative study of perception, that examines the relations between stimuli and responses and the reasons for those relations (Baird and Noma, 1978). The individual taste experience is subjective and cannot be directly compared with gustatory experiences of another person. Using several different measureable magnitudes of taste sensation, psychophysics makes it possible to compare and evaluate subjective taste perception among a group of candidates. Psychophysical measures of chemical senses are very useful in understanding the fundamental role of these senses and their dysfunction and health-related disorders (Snyder et al., 2006)

Taste perception of umami can be divided into several psychological attributes: quality, intensity, oral location, and timing (Breslin and Huang 2006). Thus, in this study, all of these attributes for salty and umami taste were carefully evaluated to classify the participants into tasters, hypotasters and non-tasters. The prototypes of these taste qualities are table salt and chicken broth, respectively. The taste intensity was the magnitude of the qualitative sensations, such as slightly salty or

strongly bitter for NaCl and pleasant or unpleasant for MSG. The location was the perceived region of the oral cavity, which gave rise to a taste sensation. The timing of taste was observed to determine whether the taste sensations arose quickly or with delay and whether they lingered on the tongue. The battery of three successive psychophysical tests used in Paper I is described below.

Test 1: quality discrimination test

In this test, participants were seated on a chair and presented with paired stimuli. The first pair was composed of water (10 mL) and a 29 mM NaCl solution (10 mL). They were asked to describe their perception and report which of the solutions was not water. Those who could not perceive the difference between water and NaCl were asked to repeat the test with a 43 mM NaCl solution (10 mL). The participants were asked to rinse with water between each presentation to eliminate any residual taste and they were asked not to swallow the solutions. The second pair was composed of 29 mM MSG and 29 mM NaCl or 43 mM NaCl, if it was shown to be necessary. The participants reporting MSG clearly being the strongest solution were considered tasters, whereas the participants who could not discriminate between MSG and NaCl were subjected to further tests. Individual sensitivities to MSG relative to NaCl were carefully compared so that the participants perceiving only the salt component in MSG were not confused with the ones perceiving both the salt and glutamate taste components in MSG (Yamaguchi, 1991).

Test 2: ranking test

Seven participants from the German population and 22 participants from the Norwegian population who could not discriminate between MSG and NaCl in the quality discrimination test (Test 1) participated in this test. These participants were presented with three cups containing 10 mL of each 29 mM NaCl, 43 mM NaCl, and

29 mM MSG solutions. The participants were asked to rank the three samples in the order of intensity. Participants were instructed to keep the stimuli in their mouth for 2 seconds before spitting it out to keep into consideration the delay in umami taste perception. Between each stimulus, participants were required to rinse the mouth thoroughly to get rid of any persisting umami taste. Participants were considered tasters if they ranked the three solutions as such: 29mM NaCl < 43mM NaCl < 29mM MSG (Lugaz et al., 2002). Participants exhibiting MSG sensitivity at isomolarity with NaCl (29 mM) were suspected not to be sensitive to the glutamate anion but only to the sodium cation in the MSG solution (Yamaguchi, 1991) and were further subjected to Test 3.

Test 3: triangular test

In this test, the participants went through 10 triangular tests and each triangular test was composed of one cup of a 29 mM MSG solution (10 mL) and two cups of a 29 mM NaCl solution (10 mL). Participants were asked to answer which solution was different from the other two to distinguish the hypotasters from potential non-tasters. Participants who could discriminate isomolar MSG from NaCl in 7 or more of the 10 triangular tests presented, were considered as hypotasters, while the rest were considered potential non-tasters. This group of potential non-tasters perceived the same taste quality (salty) but different taste intensity when they compared MSG and NaCl in that they perceived MSG less intense as compared to isomolar NaCl. Also, these participants did not experience the lingering effect of MSG, a characteristic that was distinct in the taster population. The tasters reported a delay of 2 s before they perceived the umami taste after tasting the MSG solution but no delay was observed with the NaCl solution. They also reported to perceive salt taste on the anterior part of the tongue, while the umami taste was more dominant on the lateral

ridges of the tongue and the posterior part of the palate. This group of participants could not perceive the characteristic, lingering taste quality of MSG that persists on the tongue but they perceived the salt component in it.

Concerning the methodological issue, the experimental procedure to find hypotasters and non-tasters used in this paper was relatively simple as compared to that used in the study of Lugaz et al. (2002). In an effort to obtain a maximal randomized group of individuals to take part in this study, our participants were recruited in public places such as museum and hospital. Thus, in this particular setting we were unable to retest these individuals, as they were not available for tests at another time. The study could have been made more robust by retesting the non-taster individuals as learning is an important factor for umami taste perception, which is not to be neglected (Lugaz et al., 2002). Keeping this fact in mind, we chose to characterize the subjects who could not differentiate between isomolar concentrations of MSG and NaCl as potential non-tasters.

4.2 Functional expression and calcium imaging

Functional expression is a widely used *in vitro* technique that offers a unique way to study cell behaviour and molecular mechanisms. Non synonymous single nucleotide polymorphisms (nsSNP) have been reported in the coding region of the human T1R1 and T1R3 genes (Raliou et al., 2009a) which have shown to be associated with the inability to taste MSG in non-tasters and hypotasters in a French population (Raliou et al., 2009b). Using this method, we investigated the role of identified SNP in umami taste transduction. Since binding of umami stimuli to the T1R1/T1R3 receptor leads to increased levels of intracellular calcium, calcium imaging was performed on the cells *in vitro* to monitor the umami taste transduction both in cells

transfected with wild type receptors and receptor variants identified in the previous study of Raliou and collegues (Raliou et al., 2009a).

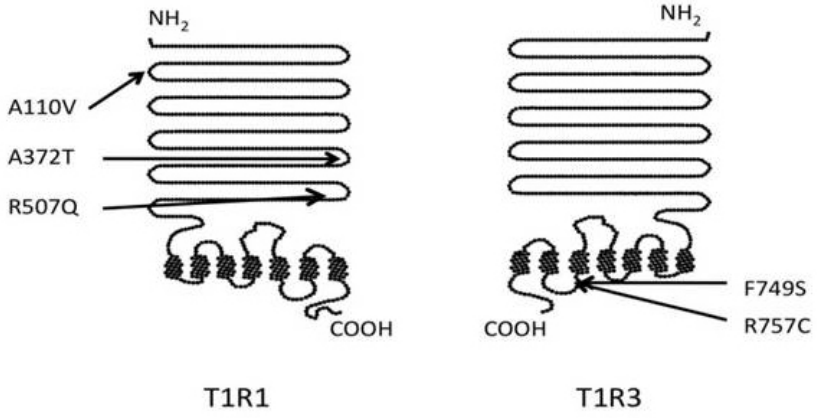

Figure 8. Functional assays were performed to elucidate the role of three SNPs in the T1R1 receptor (A110V, A372T and R507Q) and two SNPs in T1R3 receptor (F749S and R757C) in umami taste transduction. The position of these SNPs is shown in this figure (Raliou et al., 2011).

Human embryonic kidney cells (HEK293) were grown in Minimal Essential Medium without phenol red supplemented with 10% heat-inactivated fetal bovine serum (FBS) 2 mM L-glutamine and Eagle's non-essential amino acids and maintained at 37°C in a humidified incubator with 5% CO_2. The cells were then transfected with pcDNA3.1/Hygro/G16Gi3 plasmid. HEK293 derivative cells stably expressing G16Gi3 (HEK293/G16Gi3) were selected in 300 µg/ml hygromycin B amplified and frozen in several cryovials in order to use the same batch of cells over the course of the study.

T1R1 or T1R3 and their variants were transiently co-transfected in HEK293/G16Gi3 cells using 3 µg of plasmid DNA using JetPEI™. After 24 hrs, transfected cells were trypsinized and seeded at a density of $0.7x10^5$ cells/well onto a Poly-L-Lysine-

coated 96-well tissue culture plate and grown in low-glucose DMEM (Dulbecco's modified eagle's medium) supplemented with GlutaMAX and dialyzed FBS in order to minimize glutamate-induced and glucose-induced desensitization. After 24 hrs, transfected cells were rinsed twice with calcium assay buffer and loaded for 30 min at 37°C with Fluo-4 acetoxymetyl ester dye (3.5 μM) dissolved in calcium assay buffer supplemented with pluronic acid and bovine serum albumin (BSA). Further, cells were rinsed twice with calcium assay buffer and incubated for 10 min at 37°C and 1.25 hr in the dark at 25°C. The cells were stimulated by the addition of MSG using a micropipette. At the end of the experiment isoproterenol was applied as a control to stimulate the endogenously expressed β2-adrenergic receptors. Calcium imaging was monitored on an inverted epifluorescence microscope equipped with a digital camera. The data were then normalized to isoproterenol calcium responses by dividing the peak value of the MSG response by the peak value of the isoproterenol response for each cell and analyzed using SimplePCI software. The Ca^{2+} changes were expressed as fractional change in fluorescence light intensity: $F/F = (F-F_0)/F_0$, where F is the fluorescence light intensity at each point and F_0 is the value of emitted fluorescent light before the stimulus application. Data were compiled from 100 cells and represented as averaged maximal fluorescence increase of at least 5 independent experiments carried out in triplicate. Dose-response curves were fitted using SigmaPlot software.

4.3 Electroencephalogram and gustatory event related potentials

EEG derived gustatory evoked potential recording is one of the methods for assessment of gustatory function that bypasses human judgment. This non-invasive

technique that allows investigation of human neural activity by placing external electrodes on the scalp of the subject while the subject is exposed to taste stimuli. The recorded potentials predominantly reflect the activity of cortical neurons in the area underlying the EEG electrode, however, they are not receptive/ directly related to any particular neuron or a group of neurons.

EEG

When electrodes are placed on the scalp, the electrical activity along the scalp produced by the firing of neurons within the brain is recorded by an amplifier. This recording of variations in voltage is known as electroencephalography (EEG). EEG is a common diagnostic tool in the field of neurology, where electrical activity in the brain is measured in order to diagnose pathology in the brain.

ERP

When a certain stimulus is presented to the subject 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 particular stimulus. For example, we can define a section (epoch) of EEG that begins at stimulus onset and ends 1500 ms later. During this time lapse, we might observe changes that are specifically related to the brain's response to stimulus. These recordings are defined as event related potentials (ERP) or evoked potentials.

Recording gustatory ERPs

Millions of nerve action potentials are generated every moment in the human brain and all these electrical potentials added together reflect the electrical activity in the

cerebral cortex. To measure these electrical potentials metal electrodes were attached to the scalp using a conducting electrode gel. An EEG amplifier measured voltage differences between two points on the scalp. Each channel in the amplifier was connected to two electrodes where second electrode for every channel was identical, also called “reference electrode”, referenced against earlobes. Yet another electrode called the “ground electrode” was connected to the subject’s scalp. Gustatory ERP were recorded at positions Fz, Cz, Pz, C3, and C4 of the 10-20 system (Fig 9). The 10-20 is an international system of naming and position scheme for EEG measurements based on 10% or 20% proportional distances between anatomic landmarks on the skull and head (Jasper, 1958).

According to the 10-20 international system there are certain standard positions that are used as references (Fig 9). The Nasion (Ns) is the position on the bridge of the nose, and Inion (In) is the bony protrusion located in the middle of the back of the head. Preauricular points are reference points located on the earlobes called preauricular point left (PAL) on the left earlobe and preauricular point right (PAR) on the right earlobe. The point of intersection of the Ns-In line and PAL-PAR line is called Vertex. Further, the proximity to a particular region of the brain, specifies the naming and position of the electrode for example, F-frontal, C-central, P-parietal, odd numbers for the left hemisphere, even numbers for the right hemisphere and z for the midline.

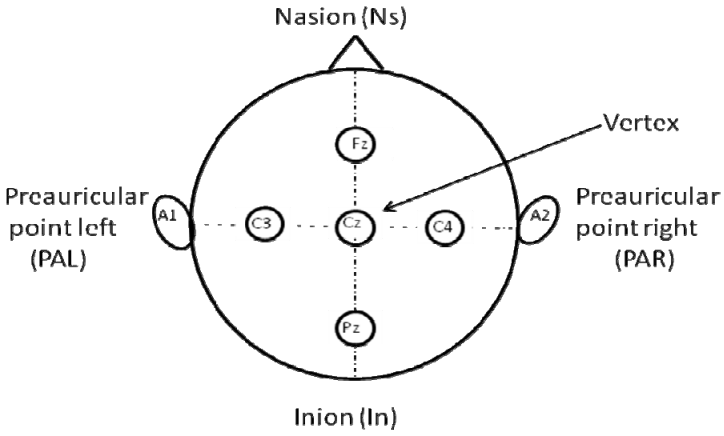

Figure 9. Some of the electrode positions of 10-20 international system.

Averaging the ERP signal

Since the ERPs represent a set of very small changes in relation to the EEG wave they are derived from, it is essential to extract the ERP recording from the EEG background. This was done by recording repeated number of EEG, time-related to repeated presentations of the same taste stimulus. The recordings were averaged to form a single wave after records contaminated with motor artifacts or blinks were discarded. A computer controlled gustometer was used to present precise repetitions of different taste stimuli and to ascertain reliable timing of multiple stimulus repetitions. An example of gustatory ERP in response to salt stimulus in a healthy individual is shown in figure 10. The amplitudes P1, N1 and P2 are marked and a white arrow shows the onset of stimulus presentation.

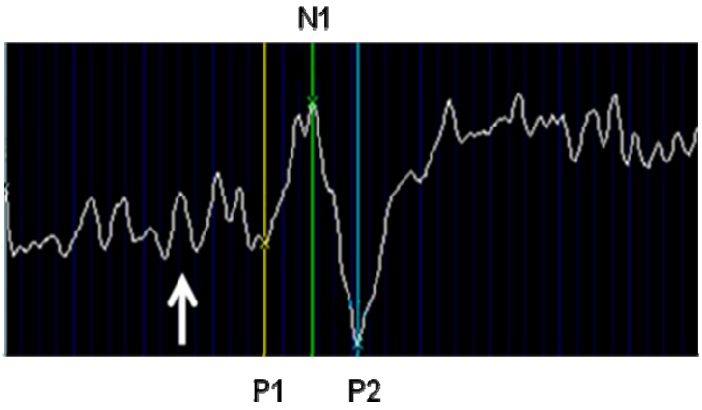

Figure 10. ERP recording to a salty stimulus from an individual subject (stimulus onset at 530 ms after onset of recording indicated by thick white arrow; maximum amplitude 25 μV; recording position Cz/A1+A2).

4.4 Functional Magnetic Resonans Imaging

Functional neuroimaging is another non-invasive technique of monitoring neural activity in humans. This technique has been very crucial in helping us understand the neural system responses to gustatory stimuli by observing and quantifying the stimulus input and related behavioural responses. ERPs measure the direct electrical activity in the cortex, whereas, fMRI signals record the indirect product of this electrical activity by measuring the hemoglobin content in the blood flow. fMRI is a functional modification of MRI, developed into an imaging method by Lauterbur (1973) (Sobel et al., 2003).

Experimental design

The fMRI paradigm was built in a block design, randomized across subjects. The subjects were placed in the fMRI scanner with tubes placed in the mouth through

which the tastants were introduced. The stimuli were presented through a computer controlled gustometer, which was placed outside the scanner room. The fMRI scans were acquired while the subjects were being stimulated with different taste stimuli. In each session subjects received the two stimuli, NaCl and MSG. The subjects were instructed during the experiment through a visual presentation on a MRI dedicated screen. The experiment started with a grey field on the screen. The pulse stimulus with the tastant was presented a few seconds after a grey field was projected on a screen. The subjects were instructed to keep the stimulus in their mouth and no movements were allowed while the gray field was presented. Then the word “swallow” guided the subject to swallow the presented stimulus. Finally the word “rinse” was projected on the screen together with a pulse of water (“w” in the graph) and the subject could rinse the mouth and swallow the water (Fig 11). Every ON-block was alternated with an OFF-block in which the pulsed stimulus was just water. The sequence was presented in a session of 6 repetitions of an ON/OFF – block (total time: 6 min) per stimulus and per side of the tongue, in a total of 4 sessions, lasting on the whole 24 min. Only the scans inside the 30s corresponding to the time when subjects received the taste stimuli (ON block) were included in the fMRI analysis. The series of scans acquired were analyzed using a software package called Statistical Parametric Mapping (SPM). SPM is a statistical method to assess the differences in brain activity related to the task performed by the participants. Functionally specialized brain responses to salt and umami were identified in an effort to determine the gustatory anatomy, which can be valuable in disease-related changes.

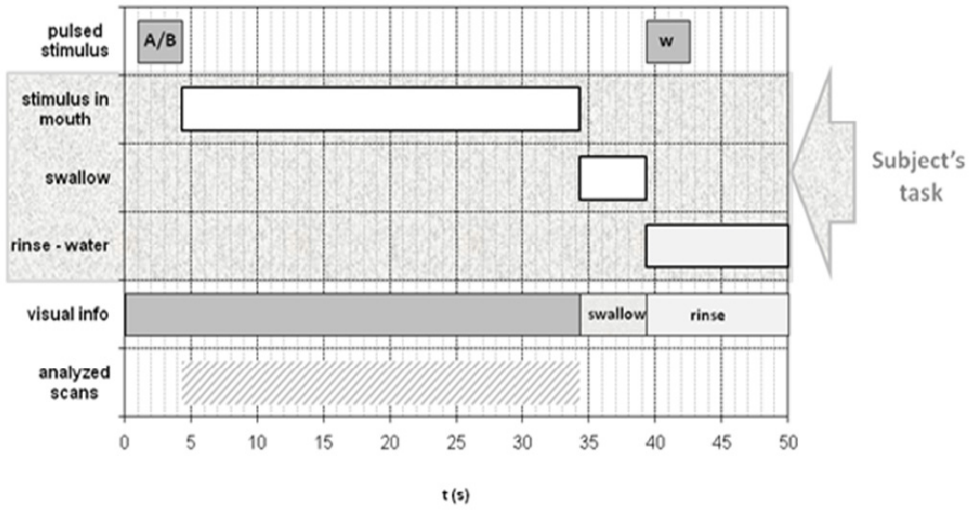

Figure 11: Time frame description of the 50s-block design applied in the fMRI sessions (ON Block)

Data analysis

The first approach in data analysis was to register the responses for salt and umami in the "taste map" of the human cortex, namely, thalamus, frontal operculum/insula and the orbitofrontal cortex (OFC) areas. These areas however, are also stimulated by other sensations like thermal, visual and auditory stimuli (Craig et al., 2000; Iannilli et al., 2008). So, the statistical parametric mapping-contrast (spm-contrast) was defined in such a way that neuronal activity specifically related to gustatory stimuli could be enhanced. In this manner we were able to scrutinize the cortical activity related to the different taste stimuli presented either on left side or the right side of the tongue and mask the unwanted thermal, visual or auditory stimulation, if

any. Futher, the laterality of gustatory pathway stimulated by salt and umami taste was assessed.

5. Summary of papers

5.1 Variation in umami taste perception in the German and Norwegian population. (Paper I)

The purpose of the study was to explore the degree of familiarity for umami taste in two European populations and to examine individual variation of sensitivity to umami taste of MSG. This study did not measure the individual threshold (e.g. detection or recognition threshold), but to demonstrate distribution pattern of tasters, hypo- and non-tasters. The study is composed of two parts 1) survey for umami taste familiarity in the Norwegian and German population, 2) psychophysical screening for inter-individual variation in the umami taste perception. Our findings from the questionnaire survey showed that a large number of German (96.2%) and Norwegian (89.7%) participants were not aware of umami taste quality. Although umami taste has been known as an independent taste and is distinct from the other four basic tastes (Yamaguchi and Ninomiya, 2000; Beauchamp, 2009; Kurihara, 2009), its quality and/or term is not fully familiar with the general population. During the survey collection, it was observed that the participants were skeptic to MSG, and they considered MSG a chemically synthesized substance and not a naturally existing component in food. It is, therefore, essential to educate people about the umami taste quality and MSG as it plays a key role in the intake of amino acids especially L-glutamate. Intake of L-glutamate, furthermore is vital for the human body for its significance in metabolism and neurotransmission (Fonnum, 1984; Newsholme et al., 2003). Moreover, results from the psychophysical screening exhibited a high inter-individual difference of sensitivity for MSG in the two populations. We divided the participants in three groups by

comparing the individual sensitivity to NaCl and MSG: tasters, hypotasters and non-tasters. Non-tasters were unable to perceive the glutamate taste, hypotasters perceived the taste of glutamate at rather high concentrations and the tasters perceived glutamate taste at low concentrations. Three successive psychophysical screening tests revealed that 3.2% of German and 4.6% of Norwegian participants were potential non-tasters who were unable to perceive MSG. In addition, 2.4% of German and 12.2% of Norwegian participants were hypotasters who perceived MSG at relatively high concentration. To our knowledge, only one similar study previously exists which has reported a multi-modal distribution of detection threshold for MSG in the French population (Lugaz et al., 2002). One of the potential reasons for this specific ageusia for MSG might be the umami taste receptor variants expressed in humans (Raliou et al., 2009a; Chen et al., 2009; Garcia-Bailo et al., 2009; Shigemura et al., 2009).

5.2 Human genetic polymorphisms in T1R1 and T1R3 taste receptor subunits affect their function. (Paper II)

Genetic factors affecting the taste receptors might be the reason behind the variation in perception of glutamate (Fuller, 1974; Lush, 1989). nsSNP in the coding region of the human umami taste receptors have been reported (Kim et al., 2006; Raliou et al., 2009a) and some of them were associated with inability to perceive umami taste (Raliou et al., 2009b; Shigemura et al., 2009b; Chen et al., 2009). In paper II, some of the taste receptor variants previously identified by Raliou and collegues were functionally expressed and their cellular response was analyzed by calcium imaging, followed by molecular modelling. In this study, candidate solely

contributed to the functional expression and calcium imaging part. Hence, only this part of the study will be discussed in this thesis. The aim of the calcium imaging part of the project was to determine whether the three T1R1 receptor variants (A372T, A110V, and R507Q) and two T1R3 receptor variants (F749S and R757C) identified in the umami taste receptor (Raliou et al., 2009a) change the function of the receptor. HEK293 cells stably expressing G16Gi3 were transiently co-transfected with T1R1 and T1R3 (wildtype and variants). After incubation for 24 hours, the transfected cells were passaged so that cells from the same batch could be used throughout the study. Further, cells were seeded in Poly-L-Lysin coated 96-well cell culture plates and allowed to grow for another 24 hours in the incubator before they were stimulated with different concentrations of MSG and the calcium response was recorded. Calcium response induced by MSG in wildtype cells was compared with calcium response in cells with receptor variants. The results demonstrated that two of the nsSNP in T1R1 receptor (A110V and R507Q) and two in the T1R3 receptor (F749S and R757C) resulted in impaired activity of the T1R1/T1R3 receptor in response to glutamate. These results reconfirm firstly, that umami taste is mediated through the T1R1/T1R3 heterodimer. Secondly, the nsSNP identified in nontasters during psychophysical screening of umami taste perception in the French population are associated with a change in function of the above mentioned umami taste receptor.

5.3 Segregation of gustatory cortex in response to salt and umami taste studied by event-related potentials. (Paper III)

Umami has been known as a specific taste for more than 100 years. Still, relatively little is known about the central-nervous processing of this taste as compared to other tastes, like salt. Umami and salt taste are mediated through different types of taste receptors. The umami taste is mediated through G-protein coupled receptors, whereas salt taste is mediated through ion channel type receptors. Moreover, psychophysical studies show that salt and umami taste are perceived very differently; umami represents a characteristic taste often described as intense, lingers on the tongue and there is a delay of about 2 seconds before the subjects perceive the taste as compared to salt. Hence, the aim of the present study was to record gustatory ERP for umami and salt taste in order to investigate the manner in which the human gustatory cortex encodes the two stimuli. A total of 17 healthy, right-handed subjects participated in the study (7 women, 10 men, age range 21-46 years, mean age 30 years). Health status in addition to olfactory and gustatory function was ascertained through a detailed medical history, the Sniffin Sticks Screening Odor Identification test (Hummel et al., 2007) and regional gustatory testing using taste strips (Mueller et al., 2003). The liquid stimuli monosodium glutamate (MSG) and sodium chloride (NaCl) were applied in two different concentrations (weak and strong: 200 mM and 400 mM, respectively). During recordings of the gustatory ERP, subjects received white noise through headphones in order to mask switching clicks of the stimulation device. Gustatory ERP were recorded at positions Fz, Cz, Pz, C3, and C4 of the 10/20 system, referenced against linked earlobes. Eye blinks were monitored via the Fp2 lead. At the end of the session subjects rated overall stimulus intensity of the MSG and the NaCl

stimuli. Results from the present study were investigated with regard to differences between the two stimulus qualities, their concentration, intensity ratings and the subjects' gender. Our results show that **a)** there was a stimulus-specific topographical distribution indicating profound differences in the processing of MSG and NaCl, **b)** larger responses were recorded on the right hemisphere compared to the left hemisphere and **c)** subtle sex-related differences were found in the processing of gustatory information, with women exhibiting slightly larger responses to taste stimuli than men.

5.4 Taste laterality studied by means of umami and salt stimuli: an fMRI study. (Paper IV)

The knowledge of human central taste pathways is mainly based on anatomical dissections and *in vivo*-electrophysiology in animals (Rolls and Scott, 2003; Simon et al., 2006). It is well established that the primary gustatory afferents from the TRCs project ipsilaterally to the nucleus of the solitary tract (Goto et al., 1983; Jyoichi et al., 1985; Nakajima et al., 1983). However, the pathway from the secondary neurons to the gustatory cortex in humans is still not clarified (Kobayashi, 2006). The aim of the present study was to investigate the laterality of the gustatory pathway for salt and umami taste using fMRI. A total of 24 subjects participated in a block-design functional magnetic resonance imaging study. The stimuli were presented in liquid form at supra- threshold concentrations and delivered through a computer-controlled gustometer. Left (L) and right (R) side of the tongue was stimulated separately with NaCl and MSG. The topography of hemodynamic activity elicited by salt and umami taste stimuli was recorded using functional magnetic resonance imaging. The

paradigm was such as AsrBsrBsrAsrBsrAsr (three repetition for tastant A and tastant B per session; s= swallow; r= rinse). A and B were pseudo-randomized across sessions and subjects. At the end of each session we instructed the subjects to move the sets of tubes from one side to the other side of the tongue, keeping body and head still to avoid invalidate the measurements by undesired movement artefacts. The site of the stimulus application was pseudo-randomized across the sessions and subjects. The whole sequence included 4 sessions with 6 block repetitions, for a total time of 24 min. After every session the subject was asked to rate the intensity of the stimulus on a scale between 0, not perceived, and 10, extremely intense. The blood oxygen level dependent (BOLD) signal was acquired through 1.5 T fMRI scanner. The fMRI data analysis was performed by means of SPM5 implemented in Matlab 7.5 R.2007b.

The results are based on a ROI analysis along the 'taste map', which, has recently been identified through Activation Likelihood Estimation (ALE) analysis (Veldhuizen et al., 2011). These areas are thalamus, insula/frontal operculum, OFC and some areas in the limbic lobe. Moreover, we defined the spm-contras in a way that the results are able to stress the neuronal connection specifically related to the taste quality presented to the left or right side of the tongue.

Our results suggest a taste dependent laterality through the thalamus, specifically an ipsilateral link for NaCl and a contralateral link for MSG (Fig 12). Moving to the frontal operculum/insula we found for NaCl applied on the left side an ipsilateral connection with the left frontal operculum/insula, while the right-sided stimulation with NaCl produced bilateral activations. MSG produced a bilateral activation after right-sided stimulation. On the contrary left-sided stimulation did not produce any activation at level of the frontal operculum/insula.

On the other hand, umami produced activations in the left OFC only after the left side stimulation of TRCs, while no activations surpassed the statistical threshold after the right side stimulation. Then remembering the crossing fibers between the NST and thalamus hypothesized from our results, and no activations in the frontal operculum/insula following left-sided stimulation with MSG, the afferent to the left OFC seems to come from the right thalamus (Fig 12). While the path (a) has been well decrypted as a possible pathway for the gustatory system (Rolls, 2000), the proposed path (b) is novel and it suggests that the involved area in the lateral OFC belong to the primary gustatory area rather than the secondary gustatory area. Finally the absence of activations inside the limbic lobe after the lateralized stimulation indicates that - at this level - the information related to the lateralized stimulation is lost.

In conclusion, the main finding of paper IV is that different pathways are followed from the solitary nucleus to the thalamus by the two taste stimuli. MSG produced a contralateral activation in the thalamus, while the results for the NaCl indicate an ipsilateral link between the nucleus of the solitary tract and the thalamus. Thus, the laterality of the gustatory system seems to be dependent on the taste quality. Moreover, our data indicate a direct link between the left OFC and the thalamus, which could suggest a primary role of the OFC in gustatory processing.

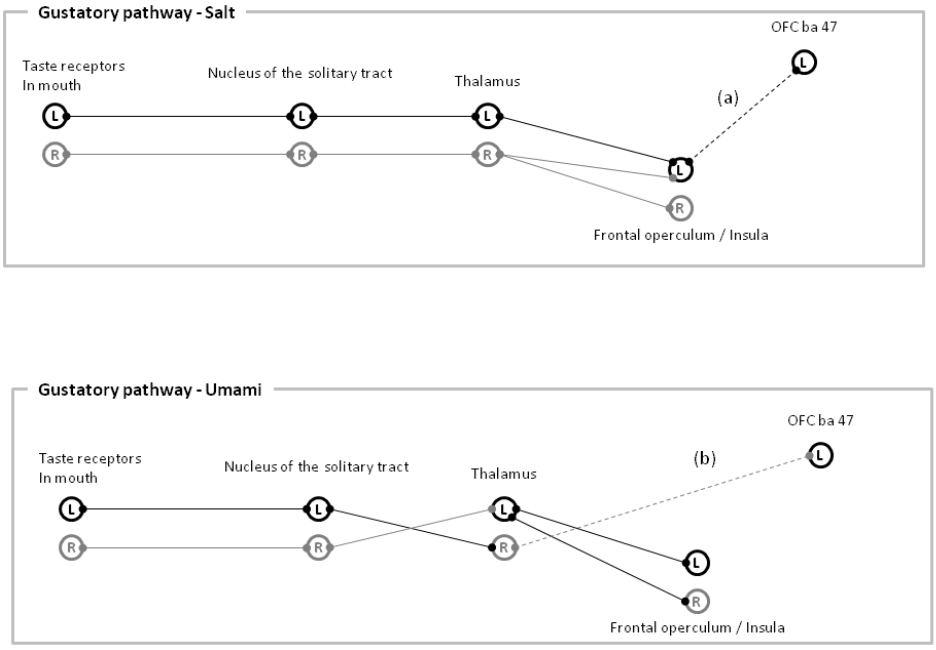

Figure 12. The fMRI results demonstrated ipsilateral pathway followed by salt taste from the taste receptor cells to the gustatory cortex. Stimulation of taste receptor cells with MSG produced contralateral activation in thalamus and bilateral activation in the gustatory cortex.

6. General discussion

Gustation is a relatively complex system as it engages a wide variety of receptor molecules, several transduction sequences, and it exhibits complex cerebral processing in humans. In this thesis, an effort was made to better understand umami taste by combining molecular biological, and neurophysiological levels of analysis with cognitive and perceptual levels of inquiry.

Psychophysical Evaluation

The analysis of chorda tympani nerve responses to glutamate has shown that there is a similarity between taste glutamate receptors and glutamate receptors of the central nervous system (Faurion, 1991). Several receptors both metabotropic and ionotropic glutamate receptors have been proposed to be involved in umami taste perception. Moreover, glutamate has also been suggested to have a neurotransmitter function between the taste cells and the innervating afferent fibres (Lawton et al., 2000; Caicedo et al., 2000). The determination of umami taste through multiple receptors could lead to inter-individual variation in perception of umami taste in healthy individuals (Lugaz et al., 2002). Population studies have previously been crucial to determine the genetic taster status for bitter taste, where individuals are classified into tasters and non-tasters of bitter compounds depending on the genetic heritable trait (Snyder, 1931; Lugg, 1966). However, in the case of umami taste, only one similar study has been performed to investigate the variation in umami taste perception (Luagz et al., 2002). Our psychophysical screening of healthy individuals from the Norwegian and German population showed that about 4% of the participants had “taste-blindness” for umami taste. These findings could help in better understanding mechanism of umami taste perception in humans,

which further might be beneficial in the clinical work to assess taste disorders. At the level of food choice behaviour in humans, the preference for glutamate addition in food has already been studied (Bellisle F, 2008), but the individual behaviour has not been related to the sensory data as these inter-individual differences of sensitivity to glutamate are of recent date. Does the absence of sensitivity or the low sensitivity to glutamate, which itself acts as a taste enhancer, result in a difference of consumption of added salt? There is a fair possibility of an increase in salt consumption in glutamate nontasters, unless physiological mechanisms reveal to be even more complicated.

One of the potential reasons for this specific ageusia for MSG observed in our study might be the umami taste receptor variants identified in umami taste receptors in humans (Lugaz et al., 2002; Chen et al., 2009; Garcia-Bailo et al., 2009; Shigemura et al. 2009). Thus, further studies were performed to correlate the variations in umami taste perception and genetic variations in the umami receptor.

Functional Expression

To understand the underlying mechanisms in glutamate non-tasters the expression pattern for gustducin, T1R1 and T1R3 was investigated in the human fungiform papillae of non-taster subjects (Raliou et al., 2009a). The hypothesis of the study was that non-tasters have lesser expression of candidate umami taste receptors as compared to the tasters and hence the inability to perceive glutamate taste. But on the contrary, no differences in expression pattern of the mentioned receptors were found in the two groups. Further, three SNPs were identified in the coding sequence of T1R1, four SNPs in T1R3 and four SNPs in mGluR1 in these subjects (Raliou et al., 2009a). Our functional assays confirmed the hypothesis that A110V, R570Q

substitutions in T1R1 and F749S, R757C in T1R3 lead to a reduced activity of T1R1/T1R3 expressed in HEK293 cells when stimulated by MSG, whereas A372T substitution in T1R1 did not reduce this activity. Furthermore, these results strengthened the role of T1R1/T1R3 heterodimer in the detection of glutamate.

Gustatory Event Related Potentials

The discovery of subjects specifically non-tasters to glutamate, and confirmation of the role of receptor variants in impairing the umami taste transduction makes glutamate taste a very tempting model for studying the neurophysiological bases of gustation in humans. The primary and secondary taste areas in the human cortex have been identified. However, it is not yet clear whether there is segregation or integration of taste processing of different taste qualities in the gustatory cortex. Moreover, the issue of hemispheric dominance for cerebral processing of taste is still unresolved. Our findings from the gERP analysis clearly demonstrated stimulus-specific topographical distribution of responses indicating profound differences in the processing of MSG and NaCl. Larger responses were recorded in the right hemisphere compared to the left hemisphere for both MSG and NaCl, suggesting right hemispheric dominance of taste processing independent of taste quality.

Functional Magnetic Resonance Imaging

During the ERP recordings, interesting stimulus-dependent differences in encoding of salt and umami taste were observed. With the help of fMRI, we set out to investigate whether similar differences were also present during the early processing of the two taste stimuli. Another goal of the study was to elucidate the laterality of the gustatory system when stimulated by MSG and NaCl. By stimulating

the left and the right side of the tongue separately, we could monitor the gustatory cerebral activity related to each side of the tongue. We expected to observe either, ipsilateral or contrallateral gustatory pathways. Surprisingly, we found completely different pathways for salt and umami processing. The gustatory pathway for salt taste followed ipsilateral ascension all the way from the TRC to the gustatory cortex. Whereas, the pathway followed by umami taste was not so simple. The fibres ascended ipsilaterally from the TRC to the nucleus of the solitary tract. After this level, the fibres crossed and contralateral activation was observed in the thalamus. Further, right sided stimulation of TRC, resulted in bilateral activation of the primary taste areas. The left sided stimulation of TRC however, produced very interesting activation: in this case, there was no connection between the thalamus and the primary taste areas (frontal operculum/insula), but between the thalamus and the secondary taste area (orbitofrontal cortex). This novel finding, of direct link between thalamus and orbitofrontal cortex, urges us to redefine the primary and secondary taste areas.

Our findings from psychophysics, functional assays, and brain imaging techniques are an important step towards further understanding the representation of taste and flavour in the humans. In this thesis, mechanisms for salt and umami taste were studied. In future, we would like to extend our studies to other taste qualities

7. Conclusion

- The data confirms that there exists “taste-blindness” for umami taste in humans.
- Molecular functional *in vitro* assays and 3D modelling of the genetic polymorphisms A110V, A372T, R507Q in T1R1 and R757C in T1R3 exhibit that these receptor variants impair the transduction of umami taste.
- Gustatory ERP recordings demonstrated that there is segregation of gustatory cortex in processing of salt and umami taste in humans. Furthermore, right hemispheric dominance for gustatory processing independent of taste quality was confirmed in the human brain.
- Functional brain imaging in response to umami and salt taste stimulation demonstrated different pathways for salt and umami taste.

8. The candidate's contribution

The candidates contribution to the work in papers I to IV is indicated by asteric as follows:

Paper I:

- study design*
- psychophysical testing*
- analysis and interpretation*
- manuscript writing*
- corresponding author*

Paper II:

- study design
- passaging and seeding cells*
- preperation of constructs
- transfection of HEK 293 cells with G protein
- cotransfection of cells with receptors & variants*
- performed functional expression and calcium imaging experiments*
- cell count and analysis*
- immunohistochemistry
- molecular modelling & glutamate docking
- analysis and interpretation
- writing of material and method section*
- manuscript prepartion
- corresponding author

Paper III:

- study design*
- experiment protocol*
- ERP recordings under gustatory stimuli*
- ERP analysis
- statistical analysis and interpretation*
- manuscript preparation*
- corresponding author*

Paper IV:

- study design*
- fMRI paradigm
- performed fMRI experiments*
- fMRI analysis and interpretation
- writing of manuscript*

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I

II

III

IV

Taste Laterality studied by means of Umami and Salt stimuli: a fMRI study

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Running title: Gustatory pathway

Abstract

The aim of the present study was to investigate the laterality in the human brain of the gustatory system under the taste quality associated with MSG, monosodium glutamate, and NaCl, a common cooking salt. A total of 24 subjects participated in a block-design functional magnetic resonance imaging study. The stimuli were presented in liquid form at supra- threshold concentrations and delivered through a computer controlled gustometer. We stimulated the left and right side of the mouth separately in order to relate the statistical parametrical map to the site of the stimulus and the specific taste quality. The results showed tastant dependency of the laterality for the gustatory system. Specifically, a contralateral activation in the thalamus was found for stimulation with MSG, while stimulation with NaCl resulted in a predominantly ipsilateral activation. Following the effects of the site of stimulus application through the insula, frontal operculum (putative primary gustatory areas) and the orbitofrontal cortex (putative secondary gustatory areas) we tried to describe the laterality of the gustatory pathway. Most interestingly, for MSG we observed the possibility of a direct connection between thalamus and orbitofrontal cortex indicating a new role for the orbitofrontal cortex in gustatory processing.

Keywords: taste, salt, umami, gustatory cortex, gustatory pathway**,** laterality, fMRI

Introduction

Knowledge on human central taste pathways is mainly based on anatomical dissections and in vivo-electrophysiology in animals (Rolls and Scott, 2003; Simon et al., 2006). What we know is that five basic taste qualities, sour, sweet, bitter, salt, and umami, stimulate the taste buds located in the oral cavity and the pharynx. Then, while it is well known that the primary gustatory afferents from the taste receptor cells project ipsilaterally to the nucleus of the solitary tract (Goto et al., 1983; Jyoichi et al., 1985; Nakajima et al., 1983), the pathway from the secondary neurons to the gustatory cortex in humans is not well established (Kobayashi, 2006). In fact some studies report an ipsilateral projection from the nucleus of the solitary tract to the thalamic nuclei (Landis et al., 2006; Shikama et al., 1996; Uesaka et al., 1998), and others report a contralateral connection through the thalamus (Fujikane et al., 1998; Lee et al., 1998; Onoda and Ikeda, 1999), while Aglioti et al. (2000; 2001) suggested a pathways bilaterally distributed with an ipsilateral predominance.

Regarding the central-nervous processing of gustatory information it is well established in primates that the primary taste cortex is located inside the anterior insula / frontal operculum (I/fO) (Rolls and Scott, 2003; Rolls et al., 1996; Scott et al., 1986; Yaxley et al., 1990) and the secondary cortical taste area is situated in the caudolateral orbitofrontal cortex (OFC) (Rolls et al., 1990). Similar relations seem to be present in humans. Using positron emission tomography Small et al. (1997b) reported taste induced activations of the fO and bilateral OFC. Frey and Petrides (1999) showed a bilateral activation in the I/fO. Kinomura et al. (1994) among several regions, found activations in the insula. By means of functional magnetic resonce imaging (fMRI) De Araujo et al. (2003b) found that stimuli such as sucrose and umami activated areas inside the I/fO and caudolateral OFC. Schoenfeld et al. (2004), found the same areas as mentioned above, with stable activations during repeated stimulation. According to Barry et al. (2001) electric taste stimulation of the tongue activated I/fO, what has also been shown in numerous other studies using natural stimuli (de Araujo et al., 2003a; Faurion et al., 1998; Small et al., 1997a).

Because of the open question of the lateralization of the processing of gustatory information, using fMRI, we wanted to study the laterality of the gustatory pathway in relation to salt (NaCl) and

umami stimuli (monosodium glutamate: MSG). While everybody is familiar with salt, the taste of umami (Ikeda, 1909) is the taste of proteins. Umami is present in palatable foods such as meat, fish, tomatoes, mushrooms, and dairy products and it has been shown to stimulate food intake in mammals (Prescott, 2004; Yamaguchi, 1991).

With a focus on the laterality of the gustatory pathway we applied salty and umami taste in liquid form, to the left and right side of the tongue / oral cavity. Based on a region of interest (ROI)-analysis we followed brain activations along the 'taste map', as defined in Veldhuizen et al. (2011) in regions such as Thalamus, the primary (I/fO) and secondary gustatory cortex, trying to answer the following questions: Is there lateralisation in the processing of gustatory information dependent on taste quality?

Materials and Methods

Participants

Twenty-four healthy, right-handed volunteers participated in this study (13 women; mean age±standard error= 28.3 ±1.4 years). The study was approved by the Ethics Committee of the Technical University of Dresden Medical School. Written informed consent was obtained from all the subjects prior to the experiment.

Stimuli

The experiment was based on two liquid tastants; NaCl and MSG. The stimuli were set at suprathreshold concentrations of 50mM for both NaCl and MSG. The chosen concentrations of stimuli were based on previous population studies performed on human umami taste determination (Singh et al., 2010), other psychophysical studies (Lugaz et al., 2002) and previous fMRI studies (de Araujo et al., 2003b; McCabe and Rolls, 2007). The stimulus was provided only on the lateral ridges of the tongue and mouth, more than 2cm away from the anterior tip. Each side of the tongue was stimulated separately.

Taste delivery system: the gustometer

The stimuli in a liquid form were delivered by means of a computer controlled gustometer (Burghart GU002 - variant GM04; Burghart instruments, Wedel, Germany). The gustometer was placed outside the scanner room; tubings for stimulation were funnelled through a dedicated opening to the scanner room. Stimuli were delivered in a pulse design (total pulse volume 1ml, total pulse duration 3.3s) at room temperature (24°C) through two Teflon™ tubes placed inside the subject's mouth. Apart from taste, stimulation was void of any cues that would have made subjects aware of the stimulus onset. The small dimension (1.3 mm inner diameter, 1.5mm outer diameter) made it possible for the tubes to be placed in a comfortable manner inside the mouth of the subject. The tubes were positioned on the lateral ridges of the tongue in order to separately stimulate the sensory cells in the taste buds located on each side of the tongue.

Screening of the subjects

Psychophysics

All the participants went through psychophysical tests prior to the scanning. The aim of this preliminary test was to assure that the participants were able to perceive umami and salt taste and they did not have any kind of dysguesia. In this test, participants were presented two pairs of stimuli. The first pair was composed of water (10 mL) and 29 mM NaCl solution (10 mL) and the participants were asked to describe their perception and report which of the solutions was not water. The second pair was composed of 29 mM MSG and 29 mM NaCl and the participants were asked to describe them in terms of intensity. All the subjects reporting MSG clearly being the strongest were considered umami tasters and were included in the study (Singh et al., 2010).

Olfactory & Gustatory screening

The olfactory function of all the subjects was invetsigated using the validated "Sniffin' Sticks" test (Hummel et al., 2007; Kobal et al., 1996). The subjects included in the study demonstrated a normal sense of smell. Similarly, the gustatory function of the subjects was evaluated by standardized taste

test kit, the “taste strips” (Landis et al., 2009; Mueller et al., 2003). All subjects included in this study had test scores within the normal range.

Experimental design

The fMRI paradigm was built in a 50s-block design (Figure 1). Following the information projected on a MR-room compatible screen (a grey field) the subjects were instructed to keep the stimulus-volume (tastant A or B) for approximately 30s in the mouth. Then the subjects received the information “swallow” and after 5s the information “rinse” was given together with 1ml of water (10.8s of duration). The paradigm was such as AsrBsrBsrAsrBsrAsr (three repetition for A and B conditions per session; s= swallow; r = rince). A and B were pseudo-randomized across sessions and subjects. At the end of each session we instructed the subject to move the sets of tubing from one site to the other of the tongue keeping body and head still in order not to invalidate the measurements by undesired movement artefacts. The site of the stimulus application (indicated in the paper as L for left and R for right) was pseudo randomized across the sessions and subjects. The whole sequence included 4 sessions with 6 block repetitions, for a total time of 20 min. After every session the subject was asked to rate the intensity of the stimulus on a scale between 0, not perceived, and 10, extremely intense.

fMRI acquisition

To detect the BOLD (blood oxygenation level dependent) signal a 1.5 T scanner (SONATA-MR; Siemens, Erlangen, Germany) was used. For each subject the functional images were a total of 168 volumes/session and they were acquired by means of 27 axial-slice mosaic 2D SE/EP sequence (TR=2500ms / TE=45ms / FA=90° / matrix=64x64 / voxel size=3x3x3.75mm^3). Moreover, a structural high resolution image was added for each volunteer dataset (3D IR/GR sequence; TR=2180ms / TE=3.93ms).

fMRI data analysis

The fMRI data analysis was performed by means of SPM5 (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 7.5 R.2007b (Math Works Inc., MA, USA). The spatial pre-processing included: slice timing to reduce the differences in slice acquisition times, realignment&unwarp to minimize movement effects and susceptibility artefacts, normalization in a stereotactic space and smoothing by means of a 8*8*8 mm^3 FWHM Gaussian kernel (Ashburner and Friston, 2003) in order to improve the signal-to-noise-ratio and reduce residual differences between subjects. Pre-processed functional data were modelled in single-subject first level analyses using the canonical hemodynamic function and its derivative set available in SPM. All the scans acquired during mouth rinsing and swallowing were excluded. Statistical parametric maps for the group-inferences were produced by the second-level random-effects analysis (Penny et al., 2003) by means of a factorial design 2x2 (two stimuli condition x two sites of stimulus application). The data from one subject were not usable due to movement artefacts, and other two subjects failed in two runs for the same reason.

Spm-fMRI assesment

The statistical parametrical maps were assessed at a voxel height threshold of $p_{FWE}<.05$ and masked in exclusion (p=.05 – spm default) by an spm-contrast. For example to assess the activations inside the insula/frontal operculum following the effect of MSG applied on the left side of the tongue (namely MSG_L) we used the t-contrast MSG, masked in exclusion by the contrast MSG_R. This was made in order to highlight only the areas that correlated with the effect of the tastant but to hide potential other factors as, temperature, that Craig et al. (Craig et al., 2000) demonstrated in their work being able to activate insular cortex, somatosensory effect, also involving areas in insular cortex and thalamus as reported in Iannilli et al (Iannilli et al., 2008), but also visual and auditory responses. Moreover, by means of this mask, we focused on brain activations related to the specific quality of tastants and directly linked with the site of application in the mouth. All reported coordinates are in MNI space. The Pick-Atlas software toolbox (Mai et al., 2004; Maldjian et al., 2003; Maldjian JA, 2004) and Atlas of the Human Brain (Mai et al., 2004)

was used to identify the brain areas. Finally all the region of interest (ROI) used in our analysis were depicted by means of Pick-Atlas software toolbox (Maldjian et al., 2003; Maldjian JA, 2004)

Results

Intensity ratings

The results of the intensity ratings of the two taste stimuli obtained during the functional neuroimaging acquisition showed that the subjects' group did not perceive a significant difference in intensity between the solution of NaCl and the solution of MSG (2-sample t-test$_{df=46}$=1.96, p=0.06), indicating that the intensity of the stimuli was well matched between the two different tastants; moreover the effect between intensity and the stimulus presentation site was not statistically significant either for NaCl ($t_{df=46}$=1.25, $p_{\text{-left-right}}$=.22) or MSG ($t_{df=46}$=.25, $p_{\text{left-right}}$=.80;).

Laterality of MSG and NaCl

To assess the laterality of the gustatory pathway, based on the hypothesis described in the introduction, we applied a ROI analysis of the fMRI imaging data choosing the following regions of interest: Thalamus, insula/frontal operculum, orbitofrontal cortex and limbic system. The thalamus receives a direct neuronal projection from the nucleus of the solitary tract, then insula/orbitofrontal cortex are the putative primary gustatory areas and orbitofrontal cortex as well as probably some areas in the limbic system are the putative secondary gustatory cortex.

Region of interest: thalamus

In order to elucidate the MSG-taste and NaCl-taste laterality we performed a ROI analysis inside the thalamus. To assess the activations related to the site of stimulus application we used the t-contrast ´tastant-A(B)' at p_{FWE}=0.05, with the factor 'site' contracted, masked in exclusion by the t-contrast ´tastantA(B)_L´(/R´) at p=0.05 (spm default). That is to look in the thalamus at the effect of NaCl applied on the left side of the tongue (NaCl_L) we used the contrast ´salt´ masked in

exclusion by the t-contrast 'NaCl_R', and similarly for the effects of the other site of application as well as for the effect of the other tastant.

Table 1 shows a summary of the results. The condition MSG_L highlighted a cluster in the left side of the Thalamus whereas right-sided stimulation with MSG produced a left-sided thalamic activation. The condition NaCl_L generated a cluster of activation in the left side of the brain, the condition NaCl_R produced no activation in the thalamus at the set statistical level, although at a lower p-value (p_{unc}=0.001, p_{FWE}= 0.06) we found an activated voxel at the right side. All together this information seems to indicate a predominantly ipsi-lateral processing for the stimulus NaCl while this is different for MSG.

Region of interest: Insula and frontal operculum

To further follow the hypothesized central taste pathways we performed a ROI analysis in the insula and frontal operculum, both defined as putative primary gustatory areas as mentioned in the introduction.

The results of the ROI analysis in the Insula and Frontal Operculum are summarized in Table 2. The contrast used were ´tastant-A(B)' at p_{FWE}=0.05, with the factor 'site' contracted, masked in exclusion by the t-contrast ´tastant-A(B)_L´(/R´) at p=0.05 (spm default), similar to the one illustrated in the previewed paragraph. We found that the application of the NaCl in the left side of the tongue highlighted only one cluster of activations in the left insula. When the same stimulus was applied on right ridge of the tongue, activations were localized in left insula (3 clusters) and one in the right operculum.

The MSG stimulus presented on the right side of the tongue produced activations in the right and left side of insula and operculum. No suprathreshold clusters were found when the MSG was applied on the left side of the tongue.

Region of interest: Orbitofrontal cortex

Following the path of the activations in the orbitofrontal cortex, which is likely to be a secondary taste cortical area (de Araujo et al., 2003b; Kringelbach et al., 2004; Small et al., 1997a), with a contrast similar to the one described in the preceding paragraph, we found that the effect of the tastants, when presented on the left side of the mouth produced an activtion in the left orbitofrontal cortex, for both MSG_L and NaCl_L. The application of the liquid stimulus on the right side of the tongue did not produce significant activation in the ROI analyzed (Table 3).

Region of interest: Limbic lobe

In the limbic lobe the effects of the lateralized stimulation assessed by means of a t-contrast as described above were not statistically significant for both taste qualities, NaCl and MSG.

Discussion

The primary goal of this study was to elucidate by means of fMRI-imaging the laterality of the gustatory system when stimulated by umami and salt. Our results are based on a ROI analysis along the 'taste map', that, as recentely indentified by activation likelihood estimation analysis (ALE) (Veldhuizen et al., 2011), are: thalamus, insula/frontal operculum, OFC and some areas in the limbic lobe. Moreover, we defined the spm-contras in a way that the results are able to stress the neuronal connection specifically related to the taste quality presented to the left or right side of the tongue (see the section **Spm-fMRI assessment)**.

Results in the thalamus region showed a contralateral activation following stimulation with MSG and a predominantly ipsilateral activation for stimulation with NaCl.

Furthermore following the fMRI-activation inside the insula/frontal operculum the exclusive effects of the taste quality was more pronounced for both tastants when they were presented to the right side of the tongue. We also observed overlap among several areas: both tastants shared an area of activation in the left side of the insular cortex (stressed in the Table 2 by an asterisk) as well as an area in the right frontal operculum (stressed in the Table 2 by an open circle).

At the level of the PFC for both stimuli we found similar activations in the left inferior frontal gyrus (L). The two areas are almost overlapping (Table 3). No suprathreshold voxels survived for right-sided stimulation in this condition.

Based on those results we can discuss laetrality in the gustatory pathway (Figure 4). It is established that afferents form the tongue project to the ipsilateral nucleus of the solitary tract (Goto et al., 1983; Jyoichi et al., 1985; Nakajima et al., 1983); then from here second order fibers project to the thalamus, but the laterality of this connection has been controversial (Kobayashi, 2006). Related to this point our results suggest a taste dependent laterality through the thalamus, and specifically an ipsilateral link for NaCl and a contralateral link for MSG (see Figure 4 for details).

Moving to the frontal operculum/insula we found for NaCl applied on the left side an ipsilateral connection with the left frontal operculum/insula, while the right-sided stimulation with NaCl gave bilateral activations. MSG produced a bilateral activation after right-sided stimulation. On the contrary left-sided stimulation did not produce any activation at level of the frontal operculum/insula.

Passing to the OFC, our results showed that the left-sided stimulation with NaCl produced a left-sided activation on the left OFC (Figure 4). This is in agreement with the definition of the OFC as a secondary gustatory area.

On the other side umami produced activations in the left OFC only after the condition MSG_L, while no activations were surviving the statistical threshold after the condition MSG_R. Then remembering the crossing fibers between the NST and Thalamus hypothesized from our results, and no activations in the frontal operculum/insula following left-sided stimulation with MSG, the afferent to the left OFC seems to come from the right thalamus (Figure 4). While the path (a) has been well decrypted as a possible pathway for the gustatory system (Rolls, 2000), the proposed path (b) is new and would move the involved area in the lateral OFC form a secondary gustatory areas in the position of primary gustatory areas. In support of this idea the connection between this part of the OFC and the thalamus has been described in humans by neuroimaging studies (Elliott et al., 2000) and specifically the most anterior section of the lateral subdivision in the OFC, exactly the

area that we found involved, seems to have pronounced connections with the mediodorsal thalamus but also the granular field of insula (Fuster, 1997; Goldman-Rakic, 1987) supporting our hypothesis.

Finally the absence of activations inside the limbic lobe after the lateralized stimulation indicate that - at this level - the information related to the lateralized stimulation is lost.

In conclusion the main finding of our work is the different pathway through the thalamus for the two taste quality. MSG produced a contralateral activation, while the results for the NaCl indicate an ipsilateral link between the nucleus of the solitary tract and the thalamus. Thus, the laterality of the gustatory system seems to be dependent on the taste quality. Moreover, besides the classical view of the gustatory pathway, our data also indicate a direct link between the left OFC and the thalamus, which could suggest a primary role of the OFC in the processing of tastes.

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Table 1

	activation site	x,y,z {mm}	p(FWE-cor)	Z	#	p(k-cor)	
MSG_L	R	21 -24 3	0.018	3.60	3	0.021	VPLN
MSG_R	L	-9 -9 0	0.030	3.45	3	0.021	VAN
NaCl_L	L	-12 -12 6	0.029	3.46	6	0.023	VLN
NaCl_R	R	9 -15 6	0.062	3.16	1	0.051	VLN
			p_{unc}=.001				

Table 2

p(k-cor)	#	p(FWE-cor)	Z	x,y,z {mm} L	R	
positive effects of NaCl_L						
0.092	3	0.018	3.88	-39 -12 15		I
positive effects of NaCl_R						
0.078	4	0.002	4.52	-36 -6 -6		I
0.040	9	0.024	3.81	-45 9 -9 *		I
0.078	4	0.026	3.78	-33 6 6		I
0.044	9	0.047	3.63		39 12 30 °	O
positive effects of MSG_L						
-	-	-	-			
positive effects of MSG_R						
0.008	26	0.001	4.59		45 15 33 °	O
0.044	9	0.002	4.50		48 0 0	I
0.035	11	0.017	3.93		42 12 -6	I
0.058	6	0.011	4.03	-42 9 3 *		I
0.067	5	0.013	3.97	-48 3 24		O
0.045	8	0.020	3.86	-45 0 -3 *		I

Table 3

p(k-cor)	#	p(FWE-cor)	Z	x,y,z {mm}
positive effect of NaCl_L				
0.027	26	0.017	4.19	-48 39 -12
positive effect of NaCl_R				
no suprathreshold voxels				
positive effect of MSG_L				
0.033	14	0.035	4.00	-48 36 -3
positive effect of MSG_R				
no suprathreshold voxels.				

Figure 1

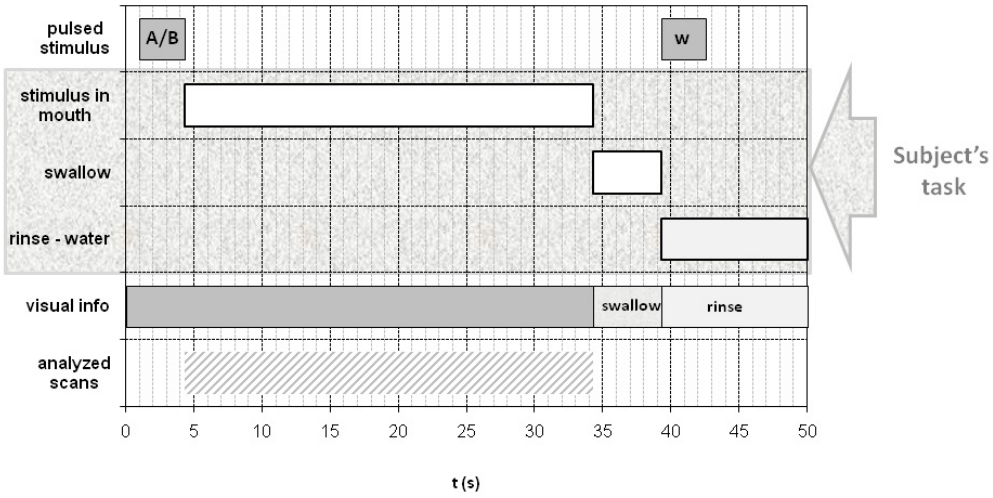
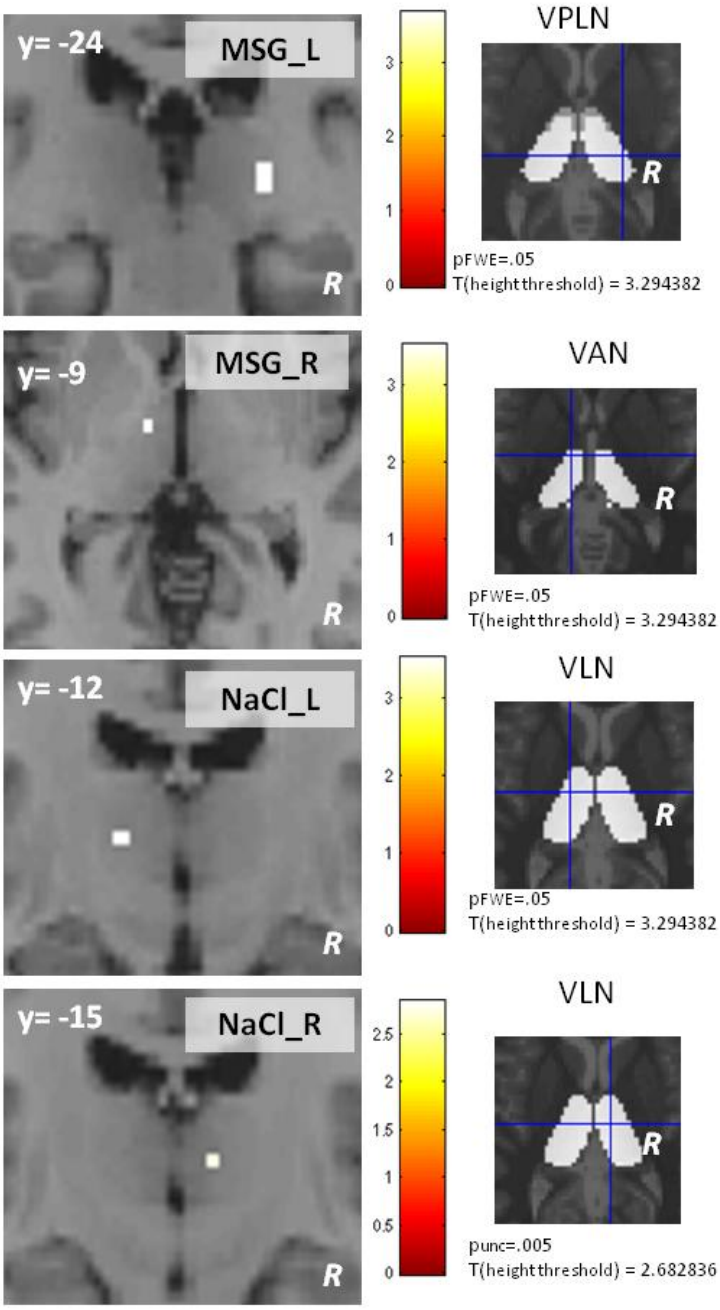


Figure 2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R
3
2
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0
VPLN
R
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y= -9
MSG_R
R
3
2
1
0
VAN
R
pFWE=.05
T(heightthreshold) = 3.294382
y= -12
NaCl_L
R
3
2
1
0
VLN
R
pFWE=.05
T(heightthreshold) = 3.294382
y= -15
NaCl_R
R
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2
1.5
1
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VLN
R
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Figure 3

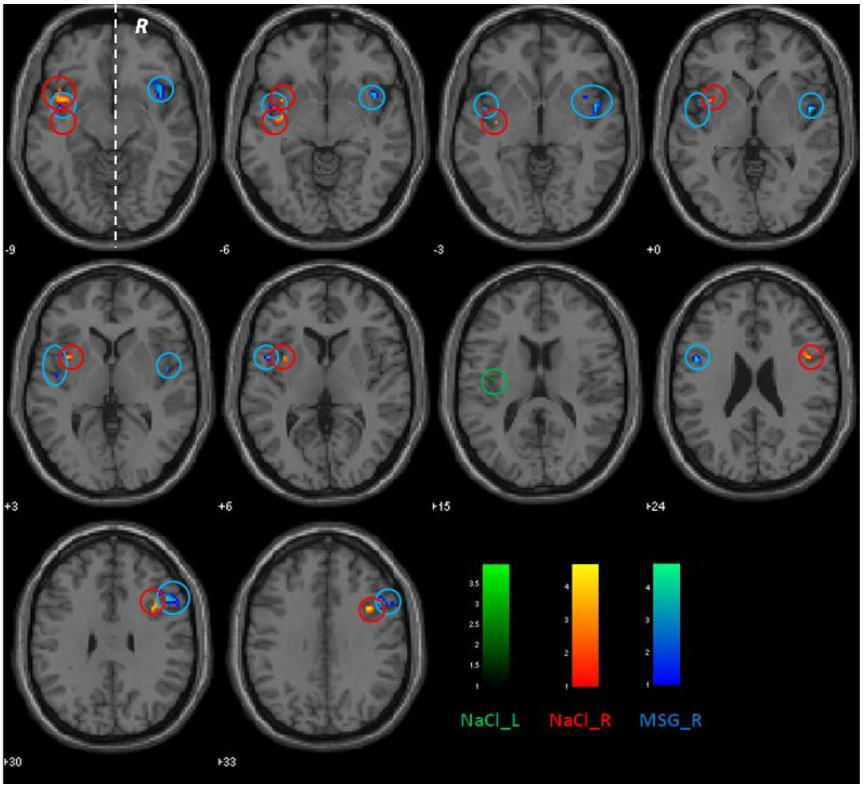


Figure 4

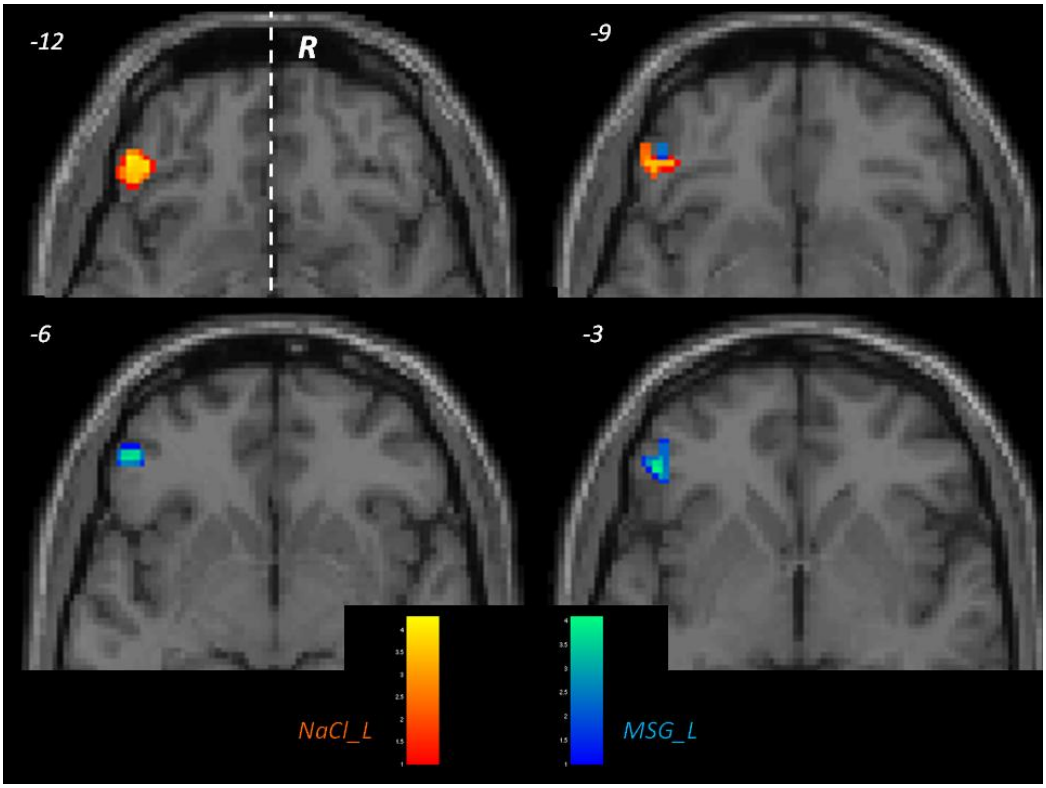
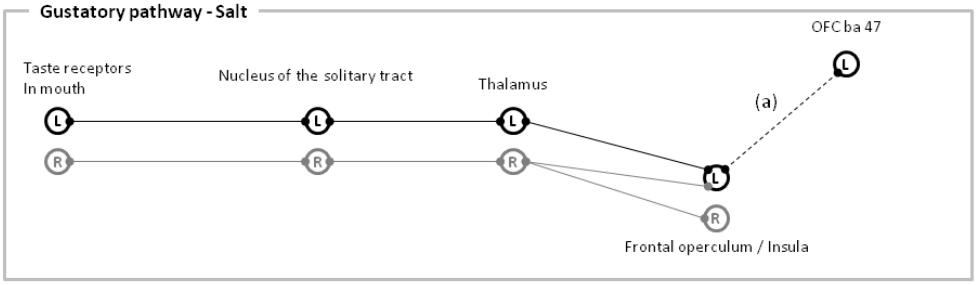


Figure 5


Gustatory pathway - Salt
OFC ba 47
Taste receptors
In mouth
Nucleus of the solitary tract
Thalamus
(a)
L
R
Frontal operculum / Insula

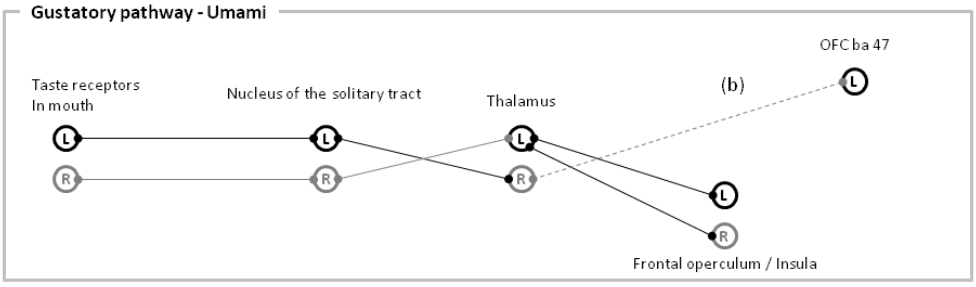

Gustatory pathway - Umami
OFC ba 47
Taste receptors
In mouth
Nucleus of the solitary tract
Thalamus
(b)
L
R
Frontal operculum / Insula

Figure captions

Figure 1
Time frame description of the 50s-block design applied in the fMRI sessions. The graph describes the subject's task highlighted in a marbled field. The pulse stimulus with the tastant was presented a few seconds after a grey field was projected on a screen. The subject was instructed to expect and keep in mouth the stimulus while the gray field was presented. Then the word "swallow" guided the subject to swallow. Finally the word "rinse" was projected on the screen together with a pulse of water ("w" in the graph) and the subject could rinse the mouth and swallow the water. Then a new block started. In the fMRI analysis were included only the scans inside the 30s corresponding to the time when subjects received the taste stimuli. The whole sequence included 4 sessions consisting of of 6 block repetitions each, which lasted a total of 20 min.

Figure 2
Activations in the thalamic ROI-analysis ($P_{FWE\text{-}corr}<.05$, cluster level=3, t-level in color coded labeled) by the stimulus conditions umami on the left side of the tongue (MSG_L), umami on the right side of the tongue (MSG_R), salt on the left side of the tongue (NaCl_L) and salt on the right side of the tongue (NaCl_R). Apart from the statistical parametric map the ROI is shown with the crosshair position in the same voxel location. The condition MSG_L activated the right VPLN, the condition MSG_R the left VAN, NaCl_L the left VLN and NaCl_R the right VLN. The reported coordinates are in the MNI space, on the pictures R=right.

Figure 3
Activations in the insular/frontal opercular ROI-analysis ($P_{FWE\text{-}corr}<.05$, cluster level=3, t-level in color coded labeled) by the stimulus conditions salt on the left side of the tongue (NaCl_L in green), salt on the right side of the tongue (NaCl_R in orange)and umami on the right side of the tongue (MSG_R in blue). The stimulus condition: umami on the left side of the tongue did not produce any significant activation at set statistical level. The reported coordinates are in the MNI space s, on the pictures R=right.

Figure 4
Activations in the OFC ROI-analysis ($P_{FWE\text{-}corr}<.05$, cluster level=3, t-level in color coded labeled) by the stimulus conditions salt on the left side of the tongue (NaCl_L in orange) and umami on the left side of the tongue (MSG_L in blue). The stimulus conditions NaCl_R and MSG_R did not produce any significant activation at the set statistical level. The reported coordinates are in MNI space, on the pictures R=right.

Figure 5
Gustatory pathway laterality reconstructed from our fMRI-results for NaCl (salt) and MSG (Umami) tastants. Interesting is the afferent fibers intersection at the thalamic level for stimulation with MSG in spite of an ipsilateral connection for salt. Moreover the OFC seems to be confirmed as a secondary gustatory cortex in path (a) –salt, but on the contrary could have also a primary role as indicated in path (b)-umami

Table captions

Table 1
Thalamic ROI analysis showing the effect of tastant applied on the left, MSG_L (NaCl_L), or right, MSG_R (NaCl_R), side of the tongue. $P_{FWE\text{-}corr}<.05$, cluster level=3. In the table the 'activation side' indicates explicitly if the activation is in the left (L) or right (R) side of the thalamus. VPLN= ventral posterior nucleus; VAN=ventral anterior nucleus; VLN=ventral lateral nucleus. Maximum coordinate x,y,z in MNI-space. P value corrected at FWE level. Z: statistical value. #= number of voxels inside the cluster. P(k-cor)= p value at the cluster level.

Table 2
Insular ROI analysis showing the effect of tastant applied on the left, MSG_L (NaCl_L), or right, MSG_R (NaCl_R), side of the tongue. $P_{FWE\text{-}corr}<.05$, cluster level=3. Maximum coordinate x,y,z in MNI-space. P value corrected at FWE level. Z: statistical value. #= number of voxels inside the cluster. P(k-cor)= p value at the cluster level. I= insula; O=frontal operculum.*= common activation in the left side-ROI. °=common activation in the right side- ROI.

Table 3
OFC ROI analysis showing the effect of tastant applied on the left, MSG_L (NaCl_L), or right, MSG_R (NaCl_R), side of the tongue. $P_{FWE\text{-}corr}<.05$, cluster level=3. Maximum coordinate x,y,z in MNI-space. P value corrected at FWE level. Z: statistical value. #= number of voxels inside the cluster. P(k-cor)= p value at the cluster level.