Taste Perception in Obesity

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List of Abbreviations

BMI= Body mass index

BOLD = Blood oxygen level dependent

EEG = Electroencephalography

ERP= Event related potentials

gERP = Gustatory event related potential

fMRI = Functional magnetic resonance imaging

MEG = Magnetoencephalography

MVPA = Multivariate pattern analysis

OFC = Orbitofrontal cortex

PET = Positron emission tomography

SNR = Signal-to-noise ratio

WHO = World Health Organization

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1. Introduction

Taste, or gustatory perception, is the sensation generated as a result of the chemical reactions between a substance and the taste receptors in an organism's mouth. In humans, five basic taste qualities have been identified: sweet, salty, sour, bitter, and more recently, savoury or "umami" (Ikeda, 2002; Lindemann et al., 2002). It is through gustatory perception that humans learn to recognize and seek nutritious food sources. For instance, carbohydrates, which are energy-rich, usually produce a pleasant sweet taste; whereas sodium plays a vital role in homeostasis and is indicated by a salty taste which is pleasant in moderate concentration. Gustatory information also aids in identifying and avoiding certain harmful substances, e.g. bitter taste often indicates toxins and is universally unpleasant even in very low concentrations. Consequently, more so than any of the other four traditionally recognized senses - audition, olfaction, somato-sensation and vision - gustation plays a central role in survival. Impaired taste sensitivity has even been associated with higher mortality rates among acutely hospitalized older individuals (Solemdal et al., 2014).

The sense of taste is additionally unique in that it does not occur in isolation. What is colloquially referred to as the "taste" of a food is usually not just the taste but the "flavour", i.e. the combination of the gustatory and retronasal olfactory sensations, produced by that food. The act of putting food in the mouth, chewing and swallowing it leads to this unitary flavour percept, which includes taste, smell, as well as other, trigeminally relayed sensations, such as the texture or temperature of the food, which are integrated in the brain (Small, 2012). At the very least, substances which produce the sensation of taste also produce oral somato-sensation upon coming in contact with the

tongue. This characteristic, among others, makes taste perception particularly difficult to measure and study.

1.1 The Human Gustatory Pathway

The taste buds present on the dorsal surface of the tongue and on the epiglottis are innervated by the facial nerve, glossopharyngeal nerve, and vagus nerve (cranial nerves VII, IX and X, respectively). When food/ other substances interact with taste receptor cells in the taste buds, these afferent fibres course to the nucleus of the solitary tract (NST) in the brainstem. From the brainstem, taste information is transmitted to the amygdala, the hypothalamus, and via the ventral posteromedial nucleus of the thalamus to cortical gustatory areas.

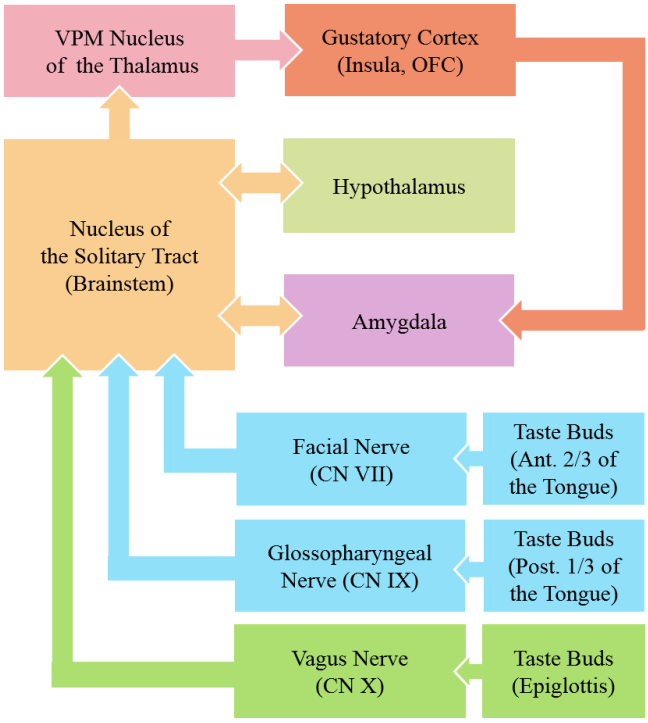

Figure 1. Schematic representation of the basic human taste pathway. Own figure based on Purves et al., 2001.

1.2 Neural Correlates of Taste in Humans

Our knowledge of the neural correlates of taste in humans is based on early lesion studies (Börnstein, 1940a, 1940b; Henkin et al., 1977), and relatively recent functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies (de Araujo et al., 2003; Frey and Petrides, 1999; Kinomura et al., 1994; Schoenfeld et al., 2004; Small et al., 1999). A meta-analysis of 10 fMRI and five PET studies, weighted by sample size, (Veldhuizen et al., 2011) showed significant activation probabilities in response to taste stimuli in bilateral anterior insula and overlying frontal operculum, mid-dorsal insula and overlying Rolandic operculum, bilateral posterior insula/parietal operculum/ postcentral gyrus, as well as left lateral orbitofrontal cortex (OFC), right medial OFC, pregenual anterior cingulate cortex (prACC) and right mediodorsal thalamus.

Of these regions, the primary gustatory area is believed to be at the transition of the insula and the overlying operculum (Kobayakawa et al. 1996), where the quality (Schoenfeld et al., 2004) and intensity (Grabenhorst et al., 2008) of taste is processed, whereas the orbitofrontal cortex is considered the secondary gustatory area, where the hedonic value of taste is processed (de Araujo et al., 2003; Kringelbach et al., 2003; McCabe and Rolls, 2007). Although the anterior insula and overlying frontal operculum have been proposed as the primary taste area (Small et al., 1999) based on its location in non-human primates (Ogawa et al., 1985), there is competing evidence suggesting that in humans, the primary taste area may in fact be at the junction of parietal operculum and insula (Kobayakawa et al. 1999).

The work of Katz et al. (Katz et al., 2002) on taste in the mouse brain emphasizes the need to understand the dynamic and distributed nature of

gustatory processing. They have shown that gustatory coding takes place via networks of feedback and feedforward pathways and that responses in the gustatory cortex are time-varying, reflecting somatosensory contact of the tastant, chemosensory processing of the tastant and multi-sensory coding of tastant palatability, in that order. A comparable account of this network in humans does not exist. The temporal resolution of fMRI and PET is in the order of seconds, and hence too low to capture dynamic neural processes that evolve within milliseconds. This problem is overcome in cognitive neuroscience through the use of magnetoencephalography (MEG) or electro-encephalography (EEG), where neural responses can be measured, in the form of magnetic fields/ electrical signals generated by post-synaptic firing, at sampling rates as high as 1000 Hz or more. Indeed, it was evidence from MEG that challenged the notion that the anterior insula and frontal operculum is the primary gustatory area, as the taste evoked responses in the parietal operculum and insula could be observed 286ms earlier than the fastest responses in the anterior insula/ frontal operculum (Kobayakawa et al., 1999). However, to date there have only been a handful of attempts at investigating taste perception with the use of MEG (Kobayakawa et al., 1999, 1996; Onoda et al., 2005). EEG, which is less expensive, less cumbersome and more flexible than MEG and has been employed more often to measure gustatory evoked responses (see Ohla, Busch, & Lundström, 2012 for review) in recent years. In the following section, I will present a brief account of the current state of and challenges particular to this research.

1.3 Gustatory Event Related Potentials

Compared to other sensory modalities, EEG studies of chemical senses in general, and gustation in particular have been few and far between. To bring a

simple yet illuminating statistic reported by Ohla et al., (2012) up to date, a search performed on the scientific publications database PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) in July 2018, using the keywords "gustatory" and "EEG", with the results restricted to "human" populations, returned 54 hits. Whereas replacing "gustatory" with "visual", "auditory", "somatosensory", or "olfactory" returned 15998, 10165, 4122, and 429 hits, respectively. This dearth of literature on gustatory EEG is a result of the challenges of stimulus control that are inherent to gustatory stimulation. While EEG records the electrical signals generated by the synchronous postsynaptic potentials of neurons, this includes the signals generated by many simultaneous brain processes, and the response to a specific stimulus is not usually visible through this ongoing signal in a single trial. Thus, the event related potential (ERP) technique looks at the averaged signal from many presentations (trials) of the same stimulus, so that the background signal is averaged out over trials, while the waveform showing the response to the stimulus of interest remains. Precisely time-locked stimuli with a sharp rise-time are required in order to average the signal from multiple trials and calculate ERPs (Coles and Rugg, 1995; Luck, 2005). This is difficult to achieve with liquid gustatory stimuli. Moreover, tactile-free taste stimulation is required in order to eliminate the oral-somatosensory component of gustatory ERPs (gERPs). An ingenious system for this purpose was devised by Kobayakawa et al., in 1996. And a similar set-up has become commercially available in recent years in the form of a "gustometer", (GU002, Burghart Messtechnik, Wedel, Germany, Fig 2) which has led to a number of gustatory EEG studies (Crouzet et al., 2015; Iannilli et al., 2014; Tzieropoulos et al., 2013). However, even the handful of existing studies are not in perfect agreement with one another in terms of the observed neural responses for the same taste quality. In the following

paragraph, I will illustrate this point by considering the two tastes most relevant to the experimental work presented here – salty and sweet – in this regard.

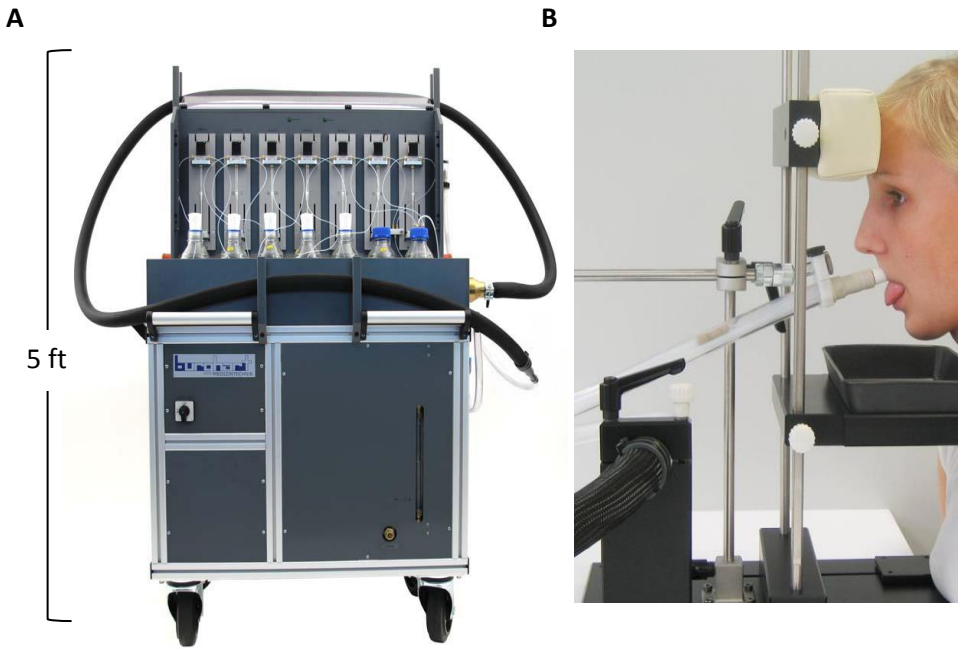

Figure 2. "Gustometer" for precise gustatory stimulation in humans **A)** Outer unit of the gustometer: Taste stimuli can be created by mixing the liquids from the five taste modules in concentrations ranging from 0-100%. The two water modules (marked by blue lids) provide a constant stream of atomised water pulses in which taste stimuli can be embedded seamlessly. In this way, the resulting evoked responses have a minimal somatosensory component. **B)** Tastant delivery apparatus inside the EEG chamber: The background water spray and stimuli are delivered through the spray-head directly on the extended anterior half of the participant's tongue. Images reproduced with permission from Burghart Messtechnik GmbH.

The gustatory P1 component, the first positive deflection seen over fronto-central electrodes, has been reported in the past with peak latencies around 130–150ms (Mizoguchi, 2002, Wada 2005) for salty taste. Using the aforementioned Burghart gustometer, the P1 peak has been reported at 178ms

(Crouzet et al., 2015) between 77-235ms (Tzieropoulos et al., 2013) for salt, although another study failed to see this component (Iannilli et al., 2014) and only saw a negative deflection, N1 around 250ms, and a late positive component (LPC), believed to be related to endogenous stimulus properties, around 650ms over central electrodes. Tzieropoulos et al., (2013) also reported an N1 at 284 and 384ms, and the LPC between 554 – 729ms. Yet another study has reported the N1 much later at 506ms (Singh et al., 2011). These examples of the variability of these deflections and their latencies in only one type of taste show the elusive nature gustatory evoked responses. These are even more difficult to capture for sweet taste, where the latencies are greater and the signal-to-noise ratio (SNR) is generally lower than that for salt (Crouzet et al., 2015), even making it impossible to compute a gERP, in some cases (Iannilli et al., 2014)

1.4 Obesity and taste perception

Obesity is defined as an excess accumulation of body fat in a way that may be detrimental to health ("WHO | Obesity and overweight Fact Sheet N 311," 2015). Being obese increases an individual's risk for developing diabetes, cardiovascular diseases, some forms of cancer, and stroke, among other physiological conditions (Haslam and James, 2005a), as well as depression (Haslam and James, 2005a; Luppino et al., 2010). Obesity can also affect an individual's social health, as individuals with obesity are often subjected to social stigmatisation and exclusion (Sikorski et al., 2011). Globally, the prevalence of obesity has nearly tripled in the last four decades, with the number of obese adults in the world currently estimated to be over 650 million. Obesity is a preventable disease. As such, it is important to understand the mechanisms of obesity, and to investigate the neuro-behavioural mechanisms

that make certain individuals more susceptible to obesity, and/ or less likely to succeed in achieving desired weight loss after the onset of obesity.

Obesity is caused by an imbalance between energy consumption and energy expenditure (Caballero, 2007). However, this process is affected by several physiological, genetic, and socio-economic variables, not all of which are understood. As mentioned earlier, taste perception is one of the central determinants of food intake. This takes place not just via nutrient-sensing, but also through some complex behavioural phenomena that depend upon taste perception. Food craving or intake can be influenced by sensory specific satiety (Rolls et al., 1981) where an individual may feel too full after consuming a particular food, but may still go on eating when presented with a different type of food, or by hedonic hunger (Lowe and Butryn, 2007), whereby an individual will feel the desire to consume food for the hedonic experience rather than in order to achieve homeostatic balance, i.e. a sense of satiety. Yet investigations of taste perception in relation to obesity over the years have provided inconsistent findings. For instance, evidence can be found for lower taste sensitivity in obesity (Proserpio et al., 2015), no effect of obesity on taste thresholds (Malcolm et al., 1980; Martinez-Cordero et al., 2015) or higher taste sensitivity in obesity in some or all tastes in children, adolescents, and older adults (Overberg et al., 2012; Pasquet et al., 2007; Simchen et al., 2006). The interpretation of these discrepancies is made difficult by the heterogeneity of methods in studies of taste perception. These are discussed in more detail in publication 1. (See also Hummel, Hummel, & Welge-Luessen, 2014; Snyder, Sims, & Bartoshuk, 2015 for an overview). Moreover, the aforementioned studies have tended to focus only on one or two measures of taste perception. A broader comparison of taste experience in obese and non-

obese individuals, including thresholds as well as supra-threshold hedonic ratings for the four basic tastes, has only been carried out once using very small sample sizes (Malcolm et al., 1980). fMRI studies of taste perception and obesity present a similarly inconclusive picture. While a BMI-dependent higher BOLD response to taste has been reported more than once in gustatory areas like the insula (Stice et al., 2008b; Szalay et al., 2012) and the rolandic operculum (Ng et al., 2011; Stice et al., 2008b; Szalay et al., 2012), it is not clear when in the perceptual process these differences occur. It should also be noted that the above studies have used relatively complex taste stimuli (e.g. milkshake), rather than basic tastants, possibly increasing the oral-somatosensory aspect of the neural responses.

1.5. Rationale for the experimental work

Deriving from the state of the literature as discussed above, we found the need to pursue the following experimental work:

To begin with, we compared lean and obese individuals on behavioural measures of taste perception. Specifically, recognising the absence of a comprehensive comparison of all dimensions of taste perception in the same sample, we included recognition threshold-estimation for four basic tastes, as well as supra-threshold measures of taste intensity and pleasantness. Following up the findings of behavioural differences found between lean and obese individuals, we investigated the neural correlates of sweet and salty taste in these two groups. To this end, we measured taste-evoked responses in these two groups using head-surface EEG. Specifically, to remove the confounding effects of oral somato-sensation that are generally inherent in gustatory stimulation, we used a gustometer [Fig 2] which delivers taste stimuli by embedding them into a constant stream of water pulses, thus habituating the

participants to the touch of the spray and minimizing the concomitant lingual somatosensory response. Measures of taste intensity and pleasantness were also acquired from all participants on all trials along with EEG to see whether any potential differences in perceived experience of taste were predictive of the observed neural response, and vice versa.

Given the lack of consistent observations in the literature, both studies were conducted in an exploratory manner. Nevertheless, specifically we intended to observe whether lean and obese individuals had differing sensitivity to taste stimuli or showed differences in perceived hedonics of taste. Furthermore, we sought to find out whether lean and obese individuals showed differential neural correlates of taste, and specifically, whether these differences occurred during the early or later phases of taste processing.

2. Experimental Work

2.1. Higher sensitivity to sweet and salty taste in obese compared to lean individuals.

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Higher sensitivity to sweet and salty taste in obese compared to lean individuals

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ABSTRACT

Although putatively taste has been associated with obesity as one of the factors governing food intake, previous studies have failed to find a consistent link between taste perception and Body Mass Index (BMI). A comprehensive comparison of both thresholds and hedonics for four basic taste modalities (sweet, salty, sour, and bitter) has only been carried out with a very small sample size in adults. In the present exploratory study, we compared 23 obese (OB; BMI > 30), and 31 lean (LN; BMI < 25) individuals on three dimensions of taste perception – recognition thresholds, intensity, and pleasantness – using different concentrations of sucrose (sweet), sodium chloride (NaCl; salty), citric acid (sour), and quinine hydrochloride (bitter) dissolved in water. Recognition thresholds were estimated with an adaptive Bayesian staircase procedure (QUEST). Intensity and pleasantness ratings were acquired using visual analogue scales (VAS). It was found that OB had lower thresholds than LN for sucrose and NaCl, indicating a higher sensitivity to sweet and salty tastes. This effect was also reflected in ratings of intensity, which were significantly higher in the OB group for the lower concentrations of sweet, salty, and sour. Calculation of Bayes factors further corroborated the differences observed with null-hypothesis significance testing (NHST). Overall, the results suggest that OB are more sensitive to sweet and salty, and perceive sweet, salty, and sour more intensely than LN.

1. Introduction

The sense of taste is important to detect nutrients and toxins in our foods. According to this notion, sweet indicates carbohydrates, salty indicates sodium, sour indicates acids and potentially spoiled foods, and bitter acts as a warning sign for potentially toxic ingredients (but also healthy compounds found in green vegetables). Impairments in taste perception and/or hedonic experience of taste can cause deviant eating behaviour, which can lead to mal- or super-nutrition, both representing major public health issues.

Overweight and obesity are defined as abnormal or excessive accumulation of body fat to an extent that may lead to negative effects on health. Body Mass Index (BMI, kg/m^2) is a simple and commonly used measure for classifying weight status (underweight, normal weight, overweight, obese etc.). According to the latest global estimates from the World Health Organisation (WHO), worldwide, prevalence of obesity has more than doubled since 1980 (WHO, 2015). WHO has also reported that an increased BMI is a major risk factor for several non-communicable diseases such as type 2 diabetes, heart disease, stroke, and some forms of cancer. Considering that obesity is preventable, it is important to understand the causes and effects of obesity in order to devise prevention and treatment strategies.

The large part of the obesity research in recent years has concentrated on 'eating behaviour', and the reward response to food or food cues (e.g. food pictures) rather than the sensory aspects of food intake, i.e. taste sensitivity and preference. Consequently, the link between taste perception and BMI is unclear (Donaldson, Bennett, Baic, & Melichar, 2009). Studies looking at BMI related sensitivity or threshold differences for sweet, salty, sour and bitter tastes have either found no effect (Malcolm, O'Neil, Hirsch, Currey, & Moskowitz, 1980; Martinez-Cordero, Malacara-Hernandez, & Martinez-Cordero, 2015), lower taste sensitivity in obesity (Proserpio, Laureati, Bertoli, Battezzati, & Pagliarini, 2015)

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or higher taste sensitivity in obesity in some or all tastes in children, adolescents, and older adults (Overberg, Hummel, Krude, & Wiegand, 2012; Pasquet, Frelut, Simmen, Hladik, & Monneuse, 2007; Simchen, Koebnick, Hoyer, Issanchou, & Zunft, 2006). A comprehensive investigation of taste experience in adults, measured with taste thresholds as well as supra-threshold hedonic ratings for the four basic tastes, found no differences between adult-onset obese, juvenile-onset obese, and never-obese women (Malcolm et al., 1980). However, the small sample sizes may have hindered the authors from detecting small differences between groups.

Research on taste perception and weight status has primarily focused on sweet taste (Bartoshuk, Duffy, Hayes, Moskowitz, & Snyder, 2006; Grinker, Hirsch, & Smith, 1972; Pepino, Finkbeiner, Beauchamp, & Mennella, 2010; Rodin, Moskowitz, & Bray, 1976; Thompson, Moskowitz, & Campbell, 1976); while bitter taste has also been investigated, studies have focused on Phenylthiocarbamide (PTC) and 6-*n*-propylthiouracil (PROP) (Goldstein, Daun, & Tepper, 2005; Tepper et al., 2008), bitter compounds that are not commonly found in foods. Salty and sour taste perception has remained largely unexplored (Donaldson et al., 2009). The combined results from these studies are inconclusive. For instance, in spite of the widespread belief that sweet foods contribute greatly to excess weight gain, no clear difference in sweet sensitivity had been seen between obese and lean individuals (Grinker et al., 1972; Rodin et al., 1976; Thompson et al., 1976). A lower sweet intensity perception was first reported in people with obesity when general Labelled Magnitude Scales (gLMS) were used instead of traditional visual analogue scales (VAS), combined with a higher sweet preference (Bartoshuk et al., 2006). GLMS are designed to be more valid than traditional VAS when comparing inter-individual subjective ratings. However, in a later study, no difference was shown between obese and normal weight groups in detection thresholds, preference, discrimination performance or supra-threshold intensity ratings, even when intensity ratings were acquired using a gLMS (Pepino et al., 2010).

An unambiguous interpretation of the literature on nutritional status and taste is further complicated by the heterogeneity of methods across studies. First of all, the current WHO definitions of weight status are: 'normal weight' = 18.5–25 kg/m^2, 'overweight' = 25–30 kg/m^2, and 'obese' $\geq$ 30 kg/m^2. But the classification for obese and non-obese groups in studies does not always adhere to these criteria (e.g. Simchen et al., 2006). Secondly, a comparison of thresholds may refer to absolute or detection thresholds, recognition thresholds, or identification thresholds, which may, in turn, be estimated in a variety of ways (Snyder, Sims, & Bartoshuk, 2015). Taste stimuli may be applied in the form of water-based taste solutions, or taste infused paper strips, cotton swabs, or discs (for an overview, see Hummel, Hummel, & Welge-Luessen, 2014). Liquid stimuli can be administered to the tongue as sprays or drops, or as larger aliquots that participants are asked to sip. There is also variability in the chemical compounds (e.g. citric acid or acetic acid for 'sour', caffeine or quinine for 'bitter)', concentration ranges, and stimulus amounts used for taste assessment. Sets of taste infused paper often use very few concentration steps (e.g. 4 for taste strips; Mueller et al., 2003) that do not readily allow detection of small differences between groups or across time. It is worth taking into account that differences in taste thresholds do not necessarily reflect differences in supra-threshold sensitivity (Bartoshuk, 1978; Webb, Bolhuis, Cicerale, Hayes, & Keast, 2015). Consequently, it is important to independently estimate supra-threshold sensitivity and preferences for taste, as human food intake generally takes place at a supra-threshold taste level. To date, measures of taste sensitivity and subjective supra-threshold perception have not been systematically assessed and compared between lean and obese individuals.

In the present study, we compared taste perception in lean and obese participants on three dimensions: recognition thresholds as an objective measure of taste sensitivity, as well as subjective intensity and pleasantness for different supra-threshold concentrations of four basic tastes.

2. Materials and methods

2.1. Participants

54 healthy participants between 18 and 35 years of age were recruited into the lean (LN) or obese (OB) group based on BMI of <25 and >30, respectively. The LN group consisted of 31 participants (Mean BMI = 21.88, range = 18.73 to 24.49; 14 women), and the OB group included 23 participants (Mean BMI = 33.8, range = 30.47 to 38.96; 12 women). All women used hormonal contraceptives. Self-report based exclusion criteria were: taste and smell disorders, smoking, substance abuse and other addictions, current or recent oral, nasal or sinus infections, pregnancy, recent (in the last 6 months) childbirth, thyroid disorders, diabetes, or weight loss of more than 10 kg in the last 3 months. All participants gave written informed consent prior to the experiment.

2.2. Stimuli

Tastants were sucrose (Sigma-Aldrich, CAS number: 57-50-1), sodium chloride (NaCl; Sigma-Aldrich, CAS number: 7647-14-5), citric acid (Sigma-Aldrich, CAS number: 77-92-9), and quinine hydrochloride (quinine; Sigma-Aldrich, CAS number: 6119-47-7) dissolved in mineral water (Volvic) creating 'sweet', 'salty', 'sour', and 'bitter' taste, respectively. Each stimulus was a 0.2 mL bolus of the tastant administered to the anterior part of the tongue. For threshold estimation, 12 dilution steps, evenly spaced on a decadic logarithmic scale, were prepared for each taste quality. The concentration ranges (Table 1) were derived from the literature, and adjusted according to preliminary testing. Tastants were stored in individual glass bottles with a spray dispenser, presented at room temperature, and kept at 5 °C in the dark for a maximum of three days when not in use.

2.3. Recognition thresholds

Recognition thresholds were estimated for each of the four taste qualities independently through an adaptive staircase procedure based on QUEST (Watson & Pelli, 1983), implemented via PsychoPy 1.80.03 (Peirce, 2007). The procedure assumed the relationship between log-transformed stimulus concentrations and perceived taste intensities to follow the shape of a Weibull function with a slope of 3.5, and the threshold as free parameter. Pilot testing showed that participants were highly unlikely to report a stimulus at very low concentrations or when pure water was presented (low false-alarm rate; FAR), and, likewise, would only rarely report not perceiving a stimulus at high concentrations (low lapsing rate). Therefore, we assumed both false-alarm and lapsing rates to be fixed at 0.01. A starting concentration and its standard deviation were provided to QUEST as a prior. These concentrations were chosen after pilot testing in such a way that they would be clearly perceptible to most participants (sucrose: 5.022 g/100 mL, NaCl: 1.615 g/100 mL, citric acid: 0.285 g/100 mL, quinine: 0.0092 g/100 mL) and presented on the first trial of threshold estimation for the respective taste quality. After each response given by the participant, QUEST updates the posterior probability density function for the threshold, and proposes the next concentration to be presented. Since we only had a limited number of stimuli

available, if the exact concentration proposed by QUEST was unavailable (which was usually the case), QUEST suggested the closest available concentration for presentation. If the newly selected concentration had already been presented in the immediately preceding trial, the next lower or higher concentration was chosen based on whether the participant had succeeded or failed in detecting it, respectively. The procedure was repeated until the 90% confidence interval of the estimated threshold was less than half (approx. width of the log-step) of the concentration presented last, or after a maximum of 20 trials. Thresholds were estimated separately for the four taste qualities in a counterbalanced order across participants. On each trial, participants were presented with a single stimulus and asked to answer whether they could perceive the target taste or not by stating 'Yes' or 'No', respectively. They were instructed to respond promptly, and only answer 'Yes' if they were certain, thereby enforcing a strict response criterion. After a response was given, participants rinsed their mouth with water, and waited for 30 s before the next presentation.

2.4. Supra-threshold perception

Participants rated four supra-threshold concentrations of each taste quality (16 stimuli) for their intensity and pleasantness using VAS anchored with labels, i.e., "no sensation" (0) and "extremely intense" (100) for intensity, and "extremely unpleasant" (−50), "neutral" (0), and "extremely pleasant" (50) for pleasantness. All participants evaluated an "Absolute High" and "Absolute Low" concentration for each taste to allow for comparison independent of individual taste sensitivity. These concentrations (39.91 and 10.02 g/100 mL for sucrose; 8.8 and 2.84 g/100 mL for NaCl; 1.67 and 0.40 g/100 mL for citric acid; and 0.0151 and 0.0055 g/100 mL for quinine) were the same for all participants. Additionally, participants evaluated a "Relative Low" and "Relative High" concentration of each taste quality. These were one and three concentration steps above the individual threshold, respectively, and thereby adjusted to each participant's individual taste sensitivity. By including both "Absolute" (for all subjects) and "Relative" (threshold adjusted) concentrations, we measured not only how participants rated a given 'high' or 'low' concentration, but also how participants rated high or low concentrations within their individual taste perceptual space.

2.5. Questionnaires

Participants also completed four questionnaires using LimeSurvey (Schmitz, 2012) in a separate session to assess levels of chronic stress (Trier Inventory for Chronic Stress; TICS; Schulz & Schlotz, 1999), depression (Beck Depression Inventory; BDI; Beck, Steer, & Brown, 1996), Inhibition, Drive, Fun-Seeking and Reward Responsiveness (Behavioural Inhibition System/Behavioural Activation System; BIS/BAS; Carver & White, 1994), and Dietary Restraint, Disinhibition and Hunger (Three Factor Eating Questionnaire; TFEQ; Stunkard & Messick, 1985). Questionnaire data from 7 participants (2 LN, 5 OB) for BDI and BIS/BAS, 10 participants (5 LN, 5 OB) for TFEQ, and 11 participants (5 LN, 6 OB) for TICS was missing due to technical difficulties.

Table 1
Taste concentration ranges used for threshold estimation.

Taste	Grams/100 mL		Log-step width
	Lowest	Highest	
Sucrose	0.0100	20.000	0.300
NaCl	0.0100	5.000	0.245
Citric Acid	0.0010	0.900	0.269
Quinine	0.0001	0.025	0.218

2.6. Statistical analyses

Unpaired *t*-tests were performed to compare mean thresholds between groups. Supra-threshold ratings and questionnaire scores were compared using the Mann-Whitney *U* test, as they did not follow a normal distribution (according to Shapiro-Wilk tests).

Along with conventional NHST, Bayes factors (BF) were calculated for taste thresholds as well as supra-threshold ratings via JASP 0.7.5 Beta2 (JASP Team, 2015; https://jasp-stats.org/) using a Cauchy prior width of 0.707. BFs indicate the likelihood ratio that expresses how likely the observed data are under the alternative (H_1) hypothesis relative to the null (H_0) hypothesis. Thus, a BF_{10} of 4.5 would mean that the data are 4.5 times more likely to be observed under H_1, whereas a BF_{10} of 0.3 would mean that the data are 3.3 (i.e., 1/0.3) times more likely to be observed under H_0.

Correlations were computed between the first and second subjective rating as a measure of within-subject consistency, and between taste thresholds and supra-threshold ratings to quantify the relation between objective and subjective measures. Pearson's correlation coefficient r is reported for data that were normally distributed and Spearman's ρ is calculated for ratings that were not normally distributed.

3. Results

3.1. Questionnaires

OB and LN yielded similar scores in all questionnaires expect for higher median Hunger scores in the TFEQ (LN = 4, OB = 5; $U = 134$, $p = 0.015$) and higher Fun Seeking in BIS/BAS (LN = 12, OB = 13.5; $U = 154.5$, $p = 0.018$) in OB compared to LN.

3.2. Recognition thresholds

OB had significantly lower thresholds for sweet ($t_{52} = 2.681$, $p = 0.01$) and salty ($t_{52} = 3.072$, $p = 0.003$) than LN, which was further corroborated by moderate evidence for H_1 (a difference in the two groups) for sweet ($BF_{10} = 4.778$), and strong evidence for H_1 for salty ($BF_{10} = 11.008$) thresholds. No significant group difference was found for sour and bitter. Threshold statistics are reported in Table 2 and Fig. 1.

3.3. Supra-threshold perception

For the supra-threshold tastants, OB tended to rate the "Absolute Low" and "Absolute High" concentrations as more intense than LN (Tables 3a and 3b, Fig. 2a and b). This difference was significant for the "Absolute High" sweet ($U = 227.5$, $p = 0.024$), "Absolute Low" sweet ($U = 201.5$, $p = 0.007$), "Absolute Low" salty ($U = 209.5$, $p = 0.01$), and "Absolute Low" sour ($U = 193.5$, $p = 0.004$) concentrations. OB also rated the "Relative High" sweet ($U = 220$, $p = 0.017$) as more pleasant than the LN.

In line, BFs provided moderate evidence for H_1, implying higher intensity ratings in OB compared to LN for the "Absolute Low" sweet ($BF_{10} = 4.689$), "Absolute Low" salty ($BF_{10} = 6.387$), and "Absolute Low" sour ($BF_{10} = 8.075$) concentrations. No significant group differences were found for intensity ratings of "Absolute High" salty, sour or bitter tastants, the "Relative High" and "Relative Low" concentrations of any of the four tastes, the pleasantness ratings "Relative High" salty, sour or bitter tastants, or any of the "Absolute High", "Absolute Low", or "Relative Low" concentrations (all $p > 0.055$). For "Absolute High" sweet intensity, and "Relative

Table 2
Unpaired *t*-tests and Bayes factors (log-transformed threshold values (g/100 mL) [a].

Taste	Lean		Obese		NHST		BF	
	Mean	SD	Mean	SD	t_{52}	p	BF_{10}	error %
Sucrose	−0.273 [b]	0.344	−0.554 [c]	0.427	2.681	0.01	4.778	2.341e−6
NaCl	−0.888 [d]	0.295	−1.170 [e]	0.383	3.072	0.003	11.008	1.423e−6
Citric Acid	−1.612 [f]	0.386	−1.711 [g]	0.406	0.905	0.370	0.390	1.586e−4
Quinine	−3.175 [h]	0.604	−3.346 [i]	0.697	0.960	0.342	0.405	1.619e−4

[a] The log threshold values correspond to the following concentrations on a linear scale.
[b] 0.5333 g/100 mL.
[c] 0.2796 g/100 mL.
[d] 0.1294 g/100 mL.
[e] 0.0676 g/100 mL.
[f] 0.0244 g/100 mL.
[g] 0.0195 g/100 mL.
[h] 0.0007 g/100 mL.
[i] 0.0005 g/100 mL.

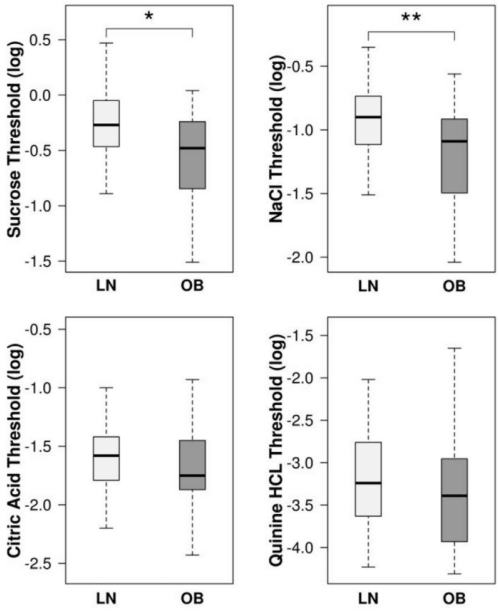

Fig. 1. Boxplots of log-transformed recognition threshold (g/100 mL) distributions for LN and OB groups for sucrose (sweet), sodium chloride (salty), citric acid (sour), and quinine (bitter). *p ≤ 0.05, **p ≤ 0.01.

High" sweet pleasantness, the BFs provided only anecdotal evidence for the alternative hypothesis ($BF_{10} = 2.757$, and $BF_{10} = 2.867$, respectively).

Notably, participants exhibited a high intra-individual consistency in their ratings as supported by significant correlations between ratings on the first and second trial of all stimuli (Spearman's $\rho = 0.911$, $p < 0.0001$) which was similar for OB ($\rho = 0.9$, $p < 0.0001$) and LN ($\rho = 0.921$, $p < 0.0001$).

Taste thresholds and subjective ratings of intensity for concentrations "Relative" to individual thresholds were positively correlated (two-tailed). These were most pronounced for "Relative High" concentrations of sucrose ($r = 0.689$, $p < 0.0001$), NaCl ($r = 0.487$, $p < 0.0001$), citric acid ($r = 0.293$, $p = 0.031$), quinine ($\rho = 0.607$, $p < 0.0001$), and weaker, yet in part significant, for "Relative Low", sucrose ($\rho = 0.456$, $p = 0.001$), NaCl ($\rho = 0.247$, $p = 0.07$), citric acid ($\rho = 0.163$, $p = 0.239$), and quinine ($\rho = 0.368$, $p = 0.006$).

"Absolute" concentrations, which were chosen independent of individual thresholds, exhibited consistently negative correlations (one-tailed) with individual thresholds; these were strongest for "Absolute Low" sucrose ($r = -0.253$, $p = 0.044$), NaCl ($\rho = -0.308$, $p = 0.012$), citric acid ($r = -0.481$, $p < 0.0001$), and quinine ($\rho = -0.649$, $p < 0.0001$), weaker for "Absolute High" sucrose ($\rho = -0.114$, $p = 0.206$), NaCl ($\rho = -0.274$, $p = 0.022$), and quinine ($\rho = -0.569$, $p < 0.001$), and stronger for "Absolute High" citric acid ($r = -0.536$, $p < 0.0001$).

4. Discussion

In the present study, we compared lean and obese participants on three dimensions of taste perception: recognition threshold as an objective measure of taste sensitivity, and intensity and pleasantness as the subjective measures, for four basic tastes. Our results indicate that obese participants are more sensitive to taste than lean participants. This notion is evidenced by significantly lower recognition thresholds to sweet and salty, indicating a higher sensitivity, and further corroborated by the observation that OB rated the same concentrations of sweet, salty, and sour as significantly more intense than LN. We did not find evidence for a difference in sour and bitter thresholds between OB and LN. It was observed that overall, participants could taste more of the lower concentrations of sour and bitter, and the final threshold estimates for these taste qualities were gathered towards the lower end of the concentration range. These concentration ranges were designed to cover the expected thresholds of the entire population with a limited number of stimuli. For taste qualities with large inter-individual differences, this implies a decrease in the granularity of the threshold estimate, i.e., lowered precision on the single-subject level. Hence, it is possible that even more narrow dilution steps are required to detect potential group differences for these tastes.

In line with lower thresholds, OB participants reported significantly higher subjective taste intensity for "Absolute Low" sweet, salty, and sour and "Absolute High" sweet, compared to LN. Notably, this group difference did not manifest in the concentrations that were adjusted to individual thresholds (i.e., "Relative Low" and "Relative High") corroborating our threshold estimates. The group differences in sweet and salty taste are of particular interest for eating behaviour and energy intake, and with that for obesity research, as these taste qualities provide information

Table 3a
Intensity ratings for supra-threshold concentrations.

Taste	Conc.	Lean	Obese	Mann-Whitney *U*		Bayes factor	
		Median (IQR)	Median (IQR)	*U*	p	BF_{10}	error %
Sucrose	Absolute High	84 (71–90.5)	94.5 (80.5–98.5)	227.5	0.024	2.757	1.019e −7
	Absolute Low	67 (56–77.5)	81 (70–89)	201.5	0.007	4.689	2.306e −6
	Relative High	45 (32–60)	36 (14.5–61)	279	0.175	0.667	7.549e −5
	Relative Low	18 (7–40)	8.5 (3–22.5)	247	0.055	1.305	3.152e −5
NaCl	Absolute High	90 (81–97.5)	93.5 (89–99)	259.5	0.089	0.935	6.086e −5
	Absolute Low	81 (66.5–85.5)	91 (77–95.5)	209.5	0.01	6.387	2.397e −6
	Relative High	55 (34.5–61.5)	45 (26–54.5)	273.5	0.146	0.690	7.556e −5
	Relative Low	20 (8–35)	16 (8–29)	321	0.535	0.359	1.527e −4
CitricAcid	Absolute High	93 (77.5–98)	95 (85.5–99.5)	274.5	0.151	0.793	7.183e −5
	Absolute Low	71 (61.5–79)	86 (76–90)	193.5	0.004	8.075	2.035e −6
	Relative High	44 (32.5–63)	47 (33–69)	325	0.582	0.296	1.459e −4
	Relative Low	14.5 (10–31)	15.5 (4–34)	339	0.759	0.286	1.459e −4
Quinine	Absolute High	79.5 (70–86)	85 (75–95)	258	0.085	0.510	1.813e −4
	Absolute Low	58 (40.5–76)	67.5 (43.5–86.5)	306.5	382	0.357	1.523e −4
	Relative High	39.5 (15–71)	36.5 (14–68.5)	319	0.512	0.297	1.459e −4
	Relative Low	18.5 (10.5–36.5)	15.5 (7.5–21.5)	308.5	0.401	0.375	1.557e −4

Table 3b
Pleasantness ratings for supra-threshold concentrations.

Taste	Conc.	Lean	Obese	Mann-Whitney *U*		Bayes factor	
		Median (IQR)	Median (IQR)	*U*	p	BF_{10}	error %
Sucrose	Absolute High	26.5 (10–36)	24 (8.5–42.5)	353.5	0.958	0.277	1.462e −4
	Absolute Low	19.5 (13.5–32)	14.5 (6.5–29.5)	281.5	0.189	0.564	1.866e −4
	Relative High	13.5 (5.5–24.5)	6 (0–14.5)	220	0.017	2.867	3.342e −8
	Relative Low	2 (0–8)	0 (0–2)	251.5	0.064	1.078	4.845e −5
NaCl	Absolute High	−36 (−42.5–−16)	−31 (−45–−15)	329	0.630	0.298	1.459e −4
	Absolute Low	−20 (−31.5–−4)	−24.5 (−34–−7)	293	0.267	0.465	1.741e −4
	Relative High	−3.5 (-14–3)	−1 (-7–2)	294.5	0.278	0.482	1.771e −4
	Relative Low	−1 (-4–0)	0 (−2.5–1.5)	292	0.255	0.277	1.462e −4
Citric Acid	Absolute High	−21 (−34–−1)	−9 (-43–5.5)	337	0.733	0.306	1.462e −4
	Absolute Low	−11 (-22–0.5)	−12 (−36.5–7)	341	0.786	0.317	1.469e −4
	Relative High	−2 (-6–5)	−4.5 (-13–2)	279	0.175	0.819	7.010e −5
	Relative Low	0 (-2–2)	0 (−3.5–0)	285	0.205	0.846	6.814e −5
Quinine	Absolute High	−33 (−41–−24)	−41.5 (−45–−28)	278	0.170	0.452	1.717e −4
	Absolute Low	−20 (−27–−15)	−27.5 (−39–−9)	294	0.274	0.420	1.651e −4
	Relative High	−10 (−24–−2)	−9 (−28–−1.5)	344.5	0.512	0.306	1.462e −4
	Relative Low	−2 (-10–0)	−5.5 (−9.5–0)	346	0.853	0.286	1.459e −4

regarding the nutritional value of food. In that sense, sweet taste indicates calories from certain carbohydrates, and salty taste signals the availability of sodium and/ or minerals. Beyond this traditional view, saltiness can be associated with the availability of energy from fat, particularly in processed foods in the Western diet. High amounts of fat are also more likely to be consumed in processed sweet foods.

The findings on subjective perception are consistent with the group difference found for taste thresholds: more sensitive participants reported “Absolute” taste concentrations, i.e. those concentrations that were identical for all participants irrespective of their threshold, as more intense than less sensitive participants. Whereas “Relative” taste concentrations, i.e. concentrations that were aligned to individual threshold levels, yielded no group differences in intensity. Previous studies have investigated the link between different - objective and subjective - measures of taste perception including detection and recognition thresholds, ratings of supra-threshold tastants, and density of fungiform papillae. While detection and recognition thresholds are commonly correlated (e.g. Webb et al., 2015; Wise & Breslin, 2013), thresholds have seldom been found to be related to supra-threshold intensity rating (Webb et al., 2015). Webb et al. (2015) concluded that these individual measures characterize different facets of the taste experience rather than providing a measure of overall taste function, and suggested that this explains conflicting data pertaining to taste function and sensitivity and its link with dietary intake. We observed consistently strong positive correlations between thresholds and intensity ratings that were aligned “Relative” to individual thresholds as expected, because the concentrations were only few concentration-steps above individual thresholds and provide a similar perceptual frame for participants. Correlation between thresholds and intensity ratings for concentrations that were chosen independent of thresholds were consistently negative, weaker, and only partially significant. Together our findings suggest a systematic link between taste thresholds and supra-threshold intensity ratings if these are aligned to individual taste sensitivity.

Our findings of heightened taste sensitivity in obese participants stand in contrast to existing literature which presents a rather diverse picture that nevertheless points to reduced taste abilities in obesity. Previous reports have suggested either no difference on sweet and salty taste sensitivity between obese and lean participants (Bertoli et al., 2014; Pepino et al., 2010; Simchen et al., 2006), or even lower sensitivity in obesity for salty (Skrandies & Zschieschang, 2015) for sour (Bertoli et al., 2014), sour and bitter (Simchen et al., 2006), or for sweet, salty, sour and bitter (Proserpio et al., 2015). An improvement of taste detection rates has also been reported after weight loss (Altun et al., 2016). However, it cannot be excluded that the choice of methods and the resolution of the

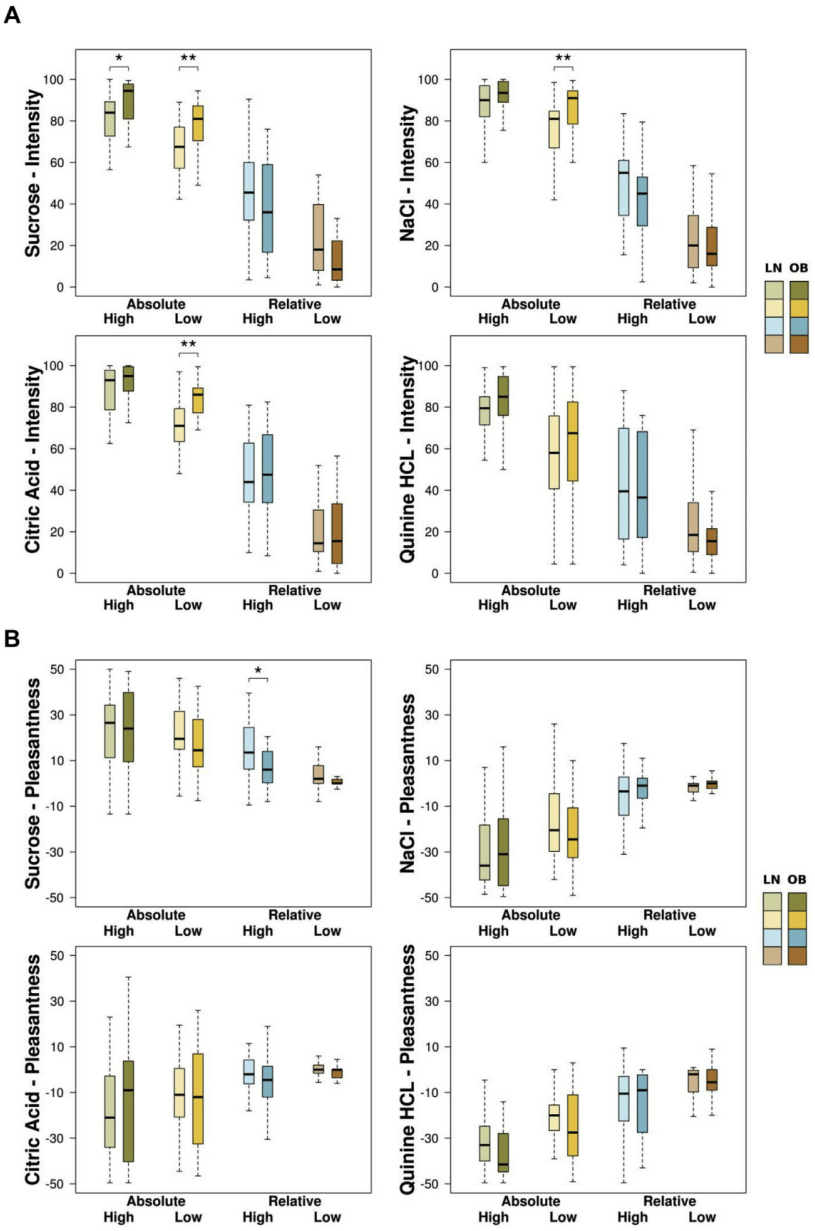

Fig. 2. (A) Boxplots showing distributions of the intensity of four concentrations of sucrose (sweet), sodium chloride (salty), citric acid (sour), and quinine (bitter), for LN and OB. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$. (B) Boxplots showing distributions of the pleasantness of four concentrations of sucrose (sweet), sodium chloride (salty), citric acid (sour), and quinine (bitter), for LN and OB. $^{*}p \leq 0.05$.

measure (i.e. number of dilution steps) to assess taste sensitivity contributed to this discrepancy.

Similarly, the supra-threshold findings of higher intensity differ from previous studies where obese individuals were reported to have perceived supra-threshold sweet and salty tastants as less intense (Sartor et al., 2011), or no different from lean individuals (Malcolm et al., 1980; Pepino et al., 2010). In our sample, taste pleasantness was significantly higher in OB compared to lean for "Relative High" sweet only. Although OB tended to rate all concentrations of sweet taste as less pleasant than LN, this model was moderately favoured by the BF calculation only for the "Relative High" sweet. Previously, there have been isolated reports of obese individuals reporting higher pleasantness for higher concentrations of sucrose (Rodin et al., 1976), reports of obese adults consuming more energy in salty foods (Cox, Perry, Moore, Vallis, & Mela, 1999), and also a correlation of BMI with liking for salty-fatty foods (Deglaire et al., 2014). One may speculate that increased liking and perceived pleasantness may therefore be an indicator of actual food intake, and liking of sweet and salty taste may foster overweight as processed foods with that taste commonly contain lots of calories.

As mentioned earlier, a comparative interpretation of existing findings is hampered by the methodological differences present across studies. These involve particularly the differences in threshold algorithms (e.g. ascending versus adaptive methods), tasks (e.g. 2- or 4- alternative forced choice; AFC), modes of stimulation (e.g. whole mouths versus localised stimulation), concentration ranges and number of dilution steps, and also the type of concentration scale (linear versus log-linear). Our use of broad concentration ranges together with the adaptive, Bayesian approach has enabled us to detect minute threshold differences that may have been missed in previous studies. Our threshold measurement procedure was specifically designed for a rapid estimation of taste thresholds. While spatial and temporal AFC tasks are typically used for that purpose, we employed a yes-no paradigm because the total time required for the procedure is greatly reduced as only a single stimulus is presented on each trial. Furthermore, this approach avoids memory effects and interval biases typically associated with AFC tasks in naïve participants, who might be more inclined to pick one interval over the other, regardless of actual stimulation (see e.g. Klein, 2001). Two major problems specific to the chemical senses are persistent habituation and carry-over effects from one stimulus to the next. To avoid these, each stimulation is followed by rinsing and a long inter-stimulus interval (ISI; typically 10s–30s). However, experiments with long ISIs are known to prevent participants from directly comparing two or more stimuli (Kaernbach, 1990), thereby introducing a major memory-related confound in gustatory AFC tasks. Presenting one stimulus per trial bypasses this problem, and allows the processing of stimuli independently from one another. Additionally, King-Smith et al. (1994) stated that for the estimation of an approximate absolute threshold, a yes-no method similar to the one used here is preferable to an AFC task, mainly because of its greater speed while still providing accurate results.

It is known that the outcome of threshold estimation procedures might not entirely reflect participants' true sensory sensitivity due to individual response criteria, specifically the decision as to how strongly a stimulus has to be perceived to elicit a "Yes" response. Commonly, stimulations with pure water (blanks) are used as control and allow for the estimation of false alarm rate (FAR), i.e., a "Yes" responses when no tastant is presented. However, adding blanks to derive a meaningful FAR inflates the number of trials required. Instead, based on pilot testing, we assumed a fixed FAR of almost zero (0.01). While we cannot rule out the possibility that LN and OB in the present study employed different response criteria on population average, any such difference should not be selective of one or more taste qualities within an individual, leaving the reported group effects for different tastes unaffected by this cognitive confound.

Another practical argument builds on observations that forced-choice procedures are unsuitable for some participants, especially in a clinical setting, as participants might be reluctant to guess the target interval when feeling unsure (Green, 1993). As extensive "practice" sessions cannot usually be performed with these populations (Jones, Moore, & Amitay, 2015), directly asking the participants whether or not they perceived a stimulus might be more suitable for naïve subjects (Green, 1993). Because the method presented here does not require any practice, doesn't strain the memory, has a very short testing duration, relies on portable stimulus material, and can even be easily adapted to allow for non-verbal responses (e.g. indicating the perceived taste by pointing out a related food item on a response chart), we suggest it is suitable also for children, elderly, and clinical populations. Future studies will have to confirm this claim of applicability.

5. Conclusion

Together, our findings suggest that higher body mass is associated with higher sensitivity to, and subjective strength experience of salty and sweet taste. While sour and bitter taste showed a similar pattern of results, this was markedly less pronounced and not statistically significant. Given that our understanding of the aetiology of obesity is in its infancy, any interpretation of the results along those lines would be highly speculative. Accordingly, the current findings are presented and discussed within the context of the existing literature on gustatory perception and BMI. Notably, these findings contradict some of the previous reports suggesting a reduced sensitivity and/or ability to detect different tastes in obese compared to lean. We believe that the discrepancies are grounded in methodological but also conceptual differences in measuring taste sensitivity. Comparisons of taste perception in lean and obese groups have also continually suffered from the drawback of small sample sizes. It should be recognised that BMI is a population level measure of obesity (WHO), and may only have a small effect compared to the total effect of other factors such as age, health, socio-economic status, current eating habits, hormone levels etc., in small samples. We have tried to control for some of these by limiting our sample to young men and women without known chronic illness, and women who use contraceptives. While we deem understanding the role of taste sensitivity in the development of obesity crucial, ascertaining the extent to which differences in supra-threshold taste experience modulate eating behaviour and weight status remains equally important.

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Shorter-lived neural taste representations in obese compared to lean individuals

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Previous attempts to uncover a relation between taste processing and weight status have yielded inconclusive results leaving it unclear whether lean and obese individuals process taste differently, and whether group differences reflect differential sensory encoding or evaluative and reward processing. Here, we present the first comparison of dynamic neural processing as assessed by gustatory evoked potentials in obese and lean individuals. Two supra-threshold concentrations of sweet and salty tastants as well as two sizes of blue and green squares were presented to 30 lean (BMI 18.5–25) and 25 obese (BMI > 30) individuals while recording head-surface electroencephalogram (EEG). Multivariate pattern analyses (MVPA) revealed differential taste quality representations from 130 ms until after stimulus offset. Notably, taste representations faded earlier and exhibited a reduced strength in the obese compared to the lean group; temporal generalization analysis indicated otherwise similar taste processing. Differences in later gustatory response patterns even allowed decoding of group membership. Importantly, group differences were absent for visual processing thereby excluding confounding effects from anatomy or signal-to-noise ratio alone. The latency of observed effects is consistent with memory maintenance rather than sensory encoding of taste, thereby suggesting that later evaluative aspects of taste processing are altered in obesity.

Obesity has been associated with altered perception of food cues, with the literature focusing primarily on visual food stimuli (see[1,2] for review). Several psychophysiological studies using either pictorial or verbal cues have pointed towards an attentional bias and augmented sensitivity to food cues in obese compared to lean individuals, irrespective of the technique used[1]. This is also corroborated with neuroimaging, as obese compared to lean participants are found to have higher blood oxygen level dependent (BOLD) responsivity to food cues, especially for high calorie foods[3,4].

Comparatively fewer studies have investigated the role of body weight in taste processing. This is surprising given its pivotal role in nutrient sensing - gustation facilitates decisions as to the edibility and spoilage of food - and food-related behaviour. The few findings regarding gustatory perception are also more ambiguous. For instance, obese compared to lean participants have been found to display a lower sensitivity to all four tastes[5], a higher sensitivity to sweet and salty[6], or no difference in taste sensitivity[7]. Seemingly mixed is the evidence from weight-loss intervention studies: higher taste sensitivity after surgery-induced weight loss was reported when using the taste-strips method[8] while taste sensitivity was unaffected in surgery-induced weight loss when measured as sensory thresholds to sapid tastants[9,10]. Some of the discrepancy may be explained by the complex nature of ingestive behaviour, the multitude of variables under investigation, and the heterogeneity of methods used for taste sensitivity assessment. Additionally, achieving chemosensory stimulation in a precise and controlled way is more challenging than visual, auditory, or somatosensory stimulation. It is partly due to this reason that researchers so far have relied greatly on verbal or visual food stimuli, rather than real foods or gustatory stimuli. Especially neuroimaging investigations of taste and obesity are few in number and present an inconclusive picture. Functional magnetic resonance imaging (fMRI) studies have reported a BMI-dependent higher

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	Lean (n = 30)	Obese (n = 25)	p-value
Age (years)	25.47 ± 3.81	26.64 ± 3.52	0.245[a]
Sex (men, women)	15, 15	10, 15	0.639[b]
BMI (kg/m^2)	22.13 ± 1.83	35.48 ± 4.53	<0.001[a,c]

Table 1. Participant Characteristics (group means and SD). [a]Two-sample t-test. [b]Pearson's chi-squared test. [c]Levene's test is significant suggesting unequal variances.

BOLD response in the rolandic operculum[11–13], and the insula[11,12] - both areas of sensory taste representation[14,15]. Whereas recently Frank *et al.*[16] reported a lower taste discrimination in the insula in obese compared to lean.

The evidence is even more complicated where the evaluative or reward related brain areas are concerned. When a palatable food stimulus (milkshake) was used, adolescent females with obesity showed a higher anticipatory reward response to the stimulus cue, but a lower striatal reward response on receipt[11]. This attenuation of consummatory food reward in the obese may be mediated by the Taq|A1 gene[17]. On the contrary, Szalay *et al.*[12] observed a higher BOLD response not only in the insula, operculum, and the OFC - the site of gustatory hedonic encoding - but also subcortical structures like the amygdala and nucleus accumbens in response to both pleasant and unpleasant tastes.

Together, the fMRI literature suggests differential activation of brain areas implicated in gustatory processing, particularly at anatomically early levels within the primary gustatory cortex, the insula and opercula. While such activation may implicate the initial, sensory activation, it could also reflect later, evaluative processes possibly through feedback from higher cortical areas. Insular activation has been implicated in both, sensory processing, e.g. in taste intensity[18,19] and quality perception[20] within only 200 ms of taste stimulation, and later evaluative processing, with top-down modulation from cross-modal cues[21]. Due to its poor spatial resolution, fMRI cannot distinguish between these explanations.

In order to elucidate the spatio-temporal dynamics of neural processes, and disentangle the potential differences between lean and obese groups in various stages of processing, a temporal resolution in the millisecond range, as that provided by electroencephalography (EEG), is required. To date, EEG studies of taste perception have been exceptionally rare due to the difficulties involved achieving taste stimulation in a way that is both temporally and quantitatively precise, and not confounded by oral somatosensation[22]. Here we present the first ever exploratory investigation of the neural response to taste between lean and obese individuals as measured by EEG and analysed using multivariate pattern analysis (MVPA).

Methods

Participants. 55 healthy adults between 18–35 years (25 men, 30 women) participated in the study, 30 of whom were lean (BMI range: 18.5–25 kg/m^2) and 25 obese, (BMI $\geq$ 30 kg/m^2). Participant characteristics are detailed in Table 1. Participants were screened via telephone interviews to exclude those with self-reported taste/smell disorders, smoking, alcohol/other addiction, stroke, depression, diabetes, hypothyroidism, oral/nasal/sinus infections, pregnancy, or recent (last 6 months) childbirth. To minimise the confounding effects of hormonal changes, only women using oral contraceptives were included. All participants gave written informed consent prior to the experiment and were free to quit the experiment at any point without giving a reason. The experimental procedures were in accordance with the Declaration of Helsinki and approved by the medical ethics committee of the University of Leipzig.

Stimuli. Tastants included sucrose (Sigma-Aldrich, CAS number: 57-50-1) and sodium chloride (NaCl; Sigma-Aldrich, CAS number: 7647-14-5) that were dissolved in mineral water (Volvic, Danone Waters Deutschland GmbH), which was also used as rinse. We created four different taste stimuli: high (0.29 M; 10 g/100 mL) and low (0.15 M; 5 g/100 mL) sweet, and high (0.43 M; 2.5 g/100 mL) and low (0.21 M; 1.25 g/100 mL) salty. Volvic mineral water has a mineral content of 130 mg/L (Calcium 12 mg/L, Chloride 15 mg/L, Sodium 12 mg/L, Potassium 6 mg/L, Silica 32 mg/L, Hydrogencarbonate 74 mg/L, Magnesium 8 mg/L, Sulphate 9 mg/L), and a pH of 7.

Visual stimuli were green and blue squares of two sizes (large: 11.9°; small: 6.0°). As this is the first study of gustatory event related potentials (ERPs) in lean and obese individuals, the visual stimuli were included as a control for the analyses of "group" (lean, obese) differences in ERPs. Any difference in visual processing between groups would indicate differential signal-to-noise levels in the recordings or anatomical differences that would then have to be considered in the interpretation of any group difference in taste processing.

Experimental procedure. Taste stimuli were presented as atomized aliquots of 210 µL delivered over 900 ms through a computer-operated gustometer (GU002, Burghart Messtechnik, Wedel, Germany; see[18,20,23]). Stimuli were embedded into a continuous 3.3 Hz sequence of water sprays to minimize oral-somatosensory responses. Visual stimuli were presented on the center of a computer screen for 900 ms on a grey background.

Participants were asked to sit in front of a computer screen (67 cm from the eyes) leaning forward against the headrest of the gustometer, and extend the anterior half of the tongue under its spray nozzle, which was positioned approx. 1.5 cm above the extended tongue.

During each trial, a taste and either none, one, two or three visual stimuli were presented sequentially (see Fig. 1 for the schematic of a single trial). On each trial, a central fixation cross appeared on the screen for 2000 ms before a taste stimulus was presented via the spray nozzle for 900 ms; the fixation remained on screen during taste stimulus delivery and 2100 ms thereafter. Next, participants were presented with an on-screen visual analog scale (VAS) to rate the intensity of the taste from 0 (no sensation) to 100 (extremely strong), followed 2 s later by

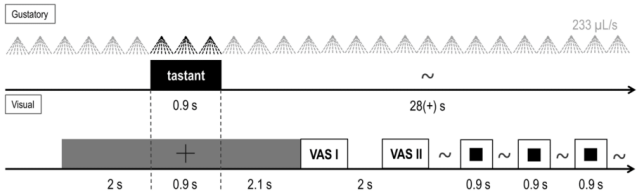

Figure 1. Schematic diagram of an experimental trial. tTop row: spray pulses of the gustometer, tastants are represented in black, water is represented in grey. Bottom row: VAS I and VAS II were visual analog scales for rating stimulus intensity and pleasantness. Filled black squares represent instances of visual stimulus presentation. Variable intervals between events are indicated with ~.

another VAS to rate the pleasantness from 50 (extremely unpleasant) to 0 (neutral) to 50 (extremely pleasant). For this, participants moved a mouse cursor along the scale and logged their rating by clicking the left button. The inter-stimulus interval (ISI) between tastes varied depending on the participants' response time for the VAS, but a minimum waiting period of 28 s was maintained between trials. During most ISIs, participants were sequentially presented up to three visual stimuli, one at a time, on the computer screen for 900 ms. No task was to be performed with the visual stimuli.

Each of the four taste stimuli was presented 48 times on average. The number of taste trials was increased from 36 to 50 after the first 10 participants to improve the signal to noise ratio of the gustatory response. The order of taste stimuli was counterbalanced, such that the same taste quality was never presented on consecutive trials.

Each of the four visual stimuli was presented 65 times. The number of visual stimulus presentations during each ISI varied between zero and three, and the order of presentation was randomised.

The experiment was divided into 10 blocks of 11–12 min. Blocks were separated by breaks of two to five minutes as needed and requested by participants. On average, the experiment lasted 120–140 minutes, and an additional 45–60 minutes were required for preparation and training. Participants went through a short training (three trials) prior to the experiment to get acquainted with the procedure and to find a comfortable posture and tongue position that could be held for the duration of a block. They were instructed to hold the tongue in the same position for the duration of the block and, if absolutely necessary, to move it only immediately after the VAS presentations, to avoid movement during epochs of interest and to have a substantial "spray-habituation" period before the next trial. The stimulation required no swallowing.

Transitor-transitor logic (TTL) pulses between the gustometer and the stimulation and EEG computers controlled the timing of stimulus onset, and also logged it into the EEG recording files. The delay between these pulses prompting the syringe plungers of the gustometer to push liquids through individual Teflon® tubes and the spray nozzle, and the atomized liquids reaching the tongue is 36 ms (SD = 2 ms) with a rise time to reach 70% of the maximum response of <15 ms (data provided by the manufacturer based on an identical setup). The separate tubes carrying the tastants and water to the spray nozzle were enveloped by a hose of warm water (39 °C), and the pressure of the spray was kept constant throughout.

EEG acquisition and pre-processing. Participants were seated in a sound-attenuated recording booth during the experiment and the gustometer was positioned outside the booth to minimize acoustic disturbance from the device. Head-surface EEG was continuously recorded with the actiCHamp amplifier system (Brain Products GmbH, Munich, Germany) from 62 silver/silver chloride (Ag/AgCl) active electrodes mounted in an elastic cap according to the extended 10–10 system; another electrode was placed under the left eye to monitor eye movements. FCz served as reference during recording only. The EEG was recorded with BrainVision Recorder Professional V. 1.20.0506 (Brain Vision LLC, Morrisville, NC, USA) at 500 Hz using analogue filters (0.01 Hz high-pass and 200 Hz low-pass). The continuous EEG data were processed offline by using custom-made scripts in MATLAB (MathWorks, Natick, MA, USA) and the EEGLAB toolbox[24]. Malfunctioning channels were interpolated manually before re-referencing data to average of all channels. The continuous data were low-pass filtered with a 44 Hz cut-off and 8 Hz transition width (order 208) and then high-pass filtered with a 0.1 Hz cut-off (order 8250) using a zero-phase Hamming windowed sinc FIR filter with a maximum passband deviation of 0.2% and a stopband attenuation of −53dB (cf.[25]). Data were then segmented into epochs of 2 s with an additional 500 ms pre-stimulus baseline period. Epochs with unique recording artefacts were rejected by visual inspection. Further, commonly observed artefacts (e.g. ocular, muscular, or vascular) were identified using Infomax independent component analysis as implemented in EEGLAB[26] and removed. Overall <2% of all trials were rejected. Epochs were separately analysed for gustatory and visual trials.

Statistical Analyses. First, global measures of evoked field strength and distribution were compared in order to get a general overview of evoked neural gustatory and visual responses and to verify that we have above baseline gustatory activation. The instantaneous topographical patterns of evoked responses were then explored further using multivariate pattern analysis on a single-trial level to see whether taste-quality information emanated differently in the lean and obese groups, and whether these two groups could be differentiated based on their neural response patterns to the same taste stimuli.

Perceptual Ratings. VAS ratings of taste intensity and pleasantness were aggregated across trials for each participant and for each condition and submitted to a repeated measures analysis of variance (ANOVA) with "taste quality" (salty, sweet) and "taste concentration" (high, low) as the within-subject factors, and "group" (lean, obese) as the between-subjects factor. The alpha level was set to 0.05 for all statistical analyses if not specified otherwise. Non-significant effects from the ANOVA were followed up with Bayesian independent samples t-tests in JASP[27] to estimate whether the data are merely inconclusive or strongly in favour of the null hypothesis.

Global Field Power (GFP) and Global Map Dissimilarity (GMD). To visualize the overall EEG activity, GFP, a reference-free index of overall field strength[28,29], was calculated. GFP is analogous to the standard deviation over the entire electric field (all electrodes) at each sampling point and was calculated in Ragu (Randomization Graphical User interface[30]) using the following formula:

$$\mathbf{GFP} = \sqrt{\frac{\sum_{j=1}^{n}\left(v_j - \bar{v}\right)}{n}}, \tag{1}$$

where v_j is the voltage measured at sensor j, n is the number of sensors, and $\bar{v}$ is the mean measurement across all sensors[30]. GFP was calculated for each participant and for each condition separately. To explore differences between experimental conditions, post-stimulus GFPs were submitted to a 3-way ANOVA with "taste quality" (salty, sweet) and "taste concentration" (high, low) as the within-subject factors, and "group" (lean, obese) as the between-subjects factor.

GMD[31] between conditions and groups was calculated as an index of differences in the scalp field (topography) generated by all electrodes, using the following formula:

$$\mathbf{GMD} = \sum_{i=1}^{c}\sqrt{\frac{\sum_{j=1}^{n}(\bar{v}_{ij} - \bar{\bar{v}}_j)^2}{n}}, \tag{2}$$

where c is the number of conditions and group, n is the number of sensors, $\bar{v}_{ij}$ is the voltage of the grand mean across subjects of condition and/or group i at sensor j, and $\bar{\bar{v}}_j$ is the grand mean across subjects and conditions of the voltage at sensor j[30]. Importantly, in order to calculate GMD independent of field strength, all data were normalised prior to the GMD calculation as recommended by Koenig and colleagues[30], using the L2-norm (least squares) function provided in Ragu[30] which sets all data to equal variance across all electrodes before analysis[30]. Post stimulus GMD was analysed in a topographical-ANOVA (t-ANOVA) with the same factors as above with 5000 permutations using Ragu[30].

As no significant effects of "taste concentration" were observed for either the GFP or GMD, the data was collapsed across the two taste concentrations, and the 3-way ANOVA was reduced to "taste quality" (sweet, salty) × "group" (lean, obese). Similarly, the GFP and GMD for the visual evoked responses were calculated with a "colour" (green, blue) × "group" (lean, obese) ANOVA. Duration thresholds for significant effects were calculated (see[30]) and applied to results from the ANOVA to correct for multiple comparisons across time.

Multivariate pattern analysis (MVPA). To test for the emergence of taste quality and intensity information in the EEG signal at the single trial level, linear support vector machine (SVM) classifiers[32] were trained and tested at each time point in a sliding time window approach. This procedure is generally referred to as multivariate pattern analysis (MVPA, cf.[33]), where the machine learning algorithm attempts to leverage predictive information with respect to the stimulus class from an instantaneous topographical pattern of the amplitudes of all electrodes. For the discrimination of stimulus category MVPA was conducted separately for lean and obese participants. Trials were then pooled across participants in order to maximize generalizability. Consequently, the cross validation (CV) schemes, which separate the data into folds of training and testing sets, were stratified for both, the stimulus class and the participants, such that the trial composition of each fold was a balanced reflection of the sample distribution. This splitting of data into training set (data the classifier learns with) and testing set (data the classifier is tested on) is commonly done in order to obtain unbiased performance estimates of the classifiers. To attenuate individual differences, we scaled all trials with the mean and standard deviation of their respective baseline periods. The baseline period was set to 200 ms up to stimulus onset for taste and 100 ms up to stimulus onset for the visual condition. To improve the signal-to-noise ratio, EEG data were re-sampled to 100 Hz and the taste data additionally low-pass filtered (−6 dB cut-off 5.5 Hz, transition width 1 Hz, order 330) using a zero-phase Hamming windowed sinc FIR filter with a maximum passband deviation of 0.2% and a stopband attenuation of −53 dB (cf.[25]) because taste information has been previously shown to be limited to this particular frequency range[34]. An L2-regularization with a C parameter value of 10^{-4} was set for the SVM classifiers to enforce small weights and greater stability. Classifier performance was estimated at each time point using the area under the receiver operating characteristic curve (AUC), a balanced accuracy metric where 50% corresponds to chance performance even with uneven class distributions. All MVPA analyses were implemented with custom scripts in R[35], using the 'LiblineaR' library for the SVM algorithm[36].

Classification of taste quality. Decoding of taste quality information was implemented with a 20-fold stratified CV, where the classifiers were iteratively trained on a randomly selected subset of 95% of the trials and tested on the independent subset of the remaining 5% of trials. Data of lean and obese participants were pooled and analysed separately to compare the classification performance between the two populations. SVM classifiers were trained to make the binary discrimination between sweet and salty taste (irrespective of the concentration) and, for the visual control condition, between green and blue squares (irrespective of stimulus size). Differences

Quality	Conc.	Group	n	Intensity		Pleasantness	
				Mean	SD	Mean	SD
Sweet	High	LN	30	53.54	18.10	7.132	7.727
		OB	25	51.07	19.37	7.124	15.174
	Low	LN	30	42.55	20.35	9.824	6.276
		OB	25	40.94	19.83	9.576	14.551
Salty	High	LN	30	67.43	14.86	−15.908	10.027
		OB	25	68.17	11.54	−16.353	11.357
	Low	LN	30	59.23	15.73	−11.116	9.435
		OB	25	59.48	12.16	−10.645	10.181

Table 2. Perceptual ratings. LN = Lean, OB = Obese.

between the classification performances of lean and obese were assessed at each time point for each modality with two-sided Wilcoxon rank sum tests; significant *p*-values (<0.05) were adjusted to a minimum duration of 100 ms. Significant decoding of category (within each group) was evaluated with one-sided binomial tests which assert above-chance performance while considering sample size.

Classification of group membership. In order to test whether lean and obese individuals can be discriminated given taste-related neural response patterns within a single category, classifiers were trained separately for each stimulus category irrespective of concentration or size (i.e. sweet, salty, green, blue). Specifically, a 10-fold stratified CV was adapted to learn generalizable patterns associated with one or the other group based on the instantaneous topographical distribution for a given trial (i.e. tasting sweet; viewing green). To disentangle group differences in gustatory processing from those unrelated to gustatory processing (e.g. due to anatomy or signal-to-noise ratio) we repeated the analyses over 1000 permutations of group membership and compared the best performing permutation, an optimistic estimate of irrelevant differences, with the observed classification performance. The permutations were constrained to be unique and within a margin of 12.5% of a perfect shuffle (i.e. when exactly half of the labels, lean and obese, were exchanged) and applied to each stimulus category. Taste-evoked neural response patterns differ between lean and obese where permutation thresholds are exceeded for the duration of at least 100 ms. The difference between the actual and the maximum permutation performance provides a direct estimate of the effect size.

Generalization across time and groups. To characterize the temporal dynamics of taste information processing and its relation between the lean and obese groups, we applied a generalization method[37], which is an extension of the common MVPA, separately for both groups (with a 20-fold stratified CV and pooling across participants). Previously, a classifier was trained at one time point and tested at that very same time point. In contrast, here the learned pattern is applied at all time points irrespective of where it was observed originally. This approach is useful to see how far the neural response pattern at a certain time point generalizes backward and forward in time, enabling the examination of correlated activation clusters. If a pattern generalizes over longer time periods one can conclude sustained activation in response to the stimulus, whereas shorter but multiple temporal clusters suggest different, independent processing steps in response to a stimulus.

The decoding result provides a matrix whose cells hold the AUC of a combination of training and testing (generalization) time, one per group. The diagonal of the generalization matrix represents the MVPA with training and testing conducted at the same time point. Performance increases along the horizontal and vertical dimensions reflect sustained neural responses because neural patterns at one time point resemble those at earlier and later time points. In contrast, performance increases limited to the main diagonal reflect distinct neural patterns at a given time. Differing dynamics in gustatory processing between groups would result in significant differences for the contrast of the two generalization matrices. Two-sided Wilcoxon rank sum tests with false discovery rate (FDR[38]) adjustment were used to evaluate statistical significance. To verify above- or below-chance generalizability across time (within one group) and differences between main- and off-diagonal performance, two-sided binomial tests were conducted.

Data availability. The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Results

Perceptual Ratings. As expected, participants rated lower concentrations as less intense ($F_{1,53} = 175.449$, $p < 0.001$, $\eta^2 = 0.768$) and preferred the lower concentration of a taste over the higher one ($F_{1,53} = 51.454$, $p < 0.001$, $\eta^2 = 0.492$). Moreover, sweet was preferred over salty ($F_{1,53} = 108.248$, $p < 0.001$, $\eta^2 = 0.671$) and salty was rated more intense than sweet ($F_{1,53} = 61.259$, $p < 0.001$, $\eta^2 = 0.534$). Lean and obese participants rated all tastes similarly (all $F < 0.9$, all $p > 0.05$). Perceptual ratings are summarized in Table 2.

As no significant effect of group was apparent, Bayesian independent samples t-tests were conducted for all ratings in order to ascertain whether the data are merely inconclusive or strongly in favour of the null hypothesis regarding the effect of "group". All Bayes factors (BF_{01}) had values between 3.318 and 3.365, suggesting that the evidence in favour of the null hypothesis (i.e. no difference in perceptual ratings between the lean and obese group) is not particularly strong.

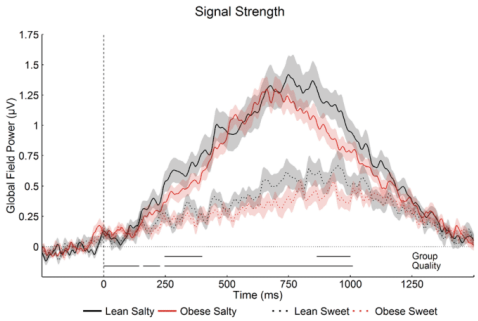

Figure 2. Global Field Power (Gustatory). Mean GFP (±1 SEM indicated as shaded area surrounding the mean) for sweet and salty tastes, in lean and obese groups (baseline removed for visualisation only). The dashed vertical line indicates stimulus onset. Stimuli were presented for 900 ms. Post-stimulus periods of significant effects ($p < 0.05$, >44 ms for "Quality", $p < 0.05$, >64 ms for "Group") are marked by horizontal black bars above the x-axis.

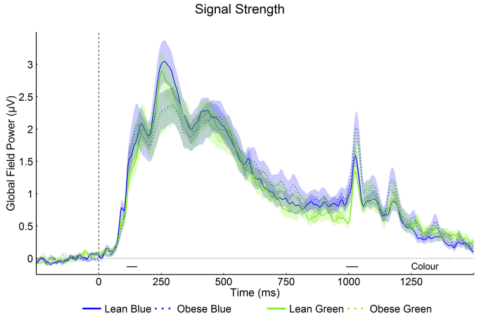

Figure 3. Global Field Power (Visual). Mean GFP (±1 SEM) for green and blue squares, in lean and obese groups (baseline removed for visualisation only). The dashed vertical line indicates stimulus onset. Stimuli were presented for 900 ms. Post-stimulus periods of significant effects ($p < 0.05$, >34 ms) are marked by horizontal black bars above the x-axis.

GFP and GMD. *Gustatory.* GFP was significantly higher for salty than for sweet at each point from 0 ms to 1010 ms post stimulus onset ($p < 0.05$, >44 ms), and significantly higher in the lean than in the obese group from 246 ms to 400 ms and from 864 ms to 1000 ms ($p < 0.05$, >64 ms). GFP for the two taste qualities and groups is shown in Fig. 2.

Electric field distributions, compared using GMD, did not differ significantly between groups ($p < 0.05$, >54 ms) and no significant group*quality interaction was observed.

Visual. GFP was significantly higher for blue than for green squares 112 ms to 154 ms after stimulus onset (from 112 ms to 154 ms; $p < 0.05$, >34 ms) and stimulus offset (from 990 ms to 1038 ms; $p < 0.05$, >34 ms). GFPs did not differ significantly between groups and no significant group * colour interaction was observed. GFP for the two colours and groups is shown in Fig. 3.

Electric field distributions differed significantly between green and blue from 100 ms to 154 ms, 446 ms to 496 ms, 506 ms to 558 ms, and 602 ms to 690 ms after stimulus onset ($p < 0.05$, >28 ms). Significant map dissimilarities differences were observed between the lean and obese groups shortly after stimulus offset (from 1034 ms to 1096 ms; $p < 0.05$, >54 ms) only. No significant group *colour interaction was found.

MVPA. *Classification of taste quality.* Taste quality information emerged rapidly in the neural response patterns after stimulus onset (Fig. 4A), reaching statistical significance at 150 ms for lean (AUC = 51.2% +/−0.6, $p = 0.041$) and at 130 ms for obese subjects (AUC = 52.0% +/−0.6, $p = 0.003$). Decoding accuracy remained continuously significant until 1260 ms for lean (AUC = 51.5% +/−0.8, $p = 0.013$) and 1100 ms for obese subjects (AUC = 51.5% +/−1.0, $p = 0.026$), clearly outlasting taste stimulation. Significant group differences (in favour of lean) between the classification performances emerged during later stages of taste processing, starting at 880 ms

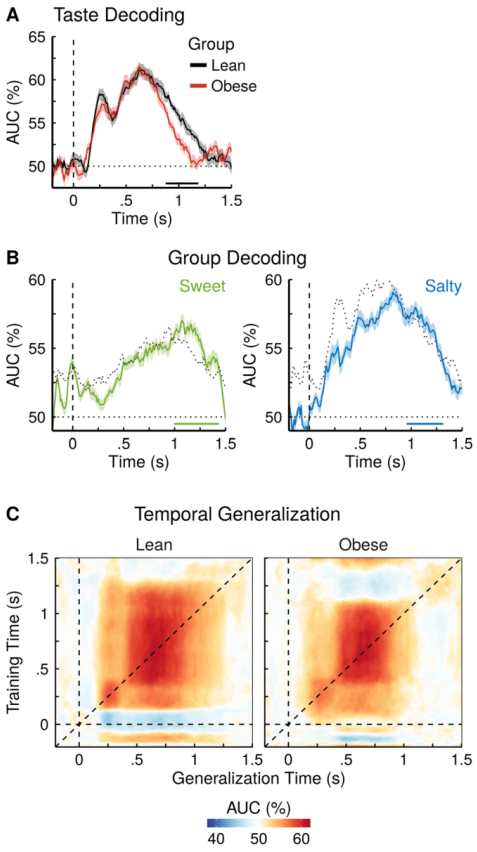

Figure 4. Decoding of taste information, group membership and temporal generalization reveals differences in taste processing between lean and obese individuals. (**A**) Binary decoding of taste quality information (salty versus sweet), separately for lean and obese individuals. Coloured solid lines show the mean performance, surrounding shaded regions show 1 SEM (dashed vertical line: stimulus onset; dotted horizontal line: theoretical chance level of an AUC of 50%). Time points with significant group differences ($p < 0.05$) are indicated by a horizontal black bar above the x-axis. (**B**) Binary decoding of group membership within a taste quality (e.g. given a sweet taste, "is this a lean or an obese person?"). The jittered black line shows the maximum performance over 1000 permutations of group membership per time point. The coloured horizontal bars above the x-axis indicate time points where the actual performance curve exceeded the permutation threshold (minimum of 100 ms). (**C**) Generalization across time. The diagonals of the matrices (same training and testing time) correspond to the performance curves in A. Square generalization patterns (i.e. symmetric increases along the vertical and horizontal dimensions) suggest sustained activity that generalizes across time.

(difference = 1.8% +/−0.9, $p = 0.049$), until 1180 ms (Difference = 2.7% +/−0.6, $p = 0.006$). The onset of this difference coincides closely with the transition from taste stimulation to rinsing (stimulation offset 700 ms, rinsing onset 900 ms). No group differences were observed for the visual control task in which the classifiers discriminated between blue and green squares (Fig. 5A).

Classification of group membership. The classification of group membership (Fig. 4B) was found to exceed chance performance for an extended period of time for both tastes (1000 ms to 1430 ms for sweet and from 960 ms to 1310 ms for salty taste). The effect lasted longer and was more pronounced for sweet (peak: 1200 ms, 2.2% above threshold) compared to salty (peak: 1170 ms, 1.4% above threshold). No such persistent group differences were found for the MVPA applied to the visual control task (Fig. 5B).

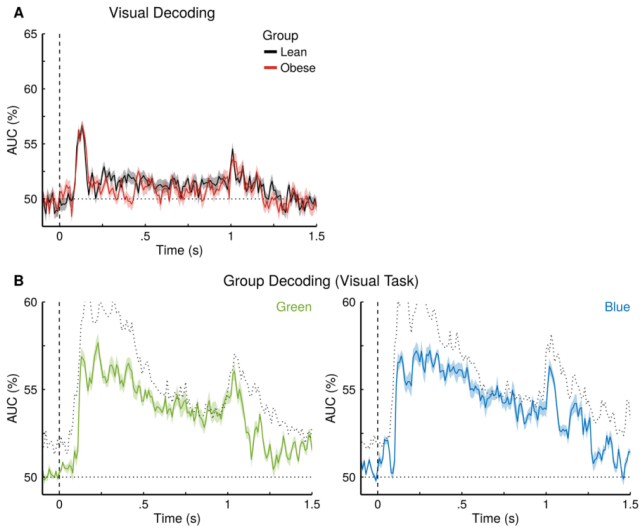

Figure 5. Decoding of visual information and group membership reveal no differences in visual processing between lean and obese individuals. (**A**) Binary decoding of colour information (green versus blue), separately for lean and obese individuals. Coloured solid lines show the mean performance, surrounding shaded regions show 1 SEM (dashed vertical line: stimulus onset; dotted horizontal line: theoretical chance level of an AUC of 50%). (**B**) Binary decoding of group membership within a colour (e.g. given a green square, "is this a lean or an obese person?"). The jittered dark grey line shows the maximum performance over 1000 permutations of group membership per time point.

Temporal Generalization. The results showed a square pattern of generalization across time for both groups (Fig. 4C), suggesting on-going, interdependent mental activity with respect to taste processing. A smaller first time window of generalization was identified within the first 400 ms after stimulus onset (peaks: lean 270 ms, AUC = 58.4% +/−0.7; obese 290 ms, AUC = 57.6% +/−0.9), whose generalization performance did not differ significantly to the peak testing time from 210 to 340 ms for lean and 220 to 400 ms for obese subjects ($p > 0.05$, uninterrupted). A second, larger time window of generalization was found with its peak at 630 ms for lean (AUC = 61.4% +/−0.6) and 610 ms for obese subjects (AUC = 61.0% +/−0.7), with no significant difference between diagonal and off-diagonal performance from 510 to 820 ms (lean) and 470 to 800 ms (obese; $p > 0.05$, uninterrupted). The latter window's peak time patterns then generalized significantly until 1230 ms and 1090 ms for lean and obese, respectively ($p < 0.05$, uninterrupted). This finding suggests that taste processing is rapidly initiated by a shorter, likely purely perceptual state before transitioning to a longer evaluative phase. Nevertheless, the first process is of relevance to the second one as its neural patterns generalize significantly until 1150 ms and 990 ms for lean and obese, respectively ($p < 0.05$, uninterrupted). A comparison of the temporal generalizability in the two groups revealed that in the lean group, the neural patterns from the time window between 1100 and 1260 ms (offset of decodability for obese and lean, respectively) generalized significantly better from ~300 up to 1080 ms ($p_{FDR\text{-}corrected} < 0.05$). In other words, the second step in the processing chain which starts around 300 ms is prolonged for lean as compared to obese. Consequently, this means the temporal generalization reveals the same number of processing steps for both groups, but with differing durations.

Discussion

In the absence of a clear account of differences in taste perception and their neural underpinnings in obesity, it is important to look at the whole perceptual process, and to disentangle the sensory and evaluative aspects wherever possible. Thus, in the present study, we analysed gustatory evoked responses in lean and obese individuals with the help of EEG, which offers temporal resolution on a millisecond time scale. We employed electro-physiological measures such as evoked field strength and topographical distribution, as well as multivariate pattern analysis which can provide avenues for further inspection even when the signal to noise ratio is not optimal.

In line with previous findings, taste quality information emerged shortly after the stimulus onset[20] and was present even after the end of stimulation in both the lean and obese groups. Remarkably, taste quality information deteriorated earlier in the obese than the lean group, suggesting that lean and obese individuals display differences during the later stages of taste processing. In line with this finding, for both taste qualities, group-membership decoding was possible only during this later stage further corroborating that the time period around taste offset differentiates between groups. Analysis of temporal generalization indeed points to similar processes between

groups that vanish earlier in the obese, rather than an additional processing step in the lean. This interpretation is also supported by the lower GFP in obese compared to lean group close to the stimulus offset, and the lack of corresponding global map dissimilarities as seen in the GMD analysis. Overall, these findings suggest consistently that lean and obese individuals process taste quality similarly, yet obese individuals exhibit shorter lasting activity within the gustatory network.

Also in line with previous findings, the GFP for salty was significantly higher than for sweet, signifying lower SNR for sweet taste[20,23]. Although this difference was already present earlier than would be expected from the latency of the gustatory evoked response, this could be due to the fact that the same taste quality was never presented twice in a row, which may have led to some effect of expectancy.

The latency of group differences in representation seen here is close to stimulus offset, and consistent with working memory maintenance rather than any previously reported sensory or evaluative components of the gustatory evoked potential[15,19–21,23,39], although this interpretation is purely speculative at this stage. Evidence from the rodent brain shows that gustatory processing takes place via networks of feedback and feedforward pathways that involve more than just the primary sensory areas[40]. Given this and the limited knowledge of the taste processing cascade in humans, we use the term "taste representations" not only with reference to the earliest sensory processing, but in a broader sense. Unlike a previous report[11] of a lower consummatory food reward to appetitive stimuli in obesity, the group differences reported here were observed for both, pleasant sweet and the somewhat unpleasant salty tastes. It is therefore unlikely that the differences between groups reflect differences in positive reward value per se. Nevertheless, it would be interesting to explore whether the shorter taste representations observed in the obese might be linked to a significantly weaker taste experience over time, perhaps contributing to the reward deficiency seen in obese individuals upon consumption[11].

Importantly, our experiment included visual control stimuli to exclude that the group differences in gustatory processing resulted from anatomical differences or differences of signal to noise ratio between the two groups. None of the effects seen in the gustatory modality were seen for the visual stimuli. Along with the method of comparing the actual decoding performance against the best performance from 1000 permutations, this presents a strong case that observed differences are in fact related to gustatory processing and cannot be explained by extraneous factors like signal-to-noise ratio alone.

The observed group differences in the electrophysiological signal occurred in the absence of statistically significant self-reported perceptual differences. In an earlier study, we found a heightened sensitivity to sweet and salty taste in obese compared to lean individuals[6]. The present data did not replicate this finding. This apparent discrepancy may be the result of the different method of stimulus administration used in the two experiments (e.g. in the previous study, stimuli were manually sprayed on the tongue with the help of a spray bottle, whereas in the current study, a gustometer was used and the taste stimuli were embedded in a continuous series of water pulses), underscoring the sensitivity of individual differences in taste perception to experimental parameters. Moreover, upon calculation of Bayes factors, the current data do not support the null hypothesis very strongly, thus, underlying perceptual differences between the two groups cannot be ruled out entirely.

Together, we present evidence for differences in gustatory neural processing between lean and obese individuals independent of subjective perceptual differences. Given that this is the first study of its kind, and the ambiguities in the existing literature, more work is needed to investigate the generalizability of the current findings across populations and methodologies. While the underlying biological mechanism behind these differences, as well as their implications for ingestive behaviour remain to be uncovered, the novel combination of electrophysiological data with MVPA offers an avenue into obesity research where the dynamics of gustatory perception and reward may be studied in greater detail.

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Author Contributions

S.H., A.V., and K.O. designed the study, S.H. performed the experiment; S.H. performed the waveform analyses; R.W. performed the classification analyses; S.H. and R.W. wrote the manuscript; K.O. and A.V. revised the manuscript.

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3. Summary

Zusammenfassung der Arbeit

Dissertation zur Erlangung des akademischen Grades Dr. rer. med.

Taste perception in obesity.

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angefertigt am : Max-Planck-Institut für Kognitions- und Neurowissenschaften, Leipzig

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October 2018

Obesity, defined as abnormal or excessive fat accumulation that may pose a risk to health (WHO, 2015), increases an individual's chances of developing cardiovascular disease, stroke, diabetes, hypertension, cancer, as well as problems of mental health (Haslam and James, 2005b; Luppino et al., 2010; Sikorski et al., 2011). Obesity is understood to be caused by increased intake of energy-dense foods, often combined with a sedentary lifestyle, resulting in an energy imbalance (WHO, 2015). But the neural, behavioural, and physiological mechanisms behind excessive energy intake have not been elucidated completely.

Since the advent of neuroimaging, a significant portion of obesity research has focussed on reward mechanisms in the brain, especially in the context of food (Kenny, 2011; Ziauddeen et al., 2015). But it is unclear whether the differences in food reward seen in obesity are accompanied, or indeed preceded by differences in the way food is perceived at the gustatory level

(Donaldson et al., 2009). Moreover, most studies of neural taste processing in obesity have relied on functional magnetic resonance imaging (fMRI; Stice, Spoor, Bohon, & Small, 2008; Stice, Spoor, Bohon, Veldhuizen, et al., 2008; Szalay et al., 2012). The low temporal resolution of fMRI leaves it unresolved whether observed differences occur during the sensory encoding, or more cognitive and evaluative aspects of taste processing. Hence, in the present work, we explored differences between lean and obese individuals in the subjective perception and neural processing of taste. To the latter end, we employed electroencephalography (EEG). The high temporal resolution of EEG makes it possible to separate the sensory and cognitive aspects of neural processing, as reflected in earlier and later event related potentials (ERPs), respectively.

Measurement of ERPs requires averaging the EEG recordings from a large number of time-locked trials (Luck, 2005). However, taste stimulation requires long inter-stimulus intervals in order to avoid habituation, making it difficult to include a large number of trials in a single session. For this reason, even though our study was exploratory, we thought it prudent to limit the stimuli to only the most relevant taste qualities. Therefore, as a foundation for the EEG study, we first tested lean and obese individuals on three behavioural measures of taste perception: recognition thresholds, and perceived intensity and pleasantness of supra-threshold tastants (study 1, Hardikar et al., 2017).

First, we compared the recognition thresholds for sweet, salty, sour, and bitter between 23 obese (BMI>30) and 31 lean (BMI<25) participants using an adaptive Bayesian staircase procedure (Watson and Pelli, 1983). Next, participants gave ratings of "perceived intensity" and "perceived pleasantness" for supra-threshold concentrations of each taste quality. Here, in order to get a

comprehensive overview of group differences, we used two types of stimulus pairs for each taste quality. One pair consisted of "absolute" high and low concentrations, which were the same for all participants, and another pair consisted of "relative" high and low concentrations, which were adjusted to each participant's taste thresholds. In this way, we were able to make between-subject comparisons both for the same concentrations of a taste, as well as concentrations that were different across participants, but comparable in terms of each participant's own taste-space.

The results showed that obese participants had lower recognition thresholds for sweet and salty taste, indicating a higher sensitivity. This difference was also present in the supra-threshold measurements, where obese participants rated the "absolute" concentrations of sweet and salty as more intense. Interestingly, no group differences were observed when the supra-threshold concentrations were adjusted to each individual's thresholds.

Based on these results, we limited the subsequent investigation of gustatory ERPs (gERPs;) to sweet and salty taste. These two tastes are also the most relevant to the study of obesity, as in everyday life, food that is classified as either sweet or salty is consumed in greater quantities than food that is sour or bitter and is more likely to be paired with edible fats, resulting in a higher caloric content. We presented two supra-threshold concentrations ("high" and "low") of sweet and salty to 30 lean and 25 obese participants while recording EEG from 62 channels (study 2, Hardikar et al., 2018). The tastes were presented with the use of a "gustometer" (GU002, Burghart Messtechnik, Wedel, Germany) especially suited to elicit gustatory ERPs without concomitant somato-sensory activation. We observed great inter-individual variability in the location, latency and strength of the gERPs [Appendix 1, Fig

3]. This presents a challenge for between-subject comparisons, as a lot of information is lost in averaging individual ERPs for the group-level analysis. It is also problematic for the selection of the time-points and electrodes of interest, as such a selection runs the risk of neural responses from some individuals being given more weight than others. Therefore, we chose to explore the responses over the whole epoch in the lean and obese group further using the "decoding" approach of multivariate pattern analysis (MVPA; see Grootswagers, Wardle, & Carlson, 2017; Kriegeskorte, 2011), where a machine learning algorithm is trained to classify the brain response patterns to two or more stimulus classes using a subset of the data, and then the decoding performance of this classifier is tested using the remaining data, thereby identifying the neural response patterns associated with specific stimuli. MVPA uses the instantaneous patterns from all electrodes, side-stepping the issue of arbitrary selection of electrodes and epochs of interest. This method has previously been employed to decode taste quality from the pattern of neural responses evoked by taste stimuli using the same protocol as the current study (Crouzet et al., 2015). Notably, we performed these decoding analyses on a single trial level. As a result, the differences reported are not driven by a few individuals within a group who display stronger signal-to-noise ratio (SNR), but rather by the characteristic patterns that are common to the whole group. In line with Crouzet et al., (2015) the results showed that taste qualities were discriminable from around 130 ms and stayed discriminable until after stimulus offset. These differential representations faded earlier in the obese group than the lean group. We investigated whether the longer representations in the lean group arose from an additional processing step or from the same processes lasting longer. For this, analysis of temporal generalization was performed for both groups, which revealed that the same "later evaluative" process that

started around 300 ms in the two groups lasted longer in the lean group and faded out earlier in the obese. We also analysed the global map dissimilarities between these two groups, which did not show any significant differences in the underlying cortical generators of the neural signal. Differences in later gustatory response patterns even allowed decoding of group membership. Importantly, group differences were absent for visual processing, and cannot be put down to group differences in the anatomy or signal-to-noise ratio alone. The latency of the observed effects suggests that later evaluative aspects of taste processing, or possibly the working memory maintenance of taste are altered in obesity. Differences in the evoked potentials were observed in the absence of significant differences in the "intensity" and "pleasantness" ratings of the two groups. However, calculation of Bayes Factors showed that the current data do not provide strong evidence for an absence of group differences.

As these analyses were exploratory in nature, future studies will have to not only replicate the results but delve deeper into the underlying mechanisms and behavioural consequences of the findings. The second study presented here is also the biggest sample of gustatory evoked potentials that has ever been reported. Given the observed variability of these potentials across individuals it seems plausible that a bigger sample introduces more heterogeneity leading to an attenuation of the group ERPs. Future gERP studies should therefore also work on ensuring a good SNR and investigate the reasons for the high variability observed in gERPs. Nevertheless, the present combination of precise taste stimulation, high temporal resolution of EEG and the exploratory potential of MVPA provides a novel and useful approach for investigating the elusive dynamics of food perception in obesity research and beyond.

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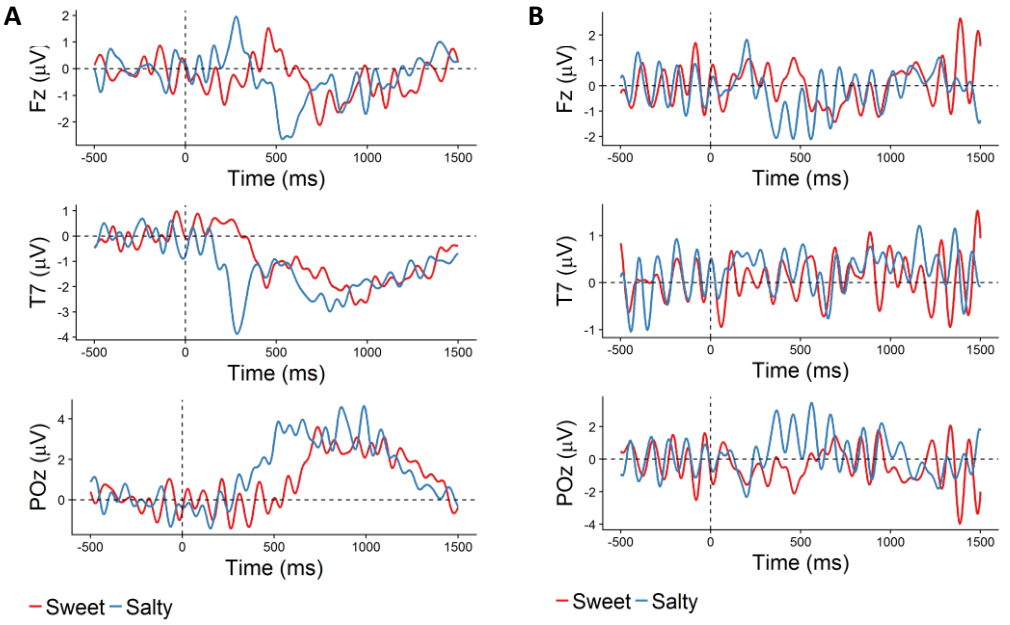
A. Appendix

A.1 Individual differences in gustatory event related potentials.



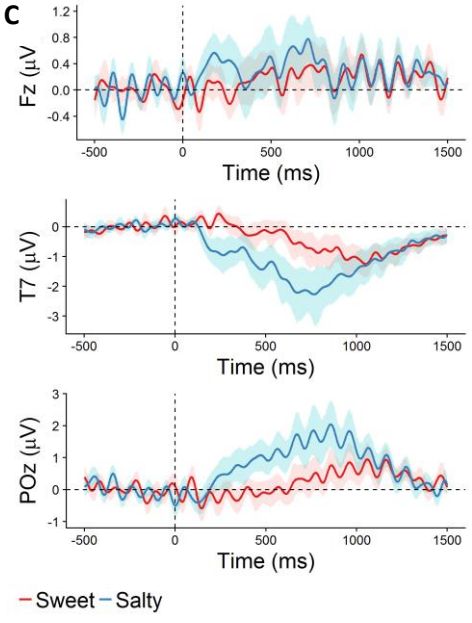


Figure 3 A) Example gERP from one participant, displaying an early sensory component in response to sweet and salty at electrodes Fz and T7, followed by a slower rising later cognitive component at POz.

B) Data from another participant does not show the aforementioned components at the same electrodes.

C) Grand averaged gERP with 95% CI. from 30 lean participants, showing much weaker deflections as compared to the single-subject data shown in 3A.

A.2 Author Contributions

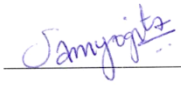
Author contributions to the journal article

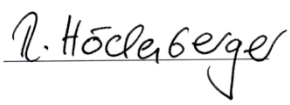
"*Higher sensitivity to sweet and salty taste in obese compared to lean individuals*" by Samyogita Hardikar **(SH)**, Richard Hoechenberger **(RH)**, Arno Villringer **(AV)**, and Kathrin Ohla **(KO)**; Appetite 111, 158-165, 2017.

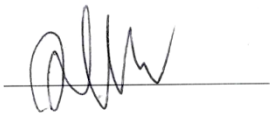
The first author SH contributed to forming the conceptual outline, study design and analysis plan. SH conducted the study and performed the statistical analysis. SH created all figures in the manuscript. SH interpreted the results and drafted the manuscript with help from RH and KO.

Author Contributions:

Conception and design of the study:	SH, KO, AV
Threshold estimation procedure:	RH
Data acquisition:	SH
Data analysis:	SH
Interpretation of results	SH, RH, KO
Figure creation:	SH
Drafting of manuscript:	SH, RH, KO
Critical revision of manuscript:	KO, AV

Samyogita Hardikar  Date 05.10.2018

Richard Hoechenberger  Date 21.09.2018

Arno Villringer  Date 5.10.2018

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Author contributions to the journal article:

"Shorter-lived neural taste representations in obese compared to lean individuals" by Samyogita Hardikar **(SH)**, Raphael Wallroth **(RW)**, Arno Villringer **(AV)**, and Kathrin Ohla **(KO)**; Scientific Reports 2018; 8: 11027.

The (co-)first author SH contributed to forming the conceptual outline, study design and analysis plan. SH conducted the study, pre-processed the EEG data and performed the waveform analysis, and contributed the corresponding sections of the methods, results and figures. SH drafted the introduction and discussion of the manuscript.

Author Contributions:

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Waveform Analysis of EEG data:	SH
Multivariate Pattern Analysis of EEG data:	RW
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Drafting of manuscript:	SH, RW
Critical revision of manuscript:	KO, AV

Samyogita Hardikar Samyogita Date 05.10.2018

Raphael Wallroth (co-first author) Date 02.10.2018

Arno Villringer Date 5.10.2018

Kathrin Ohla Date 20.9.18

A.3 Declaration of Authenticity

Erklärung über die eigenständige Abfassung der Arbeit

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar eine Vergütung oder geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren. Die aktuellen gesetzlichen Vorgaben in Bezug auf die Zulassung der klinischen Studien, die Bestimmungen des Tierschutzgesetzes, die Bestimmungen des Gentechnikgesetzes und die allgemeinen Datenschutzbestimmungen wurden eingehalten. Ich versichere, dass ich die Regelungen der Satzung der Universität Leipzig zur Sicherung guter wissenschaftlicher Praxis kenne und eingehalten habe.

Leipzig, Samyogita Hardikar

A.4 Curriculum Vitae

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Education

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10.2008- 09.2009	University of York MSc in Cognitive Neuroscience *Thesis: Viewpoint invariance in areas involved in the processing of landscape scenes*
07.2005- 08.2008	Fergusson College, University of Pune BA in Psychology
06.2003- 04.2005	Higher Secondary Certificate (Maharashtra State Board)
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Work Experience

12.2011- 03.2012	Intern MPI for Human Cognitive and Brain Sciences, Leipzig

03.2011- 01.2012	Joint organizing secretary 38th National Annual Conference of the Indian Association of Clinical Psychologists (NACIACP)
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04.2008- 06.2008	Student research associate Yellow Brick Road, Pune

Samyogita Hardikar

October 2018

A.5 List of Publications

Hardikar, S., Wallroth, R., Villringer, A., & Ohla, K. (2018). Shorter-lived neural taste representations in obese compared to lean individuals. Scientific reports, 8(1), 11027.

Hardikar, S., Höchenberger, R., Villringer, A., & Ohla, K. (2017). Higher sensitivity to sweet and salty taste in obese compared to lean individuals. Appetite, 111, 158-165.

Fritz, T. H., **Hardikar, S**., Demoucron, M., Niessen, M., Demey, M., Giot, O., ... & Leman, M. (2013). Musical agency reduces perceived exertion during strenuous physical performance. Proceedings of the National Academy of Sciences, 201217252.

A.6 Conference Contributions

Hardikar, S., Wallroth, R., Villringer, A., Ohla, K., (2017). Differences in Neural Processing of Taste between Lean and Obese Individuals. 23rd Annual Meeting of the Organisation for Human Brain Mapping (OHBM), Vancouver, Canada.

Hardikar, S., Wallroth, R., Villringer, A, Ohla, K. (2016). Differences in Neural Gustatory Processing between Lean and Obese Individuals Revealed by Evoked Responses. 46th Annual Meeting of the Society for Neuroscience (SfN), San Diego, USA.

Hardikar, S., Villringer, A., Ohla, K. (2015). Higher sensitivity to sweet and salty tastes in obese adults, 57th Conference of Experimental Psychologists (TeaP), Hildesheim, Germany.

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