The interplay between mouth and mind

Explaining variation in taste-related brain activation

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Contents

Chapter 1

General introduction

Food is one of our primary needs and essential for survival. Hence, evolution programmed us to find the act of eating pleasurable. Therefore, it is no surprise that eating plays such a dominant role in our lives. Besides our daily meals, we use food to celebrate, relax and to comfort and reward ourselves. The taste of food, including all oral sensations, is one of the main drivers of eating because of its rewarding properties. Next to taste, food nutrient content might also contribute to the oral experience in a more implicit manner. In the brain, sensory signals are integrated with other factors such as personality characteristics, cognitions, metabolic state and food preferences. The final consumption experience can therefore be considered as an end product of the interplay between mouth and mind. In this thesis, we provide more insights into what role several of these factors play in taste-related brain activation.

The research presented in this thesis was done in the context of an European Regional Development Fund (EFRO) innovation project entitled 'Food and Cognition Model systems' (FOCOM). The overall aim of the FOCOM project is to gain better understanding of consumers buying and eating behavior and to use this information to develop food products that are better equipped to consumers wishes and needs. Liking scores are not always a good predictor of product failure or success. Therefore, the potential of implicit behavioral and brain measures was investigated. This thesis describes part of the functional magnetic resonance imaging studies done within FOCOM.

Food intake

Cephalic and gastric phase

There are two phases in food intake: the cephalic and gastric phase. The cephalic phase lasts from the moment that anticipation of ingestion starts until the food is completely swallowed, and can be subdivided in a pre-ingestive and an ingestive part [1]. The pre-ingestive cephalic phase is initiated by exposure to a food cue (i.e. the thought, smell or sight of food) which leads to anticipation of ingestion, and ends with meal initiation (the first bite or sip). During this period, food cues activate receptors in the head, which in turn, trigger a cascade of hormonal and neural responses including, but not limited to, salivation, gastric acid secretion, and insulin release [2], which are referred to as cephalic phase responses. These cephalic phase responses prepare the body for food ingestion and absorption.[1] The ingestive cephalic phase and the gastric phase both start with ingestion and end respectively with meal termination and full digestion (the moment when no food is left in the stomach). During ingestion, oral sensations continue to stimulate cephalic phase responses.[1] During the gastric phase, stomach distention most likely acts as a direct satiation signal, by communicating to brain feeding centers via the vagus nerve [3–8]. Satiation can be defined as the feeling that gradually increases during

the course of a meal and finally brings it to an end. In contrast, satiety starts after meal termination and lasts until the next eating episode.[9] Both oral and gastric stimulation are necessary to achieve optimal control of appetite [10–14].

Homeostatic eating

During adulthood, body weight of the majority of the population remains within a balanced range [15–17]. This indicates that body weight is tightly regulated. The traditional view of food intake and body weight regulation involves the integration of internal feedback signals by the brainstem and hypothalamus, which in turn, trigger regulatory signals [15]. Internal feedback signals include circulating metabolites from the gut and circulating hormones such as leptin, insulin, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and ghrelin [15,18,19]. Feedback signals can be categorized as long-acting adiposity signals and short-acting gastro-intestinal signals [5,20]. In addition, besides long and short term signals, factors that have both long and short term capacities such as ghrelin and peptide YY have also been identified [5,20]. Long-acting signals are involved in the regulation of body weight and fat storage. Leptin and insulin classify as long term signals and are thought to have an important role in communication with the hypothalamus [17,21–23]. They circulate in the bloodstream in concentrations proportional to body fat content and energy expenditure and act, among others, on neurons in the arcuate nucleus of the hypothalamus. Here, high levels of insulin and leptin decrease food intake by stimulating the activity of catabolic pathways and by inhibiting the activity of anabolic pathways.[21] Short-acting signals are hormonal secretions from the gut triggered by the presence of food such as CCK and neural signals conveyed via the oral cavity and gastrointestinal tract [20]. The latter includes oro-sensory signals and mechanical and chemical signals in the stomach and intestine that are sent to the nucleus of the solitary tract (located in brainstem) via facial and cranial nerves [24,25]. These signals also reach the hypothalamus [15]. Output from the hypothalamus comprises behavioral signals (e.g. locomotor activity and wakefulness are increased during hunger to enhance the likelihood of feeding behavior) and autonomic and endocrine signals (e.g. pancreas innervation to regulate secretion of insulin and glucagon) [26]. The above mentioned mechanisms, with a central role for eating when hungry and when it is biologically relevant, are referred to as homeostatic eating. However, if this was the whole picture, we would all be in perfect balance and problems like overweight and obesity would not exist.

Hedonic eating

Hunger is not the only reason for meal initiation, and fullness not the only reason to stop a meal [27,28]. Food hedonics are also important in this process, particularly in facilitating a shift towards a higher

body weight [29,30]. The brain reward system, involved in coding food reward, entails dopaminergic projections from the ventral tegmental area and substantia nigra (midbrain) to the nucleus accumbens (ventral striatum) and putamen, pallidum and caudate (dorsal striatum). Via the striatum, projections reach other parts of the limbic system such as the orbitofrontal cortex, amygdala, anterior cingulate cortex and insula [31,32]. This system is commonly referred to as the mesolimbic dopamine pathway [32,33]. Homeostatic and hedonic systems are thought to interact to control eating behavior [22,29,34]. This view is supported by the finding that food pleasantness can vary as a function of hunger state [35–37], and that leptin and insulin (homeostatic hormones) can interact with the hedonic system to decrease food reward [30,38]. Figure 1.1 shows a simplified overview of the interconnected relationship between the

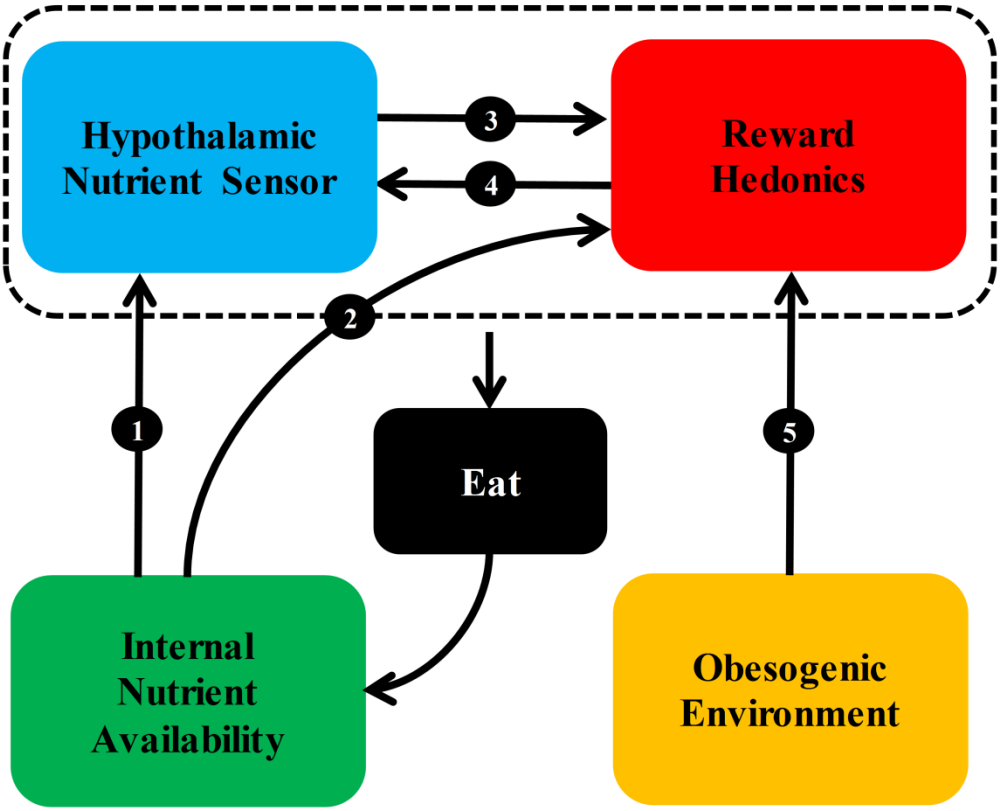

Figure 1.1 Schematic representation of the integration of the homeostatic and hedonic regulation of eating behavior and body weight. Internal nutrient availability is sensed by both the hypothalamic nutrient sensor (1) and reward processing centres (2), which communicate with each other (3 and 4) to regulate eating behavior. This figure is reprinted from Münzberg H, Qualls-Creekmore E, Yu S, Morrison CD and Berthoud H-R. Hedonics Act in Unison with the Homeostatic System to Unconsciously Control Body Weight. *Front Nutr*. 3. February. 6. 2016 [29]. Copyright © 2016 Münzberg, Qualls-Creekmore, Yu, Morrison and Berthoud.

homeostatic and hedonic systems. In this integrative model, both the hypothalamus and the brain reward system sense internal nutrient availability, and in turn, communicate with each other to regulate eating behavior. Factors such as food availability and palatability act on the brain reward system.[29] Finally, top-down influences such as habits, memory, cognitions and emotions are processed

in the corticolimbic system (e.g. prefrontal cortex, amygdala, anterior cingulate cortex and hippocampus), which is thought to act on regulatory mechanisms in the hypothalamus and brainstem [29,39]. However, until now, little is known about the neurological pathways involved in the modulating effects of top-down factors [39].

Oral processes

Food consumption starts with the first bite followed by mastication. Mastication or chewing serves to fragment the food, to enhance the release of flavor and aroma and to lubricate the food with saliva [40,41]. Saliva contains enzymes that begin with the digestion of starches and lipids [42]. Furthermore, lubrication with saliva facilitates bolus formation; the lubricated food particles can be pressed together by the tongue against the hard palate to form a bolus that can be swallowed [43]. When the food particles in the bolus are in optimal cohesion, swallowing of the bolus is triggered [43].

Taste

One of the chemical processes that occurs in the mouth is taste transduction. Humans perceive five basic tastes namely sweet, salty, sour, bitter and umami. The existence of a sixth primary taste quality, namely the taste of fat (free fatty acids), is still under investigation. However, given that the concentration of naturally occurring free fatty acids in a human diet is low, it is likely that the presence of fat is more dominantly signaled by textural components. [44] From an evolutionary perspective, taste serves to stimulate the ingestion of nutritious food, to inform the body about the upcoming nutritious load and to warn against potentially harmful substances.[45] More specifically, sweet and umami tastes signal for the presence of respectively sugars and amino acids, salty taste functions to maintain electrolyte balance, and bitterness and sourness notify the body of noxious or poisonous chemicals.[45,46] Gustatory epithelium is present on the tongue, soft palate and the pharynx [47]. In this epithelium, taste receptor cells are clustered together in taste buds, which in turn are located in folds of the tongue (papillae) [47,48]. When food is present in the mouth, taste receptors bind to taste molecules and stimulate nerve fibers to send signals to the brainstem [48]. T2Rs binds to compounds such as quinine and saccharin and is responsible for bitter taste transduction. The umami taste receptor is called T1R1 + T1R3 and is responsive to glutamate and other amino acids. [45–48] The sodium channel ENaC and the acid sensing ion channels ASiCs are possibly involved in respectively salty and sour taste signaling [47,48]. Finally, the sweet taste receptor (T1R2 + T1R3) responds to sugars and artificial sweeteners [45,49].

The gustatory pathway

Taste signals travel to the brain via the facial, glosso-pharyngeal and vagus nerve [47]. In the brain, signals follow the so-called 'gustatory pathway', which is reasonably well established in primates. Afferent nerves first arrive in the nucleus of the solitary tract (brainstem), then synapse on to the thalamus, anterior insula/frontal operculum (primary taste cortex) and the orbitofrontal cortex (OFC, secondary taste cortex) [50,51]. In monkeys, the OFC projects to many other regions including the hypothalamus, striatum, amygdala and anterior cingulate cortex [50,52–54]. Furthermore, patterns of coactivation were observed in a meta-analysis between the human OFC and the amygdala, striatum and thalamus [55]. Even though these areas are not part of the gustatory pathway, they are probably closely related and often found in taste fMRI studies in humans (see for example: [56–60]).

Identifying functional specialization of taste regions in humans is challenging because taste is a multi-dimensional sensation. The perception of taste comprises 5 dimensions, i.e. intensity, quality, spatial localization, hedonics and temporal dynamics, that combined form the final taste sensation [47]. For quality, intensity and pleasantness representation, accumulating evidence suggests a role for the insula [61–64]. Besides the insula, other regions have also been reported to code food pleasantness such as the OFC, anterior cingulate cortex (ACC), striatum and amygdala [56,65–69]. Little knowledge exists about the representation of the other taste dimensions in the brain.

Flavor

Besides taste, smell, texture and temperature of a food are also detected in the oral cavity. Hence, the integration of taste (that is, the chemical stimulation of taste receptors in the mouth) with oro-sensory and retronasal olfactory signals in the brain results in the actual consumption experience, which is referred to as flavor [50,70,71]. The sensory inputs that make up a flavor are thought to be integrated in the anterior insula, from where they accordingly travel to connected brain regions such as the brainstem, thalamus, amygdala, OFC and ACC. Here, they are combined with additional appetitive signals to control eating behavior. [70]

Several studies have shown the interdependent relationship between taste and odor [72–76]. Stevenson et al. (1999) found that an orthonasally administered caramel odor suppressed the sourness of citric acid and enhanced the sweetness of sucrose. Moreover, others found that odors were perceived as sweeter when they had retronasally been paired with a sucrose compared to a salty solution [73]. Furthermore, also in real products, adding flavor by the removal of a nose-clip during consumption, modulated taste perception [74]. Finally, the addition of a sweet odor to a sucrose solution in comparison to a tasteless solution increased salivary responses [76]. The above studies are illustrative of the strong interaction

between taste and odor. The latter study additionally indicates that congruency between odor and taste, i.e. when they are commonly combined in food products, could be important for optimal digestion.

Energy sensing

Next to taste, recent findings indicate that energy is also sensed in the oral cavity. Behavioral studies show that mouth rinsing with a sweet carbohydrate solution in comparison to a sweet non-caloric solution enhanced exercise performance [77,78]. Moreover, mouth rinsing with a sweet carbohydrate solution in comparison to a sweet non-caloric solution also increased activation in the primary motor cortex during physical activity [79]. Interestingly, a recent study reported that humans, like rats [80,81], are able to taste glucose polymers such as maltodextrin [82]. This serves as evidence for the hypothesis that there is an unknown oral carbohydrate receptor, that functions independently of the sweet taste receptor [77]. In addition, fatty acid sensing may also occur on the tongue. Presumably by oral receptors such as CD36 and GPR120 [44,83–85].

Consumption experience

Consumption experience, as referred to in this thesis, is defined as the combination of explicit and implicit oral sensations that are evoked by the presence of food in the oral cavity. Recent studies indicate that explicit and implicit processes of food reward are not necessarily in agreement with each other within one person [86–88]. For instance, explicit attitudes are thought to be more associated with controlled/reflective behavior, whereas implicit attitudes may be more involved in uncontrolled/impulsive behavior [89,90]. However, most likely reflective and impulsive processes interact and together determine behavioral output [90]. Measurements of the consumption experience in the brain – e.g. by fMRI measurements during the period food is in the mouth – are more informative than behavioral measurements (e.g. explicit ratings or implicit reaction time tasks) alone [91–93]. Behavioral measures can be used to separately obtain explicit and implicit attitudes, but are unable to show how these processes interact. In contrast, brain responses do reflect this interaction between explicit and implicit processes of food reward. In addition, brain in comparison to behavioral measures, serve as the most direct way to measure the consumption experience. Therefore, in the experiments described in this thesis, the consumption experience is measured in the brain.

The consumption experience is a dynamic process that varies from person-to-person. For example, brain responses to the same tastant vary with gender, age and body mass index (BMI) [36,59,94–97]. Moreover, consumption experiences may also differ within a person, depending on the situation. A good example is hunger state; Eating chocolate when hungry compared to satiated elicits different

responses in the brain [98]. In line with this, many others also found that hunger state modulates brain response to food cues [36,59,99,100]. An overview of potential internal and external factors that may influence the consumption experience is listed in Figure 1.2 (see also [101]). The factors addressed in this thesis are discussed below.

First, a multitude of cognitive effects such as expectations and selective attention can affect taste and flavor perception. Visual and olfactory food cues trigger beliefs about anticipated taste and satiation properties [102]. Product packages and the information they contain, such as pictures, price, caloric content and health claims, are visual cues that create expectations. For few of these factors the effect on the consumption experience has been investigated. One study reported that increasing the price of a wine resulted in higher liking scores and greater taste activation in the medial OFC [103]. Most other neuroimaging studies examined packaging effects during food anticipation by means of food images rather than by food consumption [97,104–106]. Linder et al. (2010), for example, found that food viewing activation in the ventral striatum increased when a product was presented as organic compared to regular [106].

On a related note, product labels may modulate the consumption experiences via selective attention biases. In the supermarket, attention of the consumer is often drawn towards one specific aspect of a product such as its health benefits or its palatable taste by means of claims or statements on the package. Several neuroimaging studies looked into such attentional effects. They found that neural processing of visual and gustatory food stimuli is modulated by preceding verbal descriptions that attach a particular value to the stimulus [67,107,108]. Verbal descriptors varied from the words "treat" versus "healthy" [108] to positive versus negative words [107] and sentences such as "rich and delicious flavor" versus "boiled vegetable water" [67]. Like verbal descriptors, task instructions such as "try to detect a taste" versus passive tasting were also found to influence brain activation evoked by a taste stimulus [109,110]. Finally, one study showed that focusing on specific product characteristics, i.e. pleasantness and intensity, also resulted in different taste-induced brain responses [111].

A second factor shown to modulate brain responses to food cues comprises a persons' beliefs, attitudes and personality [112–116]. Personality traits relevant for eating behavior are for example impulsivity and reward sensitivity [101]. Kerr et al. (2014) showed that impulsivity is associated with increased activation in the ACC and amygdala during anticipation of a rewarding taste [114]. In another study, trait reward sensitivity was correlated with the neural response to images of palatable relative to bland foods in reward areas such as the ventral striatum [113]. To date, little research exists with regard to personal beliefs or attitudes important for eating behavior, e.g. attitude towards healthy [117,118] or environmental friendly foods [119,120], and how these modulate brain responses to food cues.

Finally, oral food nutrient content may stimulate brain regions independently of taste. Accumulating evidence for this was found in particular for energy content. Several fMRI studies reported that the human brain responds differently to sips of similarly tasting sugar sweetened and artificially sweetened beverages [121,122]. This suggests that energy content per se may alter the consumption experience of food. As was indicated in the section "Energy sensing" carbohydrates may be sensed in the oral cavity by an until now unidentified carbohydrate receptor. However, whether oral exposure to different types of carbohydrates, e.g. glucose and fructose, induces different brain responses has not been investigated.

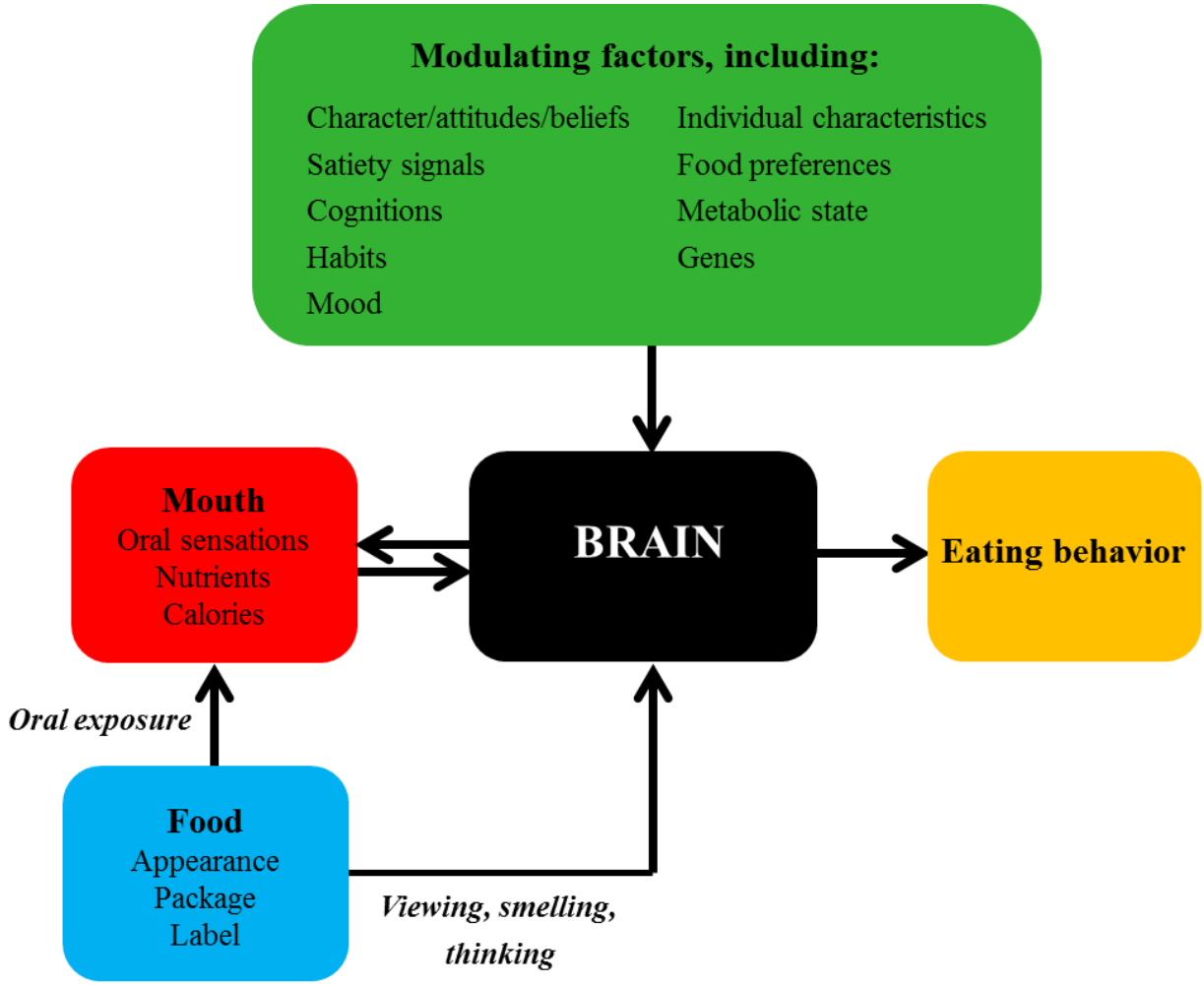

Figure 1.2 Schematic representation of food-mouth-brain interactions in relation to eating behavior. This figure is based on Smeets PAM, Charbonnier L, van Meer F, van der Laan LN and Spetter MS. Food-induced brain responses and eating behaviour. *Proc Nutr Soc.* 71. 04. 511-520. 2012 [101] with permission (Cambridge University Press).

Measuring brain activation

The technique used to study taste-related brain responses in this thesis is functional magnetic resonance imaging (fMRI). Upon a task-related event, e.g. a taste event, neuronal firing is triggered and oxygenated blood rapidly flows to the site of action. Moreover, the concentration of deoxygenated hemoglobin rises due to the rapid usage of oxygen. Deoxyhemoglobin has different magnetic properties as compared to oxyhemoglobin and the surrounding tissues [123]. The changes in blood oxygen level can therefore be indirectly measured by means of fMRI.[124,125] The signal obtained via fMRI is referred to as blood oxygen level dependent (BOLD) signal [124].

Plotted over time, the BOLD signal follows a so-called hemodynamic response. The BOLD signal starts approximately 2 seconds after neuronal activity begins, and reaches its peak after 7 to 10 seconds. The signal remains high until neuronal activity ends. Hereafter, the BOLD signal returns to baseline in approximately 8 to 11 seconds.[126] Because of the lengthiness of the hemodynamic response, fMRI lacks a high temporal resolution. However, fMRI also has many advantages in comparison to other neuroimaging techniques such as its high spatial resolution, possibility to image deeper brain regions and low invasiveness. [127] Therefore, fMRI qualifies as most suitable for measuring taste-related brain responses.

For illustrative purposes the typical study setup used in this thesis is depicted in Figure 1.3. Participants were positioned in a 3 Tesla MRI scanner in a supine position. Liquid stimuli were administered by means of a gustometer via small tubes that were attached to the mouth. Visual stimuli were projected on a screen at the back end of the bore and were visible for the participants via a mirror that was positioned on top of the head coil.

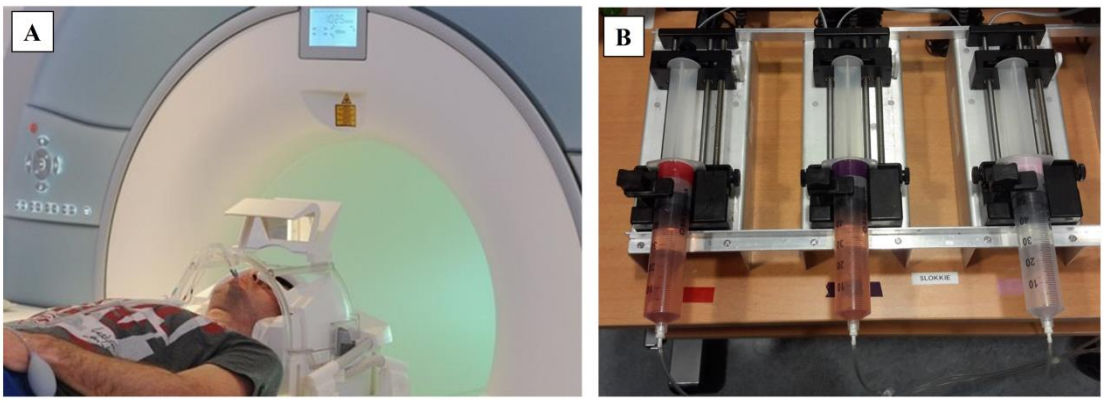

Figure 1.3 Typical fMRI study setup as used during this thesis; a participant in the scanner (A) and the gustometer (B). Copyrights of picture A belong to Wageningen UR and CAT-AgroFood, reprinted here with permission.

Aim and thesis outline

The way we experience our food is dynamic and is affected by many internal and external factors. Our brain is responsible for weighing and integrating these factors and forms the final consumption experience. Mapping the impact of all factors that influence the consumption experience is of fundamental importance for understanding why we eat the way we eat. Important drivers for food consumption are its rewarding capacity, healthiness and caloric content. In this thesis, we focus on the influencing effects of individuals reward sensitivity and health interest, and on the effect of food energy content independent of sweet taste. Furthermore, in the current supermarket environment, advertisements and food claims are omnipresent. For this reason, we assess the influence of two cognitive effects, i.e. labeling/claim effects and selective attention on the consumption experience. The overall aim of this thesis was to assess the effect of food content, cognitive effects and character on brain activation during tasting.

Specific questions addressed in this thesis are:

- Are calories sensed in the oral cavity independent of sweet taste?
- Do reward sensitivity and health interest modulate taste activation induced by beverages varying in respectively actual and perceived caloric content?
- How does selective attention to food properties modulate taste activation?

To begin with, we focus on the relation between food content and taste-related brain patterns **(Chapter 2 & 3)**. A study was performed in which brain responses to several simple solutions containing carbohydrates, artificial sweeteners or both (glucose, fructose, maltodextrin, sucralose and maltodextrin + sucralose) were obtained. With these data, we first explored whether oral exposure to caloric and non-caloric stimuli elicits discriminable brain responses (**Chapter 2**). Moreover, we assessed in how far these responses are modulated by hunger state. Secondly, in **Chapter 3**, we compared the glucose and fructose data to examine whether oral exposure of these two sugars evokes differential neural responses. Again, also the modulation by hunger state was assessed. In **Chapter 4 and 5**, the relations between individual characteristics and taste activation are explored. The data of the first study was used to explore whether trait reward sensitivity modulates brain responses to oral exposure to calories during hunger and satiety **(Chapter 4)**. In **Chapter 5**, we describe the results of a second study investigating the effect of both an individual characteristic, health interest, and a cognition, food claims. Here, the modulating effect of health interest on taste activation was assessed with a lemonade that was labeled as either low- or high-caloric. In **Chapter 6**, we report the results of a third study on the effect of selective attention to hedonics, intensity and caloric content on brain responses during tasting. Finally, in **Chapter 7**, the main findings of this thesis are discussed.

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Chapter 2

Tasting calories differentially affects brain activation during hunger and satiety

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Abstract

An important function of eating is ingesting energy. Our objectives were to assess whether oral exposure to caloric and non-caloric stimuli elicits discriminable responses in the brain and to determine in how far these responses are modulated by hunger state and sweetness. Thirty women tasted three stimuli in two motivational states (hunger and satiety) while their brain responses were measured using functional magnetic resonance imaging in a randomized crossover design. Stimuli were solutions of sucralose (sweet, no energy), maltodextrin (non-sweet, energy) and sucralose + maltodextrin (sweet, energy). We found no main effect of energy content and no interaction between energy content and sweetness. However, there was an interaction between hunger state and energy content in the median cingulate (bilaterally), ventrolateral prefrontal cortex, anterior insula and thalamus. This indicates that the anterior insula and thalamus, areas in which hunger state and taste of a stimulus are integrated, also integrate hunger state with caloric content of a taste stimulus. Furthermore, in the median cingulate and ventrolateral prefrontal cortex, tasting energy resulted in more activation during satiety compared to hunger. This finding indicates that these areas, which are known to be involved in processes that require approach and avoidance, are also involved in guiding ingestive behavior. In conclusion, our results suggest that energy sensing is a hunger state dependent process, in which the median cingulate, ventrolateral prefrontal cortex, anterior insula and thalamus play a central role by integrating hunger state with stimulus relevance.

Introduction

An important function of eating is ingesting energy. From an evolutionary perspective, the ability to sense energy in the oral cavity therefore seems useful. Possibly, oral energy sensing occurs in two ways, namely (1) the sensing of energy due to a conditioned link between sensory properties of a food and the post-ingestive consequences (indirect energy sensing) and (2) the direct binding of a caloric ligand to a receptor in the oral cavity (direct energy sensing). Nowadays, there are several serious candidate receptors (such as CD36 and GPR120), that are proposed to be involved in direct fat sensing in the oral cavity [1–4].

On the contrary, although there is some indirect evidence, there is no proposed mechanism for direct oral carbohydrate (CHO) sensing. In a traditional human diet, the taste of sweet foods is usually produced by the binding of CHOs to the sweet taste receptor (a receptor to which also other compounds such as artificial sweeteners bind [5]). Therefore, there is a strong learned association between sweet taste and energy from CHO. This makes it very difficult to distinguish between direct CHO sensing and indirect sensing through sweet taste in the oral cavity. At the same time, our diet includes many common starch-rich foods like potatoes and rice, which are high in CHO but do not taste sweet. These could be used to investigate the coupling between sweetness and energy. To our knowledge, however, no functional magnetic resonance imaging (fMRI) studies on oral CHO sensing have used this approach.

In mice, the coupling between sweetness and CHO can be bypassed by knocking out the sweet taste receptor [6–8]. Damak et al. (2003) [6] and Zhao et al. (2003) [7] showed that knockout mice lose their preference for artificial sweeteners, but partly retain their preference for glucose and sucrose. In line with this, both studies demonstrated that application of the taste stimuli on the tongue, resulted in near zero gustatory nerve responses for artificial sweeteners and diminished or normal responses for the CHOs. These findings point to the existence of direct CHO sensing in the absence of sweet taste transduction.

Earlier research speculated that rodents have a maltodextrin receptor in the oral cavity, based on their avid ingestive response of a maltodextrin solution compared to solutions of different mono/disaccharides [9,10]. Whereas in rodents, maltodextrin has a salient and pleasant taste, in humans it appears to be tasteless [11]. However, in humans, behavioral studies also substantiate the existence of an unidentified CHO receptor which facilitates direct oral CHO sensing [12,13]. Mouth rinsing with a sweet CHO solution, but not with a sweet non-caloric solution, improved exercise performance [13,14]. For example, mouth rinsing with a solution containing sucrose and glucose compared to a placebo

solution containing aspartame, resulted in shorter time to complete a standard cycle trial [15]. Recently, a similar paradigm was tested in a neuroimaging setting and provided additional evidence for this phenomenon by showing larger CHO induced changes in the primary sensorimotor cortex compared to an equisweet non-caloric placebo when contrasted with a control solution [16]. The above results suggest that humans have an oral maltodextrin receptor similar to that of rodents.

Neuroimaging research has corroborated this hypothesis by demonstrating differences in taste activation between caloric and non-caloric solutions which were matched on sweetness [12,17–19]. For instance, Frank et al. (2008) [17] showed that primary taste areas (the anterior insula and frontal operculum) as well as frontal regions (prefrontal cortex) and regions involved in reward (striatum and anterior cingulate cortex (ACC)) responded stronger to tasting a sucrose than to tasting an equisweet sucralose solution. Similarly, Chambers et al. (2009) [12] looked at glucose and saccharin (non-caloric) and found that oral glucose, but not oral saccharin, activated the striatum and the ACC. In addition, others compared caloric and non-caloric soft drinks, sweetened with either sucrose and sucralose or a mixture of artificial sweeteners, and reported divergent activation in areas such as the amygdala, median cingulate, precentral gyrus, rolandic operculum and thalamus [18,19].

Physiological responses to oral energy, including brain activation, can be modulated by hunger state. That is, mouth rinsing with CHO in fed state did not improve exercise performance [20]. Furthermore, Smeets et al. (2011) [18] found striatal activation before, but not after consumption of 450 mL of caloric orangeade, during tasting of small sips of this same caloric orangeade. Tasting a non-caloric orangeade elicited no activation in this area, neither before nor after consumption of non-caloric orangeade. In contrast, in a study of Haase et al. (2009) [21], brain areas in which activation was greater during hunger compared to satiety in response to tasting a caloric (sucrose) and non-caloric (saccharin) stimulus partly overlapped; activation was significantly greater in the thalamus and hippocampus. Additionally, they demonstrated that during hunger, regions involved in salience (amygdala), memory (hippocampus) and maintaining energy balance (hypothalamus) were more activated than regions involved in tasting (primary and secondary taste regions such as the insula and inferior orbitofrontal cortex). During satiety, this was the other way around.

In conclusion, there is a lack of consensus about the existence of direct CHO sensing in the oral cavity in humans, the brain areas involved in this process and the modulation of energy sensing by hunger and sweetness. In the current study we intend to strengthen the evidence for direct oral CHO sensing in humans. Our main objective was to assess whether oral exposure to caloric and non-caloric stimuli elicits discriminable responses in the brain. In addition, we aimed to determine in how far these responses are modulated by hunger state and sweetness. To be able to distinguish the effect of energy from sweetness we included a stimulus containing only sweetness (sucralose solution), a stimulus

containing only energy (maltodextrin solution) and a stimulus combining both (sucralose + maltodextrin solution). We hypothesize that oral exposure to caloric and non-caloric stimuli elicits differential responses in regions involved in salience (amygdala), memory (hippocampus), energy balance (hypothalamus), tasting (insula, frontal operculum and inferior orbitofrontal cortex) and reward (striatum and ACC). We expect that these differences are more pronounced during hunger compared to satiety and when sweetness and energy are combined.

Materials and Methods

Participants

We recruited healthy, normal-weight (BMI between 18.5-25 kg/m^2), right handed female participants (age between 18-35 y), who consumed artificially sweetened beverages at least two times per month. Only women were included because structural [22] and functional [22–24] brain differences exist between both sexes. Exclusion criteria were: a restrained eating score higher than 2.80 (Dutch Eating Behavior Questionnaire [25]), an energy restricted diet during the past two months, change in body weight of more than five kg during the past two months, lack of appetite, stomach or bowel diseases, diabetes, thyroid disease or any other endocrine disorder, having a history of neurological disorders, use of daily medication other than oral contraceptives or paracetamol, having difficulties with swallowing and/or eating, having taste or smell disorders, being allergic and/or intolerant for products under study, smoking more than one cigarette/cigar a day, having a history of or current alcohol consumption of more than 28 units per week, exclusive consumption or avoidance of light versions of beverages, being pregnant or lactating or having any contra-indication for MRI scanning. Before enrollment, participants were screened on inclusion and exclusion criteria via a questionnaire and completed an fMRI training session in which they were familiarized with the fMRI procedure. Of the 31 enrolled participants, one dropped out during the first scan session (hunger session) because of nausea. Thirty female participants with a mean±SD age of 22±3 y and a BMI of 22.6±1.4 kg/m^2 completed the study. Participants were on average at the same point in their menstrual cycle during both the hunger (mean±SD = 9±11 days) and satiety session (mean±SD = 10±9 days). This precludes biases in brain activation due to menstrual cycle phase, as sometimes seen in literature [26–28]. All participants gave written informed consent. This study was conducted according to the principles of the Declaration of Helsinki, approved by the Medical Ethical Committee of Wageningen University and registered in the Dutch Trial Register (NTR 3749).

Study design

The study had a randomized crossover design in which participants were scanned on two occasions, once during hunger and once during satiety. During the two scan sessions participants tasted fixed amounts of a control stimulus (water) and five stimuli that contained either CHOs, artificial sweeteners or both (sucralose, maltodextrin, maltodextrin + sucralose, glucose and fructose solutions), while their brain responses were measured using functional MRI. Here we analyze the responses to three of these stimuli, namely, a non sweet caloric (maltodextrin), a sweet non-caloric (sucralose) and a sweet caloric (sucralose + maltodextrin) solution. Subjective scores of hunger, liking, wanting, sweetness and viscosity for each stimulus were given during the fMRI training session (viscosity) or scan sessions.

Stimuli

Stimuli varied in sweetness and energy content and were a non sweet caloric, a sweet non-caloric and a sweet caloric solution; made by dissolving respectively maltodextrin (158.2 g Nutricia Fantomalt (90% polysaccharides - DE 19, 6% mono/disacharides) per liter, 2541 kJ / 607 kcal per liter), sucralose (Brenntag specialties, 0.254 g SPLENDA® Sucralose per liter, zero kJ / zero kcal per liter) and maltodextrin + sucralose (158.2 g Nutricia Fantomalt (90% polysaccharides - DE 19, 6% mono/disacharides) + 0.140 g SPLENDA® Sucralose per liter, 2541 kJ / 607 kcal per liter) in demineralized water. In addition, demineralized water was used as a control stimulus to be able to subtract out all general taste effects, such as the sensation of a fluid in the mouth, and tongue movements. Both sweet stimuli were equisweet, with a sweetness intensity comparable to that of a 10% sucrose solution. Concentrations were established in a pilot study using the method of constant stimuli (n=10). Both energy containing stimuli were isocaloric.

Prior to testing, sweetness intensity of the stimuli was scored by a trained sensory panel (n=12, Essensor B.V., Ede, The Netherlands) (Supplementary Figure 2.1, Appendix). As judged by this panel, sweetness of the sucralose + maltodextrin (mean±SEM = 68.9±4.0 mm) and sucralose (mean±SEM = 66.6±4.2 mm) solutions was equal. Sweetness of the non sweet stimulus, maltodextrin (mean±SEM = 21.2±1.9 mm), was rated significantly lower than sweetness of the sweet stimuli (P<0.0001).

Experimental procedure

Participants arrived between 10:25 h and 14:00 h at the test location (Hospital Gelderse Vallei, Ede) after a fast of at least 3 h (no food, only water). Participants were instructed to eat a small self-chosen breakfast, prior to the 3 h fast. During the hunger session participants started with tasting 5-10 mL of each stimulus and rating it on liking, wanting and sweetness, followed by rating appetite (hunger, fullness, desire to eat and prospective consumption) and thirst (Figure 2.1). Rating was done on a 9-point scale. Hereafter participants were placed in the MRI scanner and scanned while tasting the stimuli several times. After scanning, participants again rated their appetite and thirst and tasted and rated the stimuli on liking, wanting and sweetness. During the satiety session participants started with subjective appetite ratings and an ad libitum lunch consisting of bread rolls (1063 kJ / 254 kcal per 100 g), full fat cheese (1570 kJ / 375 kcal per 100 g), boiled eggs (645 kJ / 154 kcal per 100 g), butter (1549 / 370 kcal per 100 g), sandwichspread (984 kJ / 235 kcal per 100 g), cucumber, tomato, orange juice (167 kJ / 40 kcal per 100 g) and skimmed milk (197 kJ / 47 kcal per 100 g). Participants were instructed to eat until comfortably full. After lunch, the same order of events was followed as during the hunger session (Figure 2.1).

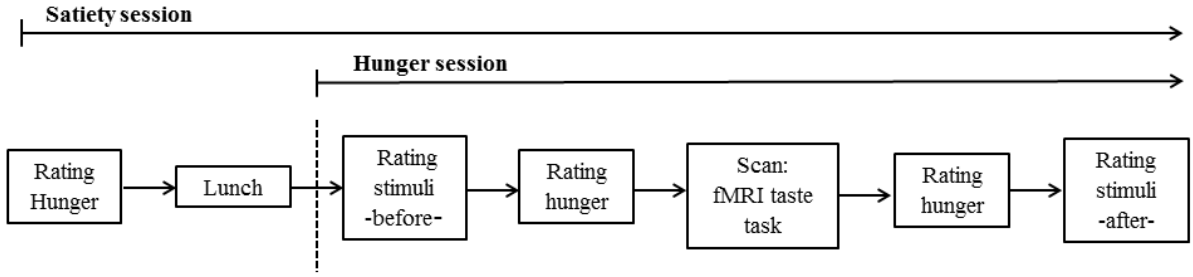

Figure 2.1 Schematic overview of the satiety and hunger session.

Scanning procedure

A scan session consisted of 3 functional runs during which 300 functional volumes were acquired using a T_2*-weighted gradient echoplanar imaging sequence (TR=2140 ms, TE=25 ms, 90° flip angle, FOV=192x192 mm, 43 axial slices, descending order, voxel size 3×3×3 mm) on a 3-Tesla Siemens Magnetom Verio (Siemens, Erlangen, Germany). The stack was tilted at an oblique angle of 30° to the anterior-posterior commissure line to reduce signal dropout in orbitofrontal cortex and ventral temporal lobe [29]. Additionally, a high-resolution T_1-weighted anatomical scan was acquired (MPRAGE, TR=2300 ms, TE=2.98 ms, 9° flip angle, FOV=256×256 mm, 192 sagittal slices, voxel size=1×1×1 mm).

In Figure 2.2, a schematic overview of the trial structures in a functional run can be found. During each functional run every stimulus was tasted 4 times, resulting in a total of 12 taste trials per stimulus per scan session. Stimuli were offered as 2 mL sips in a semi-random order. Each taste event (11 s) was followed by a 3-s swallow, a 4-s rinse with water, a 3-s swallow and a 3 to 5-s rest (one trial). During each functional run, participants rated liking once for every stimulus on a 9-point scale. Liking ratings were given directly after swallowing the taste stimuli. Instructions to either taste, swallow, rate, rinse, or rest were given to participants via visual cues on a screen placed in the bore at the back end of the scanner. Stimuli were administered with the use of programmable syringe pumps (New Era Pump Systems Inc,Wantagh, NY) at 50 mL/min.

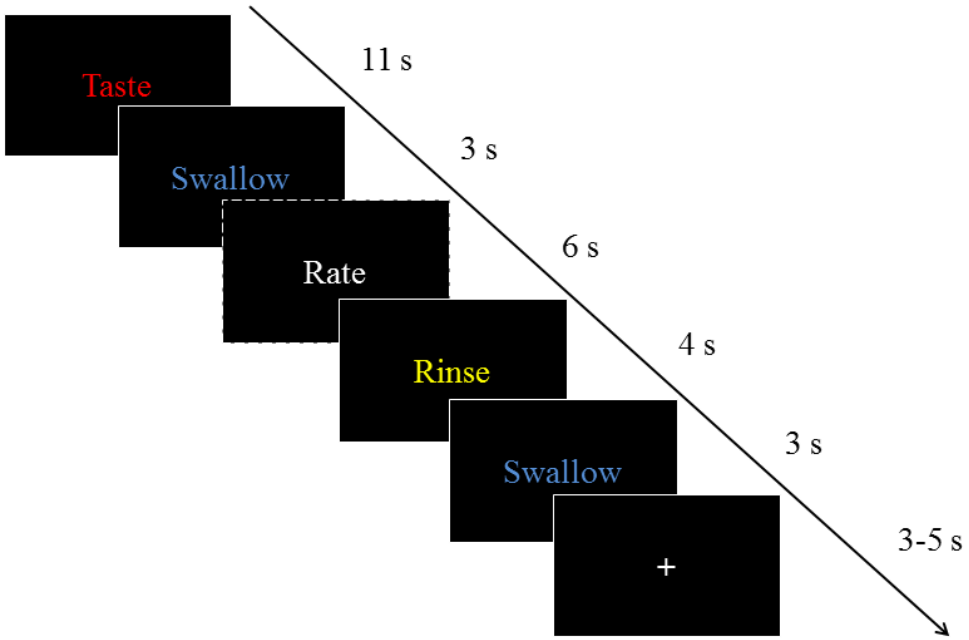

Figure 2.2 Schematic overview of trial structures in a functional run.

Analysis

fMRI data were preprocessed and analyzed with the SPM8 software package (Wellcome Department of Imaging Neuroscience, London, UK) in conjunction with the MarsBar toolbox (http://marsbar.sourceforge.net/) run with MATLAB 7.12 (The Mathworks Inc, Natick, MA).

The functional volumes of every participant were slice time corrected, realigned to the first volume of the first run, coregistered to the anatomical image, globally normalized to the Montreal Neurological Institute space (MNI space), and spatially smoothed with a Gaussian kernel of 6 mm full-width at half-maximum. A statistical parametric map was generated for every participant by fitting a boxcar function to each time series, convolved with the canonical hemodynamic response function. Data were high-pass filtered with a cutoff of 128 s. Nine conditions were modeled: delivery of sucralose,

maltodextrin, maltodextrin + sucralose, glucose, fructose and water, swallowing, rinsing and stimulus rating. Responses to swallowing, rinsing and rating were not included in further analyses and responses to glucose and fructose are not under study in the current analyses. To account for motion-related variance, realignment parameters were added to the model as regressors. For every participant, parameters were estimated for every tastant minus control (water) administration using a T-test for both the hunger and the satiety session.

For the group analyses we used a functional region of interest (fROI) approach in which a priori anatomical regions of interest are combined with clusters of activation resulting from an F-test testing for any effect of oral exposure to the stimuli, to form fROIs. This approach avoids problems of circularity and has the advantage of allowing more complex analyses than is possible with voxelwise analysis of variance as implemented in fMRI analyses packages, see e.g. [19,30–32]. A priori anatomical regions of interest were selected from literature and included regions involved in reward, salience, memory, energy balance and tasting or that were found to be modulated by hunger state: Striatum (caudate nucleus, putamen and pallidum), amygdala, orbitofrontal cortex, frontal gyri, opercula, insula, cingulate gyri, ventral tegmental area, hypothalamus, thalamus, parahippocampal gyri, hippocampus, fusiform gyri, pre and postcentral gyri, temporal gyri and parietal gyri. Individual masks of these a priori anatomical regions of interest were dilated with one voxel to accommodate between subject variability and used to create a bundled anatomical mask with the use of the WFU Pickatlas [33]. A whole brain statistical F-map was created by performing an ANOVA with stimuli (maltodextrin, sucralose and maltodextrin + sucralose) and hunger state (hunger and satiety) as independent variables. This F-map was masked with the bundled anatomical mask and thresholded at a significance level of $P<0.001$ (uncorrected for multiple comparisons) and a cluster size of $k>8$ contiguous voxels.

FROIs were formed by multiplying the clusters in the F-map with the individual anatomical masks. The average beta values of all voxels within each identified fROIs were extracted and analyzed in two separate MANOVAs (GLM) in SAS 9.3: one with the independent variables hunger state and energy content and the other with independent variables hunger state and stimulus. This allowed us to look at the effect of energy content and to the effect of adding sweetness to energy (by looking at the separate stimuli) during hunger and satiety. Liking (obtained during scanning),viscosity and sweetness ratings were added as covariates to the models.

Table 2.1 Identified fROIs and results of MANOVA with liking, viscosity (and sweetness) as covariates of mean parameter estimates in each fROI for tasting caloric and non-caloric stimuli during hunger and satiety.

fROI	Peak voxel coordinate of fROI			Main effect energy[a]	Energy x hunger[a]	Main effect stimulus[b]
	x	y	z	p-value	p-value	p-value
Frontal cortex						
L superior frontal gyrus, medial	-9	56	4	0.13	0.40	**0.03**
R superior frontal gyrus, medial	15	68	13	0.06	0.71	0.07
L superior frontal gyrus, dorsolateral	-15	-1	70	0.81	**0.03**	0.38
L inferior frontal gyrus (triangular part)	-39	11	25	0.83	**<0.01**	0.09
R inferior frontal gyrus (triangular part)	36	29	28	0.35	**<0.05**	0.21
R inferior frontal gyrus (opercular part)	42	14	28	0.55	0.07	**0.04**
L middle frontal gyrus	-33	29	31	0.71	0.08	0.65
R middle frontal gyrus	36	5	55	0.32	**0.03**	0.25
Orbitofrontal cortex						
L medial frontal gyrus, orbital part	-15	65	-2	0.17	0.62	**0.04**
L inferior frontal gyrus, orbital part	-42	44	-2	0.33	0.08	0.20
R inferior frontal gyrus, orbital part[c]	48	20	-5	0.62	**0.04**	0.67
R inferior frontal gyrus, orbital part[c]	51	44	-11	0.96	0.13	0.67
R middle frontal gyrus, orbital part	39	53	-8	0.71	0.21	0.91
Thalamus						
L thalamus	-15	-22	7	0.30	0.07	0.82
R thalamus	15	-25	7	0.52	**<0.05**	0.83
Cingulate gyrus						
L anterior cingulate	-3	35	7	1.00	0.10	0.49
L median cingulate	-9	-37	55	0.93	**0.02**	0.65
R median cingulate	6	-13	46	0.65	**0.02**	0.68
Insula						
R insula[c]	39	23	-2	0.22	**0.04**	0.42
R insula[c]	42	5	-11	0.80	0.79	0.71
Pre and postcentral gyrus						
L postcentral gyrus[c]	-33	-34	61	0.20	**0.04**	0.14
L postcentral gyrus[c]	-63	-7	25	0.67	0.48	0.83
R postcentral gyrus	42	-25	52	0.86	**<0.01**	0.39
L precentral gyrus[c]	-33	-1	64	0.99	**<0.01**	0.10
L precentral gyrus[c]	-21	-25	61	0.53	**0.02**	0.09
Fusiform gyrus						
L fusiform gyrus	-48	-58	-17	0.95	**<0.01**	0.99
R fusiform gyrus	36	-76	-17	0.91	**<0.01**	0.92
Parietal gyrus						
L inferior parietal gyrus	-30	-55	46	0.44	**0.03**	**0.03**
R inferior parietal gyrus	36	-49	49	0.24	0.07	0.08
L superior parietal gyrus	-24	-40	64	0.37	0.06	0.12
Temporal gyrus						
L inferior temporal gyrus	-54	-52	-8	0.82	**0.02**	0.77

L superior temporal gyrus	-57	-1	-2	0.28	**<0.01**	0.60
R middle temporal gyrus [c]	57	-46	13	0.99	**<0.01**	0.46
R middle temporal gyrus [c]	60	-43	-2	0.69	0.10	0.45

[a]Results from MANOVA with liking, viscosity and sweetness as covariates.
[b]Results from MANOVA with liking and viscosity as covariates.
[c]Two identified fROIs in one anatomical ROI.

Results

Identified functional regions of interest

All identified fROIs can be found in Table 2.1. Some of the hypothesized regions, such as the amygdala and caudate nucleus, were not identified as fROIs. This means that in these areas none of the stimuli differed from the control condition (water).

Energy sensing

There was no main effect of energy content in any of the fROIs (Table 2.1). For illustrative purposes, taste activation for caloric and non-caloric stimuli in an fROI in the right insula is shown (Figure 2.3).

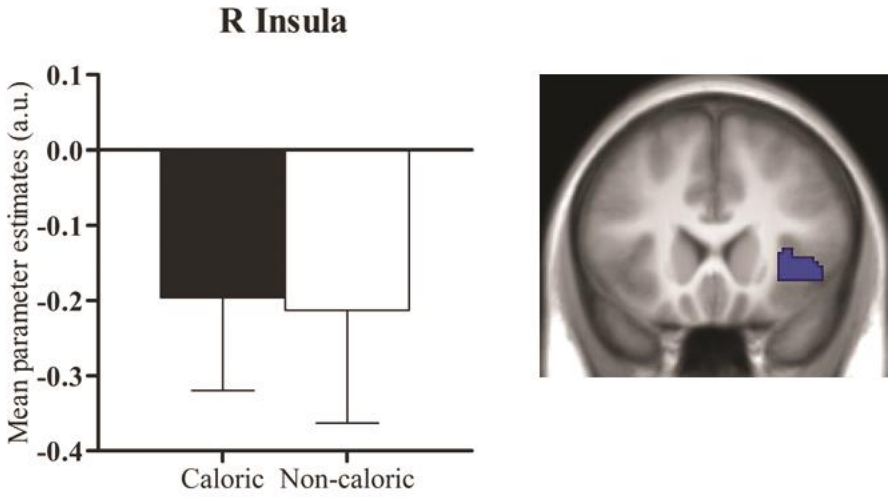

Figure 2.3 Mean ± SEM parameter estimates (arbitrary units) in comparison to the control condition after tasting caloric and non-caloric stimuli in the right insula (MNI: 39, 23, -2).

Effect of sweetness

To determine the effects of energy content, sweetness and their combination, we compared taste activation induced by maltodextrin, sucralose and maltodextrin + sucralose (when liking and viscosity ratings were added to the model). We found a main effect of stimulus type in the left inferior parietal

gyrus, the left superior frontal gyrus (medial part), the left medial frontal gyrus (orbital part) and the right inferior frontal gyrus (opercular part) (P<0.05) (Table 2.1 and Figure 2.4). In all these areas, tasting maltodextrin resulted in significantly different activation than tasting maltodextrin + sucralose (P<0.05). Taste activation did not interact with hunger state in the above mentioned areas. Sucralose and maltodextrin + sucralose activation did not differ significantly in any fROI. In addition, when sweetness was added to the model as extra an covariate, the main effect of stimulus type in the above mentioned areas disappeared (Supplementary Table 2.1, Appendix).

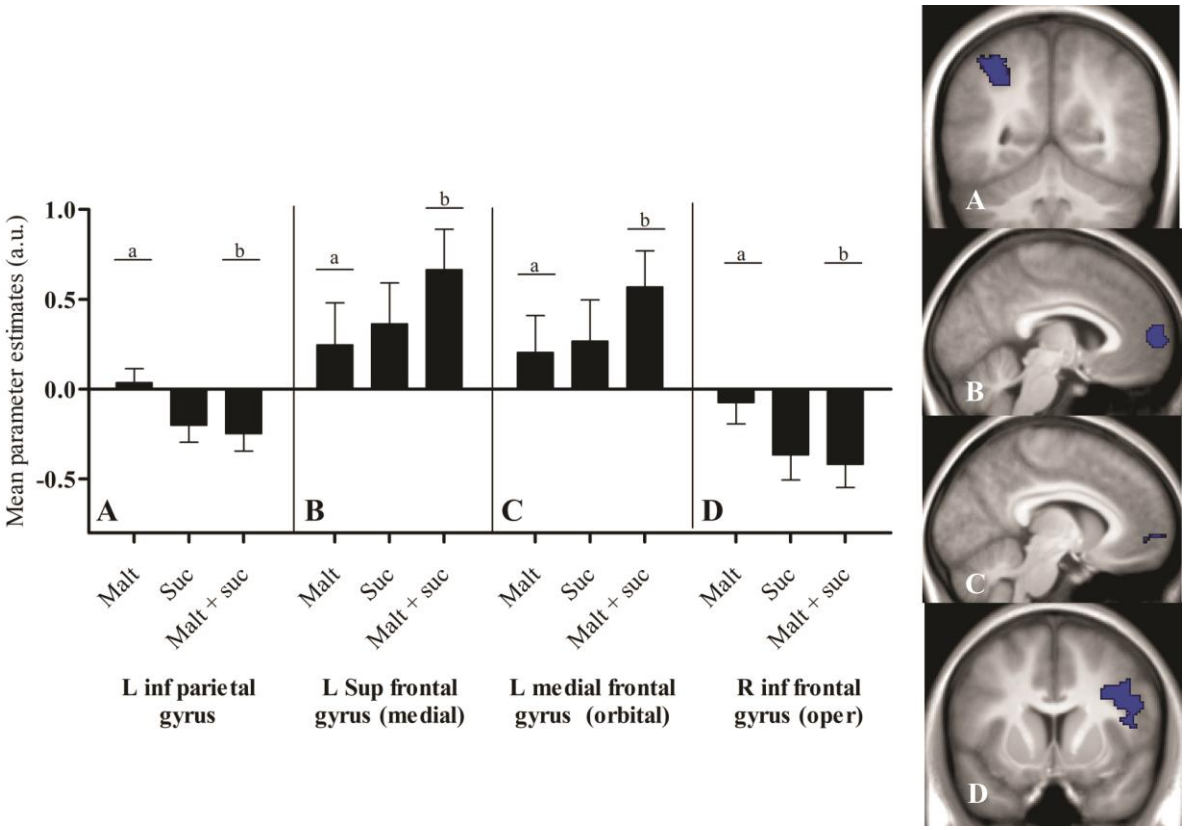

Figure 2.4 Mean ± SEM parameter estimates (arbitrary units) in comparison to the control condition after tasting maltodextrin, sucralose and maltodextrin + sucralose solutions in fROIs that showed a main effect of stimulus (P<0.05): the left inferior parietal gyrus (A), left superior frontal gyrus (medial part) (B), left medial frontal gyrus (orbital part) (C) and the right inferior frontal gyrus (opercular part) (D).

Interaction between energy content and hunger state

As previously mentioned, there was no main effect of energy content. However, there was an interaction between hunger state and energy content in the median cingulate (bilaterally), fusiform gyrus (bilaterally), pre and postcentral gyrus, right thalamus, right insula, and parts of the (orbito)frontal, parietal and temporal gyrus (P<0.05) (Table 2.1 and Supplementary Table 2.2 (Appendix)). The main effect of hunger can be found in Supplementary Table 2.3 (Appendix).

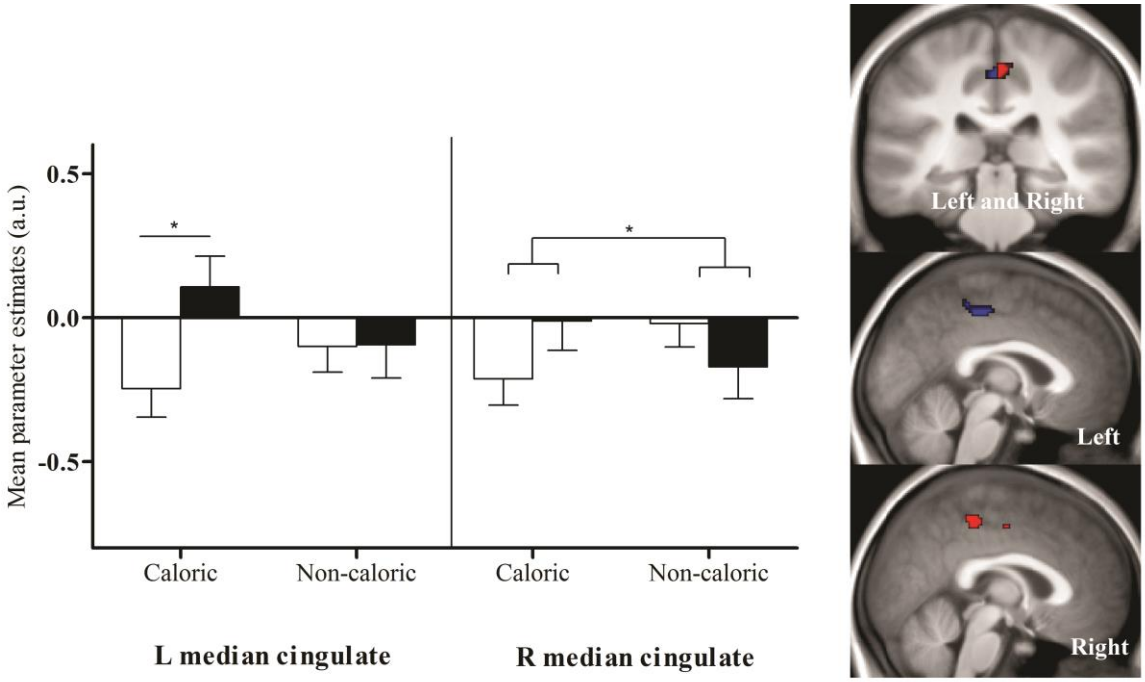

Figure 2.5 Mean ± SEM parameter estimates (arbitrary units) in comparison to the control condition after tasting caloric and non-caloric stimuli during hunger (□) and satiety (■), in the left (blue) and right (red) median cingulate.

In both the left and right median cingulate, tasting energy during satiety was associated with greater activation than tasting energy during hunger. This interaction was significant in the left median cingulate (P<0.01) (Figure 2.5). For the non-caloric stimulus there were no significant differences between hunger and satiety in both the left and right median cingulate. In addition, in the right median cingulate, the difference in activation between hunger and satiety was significantly greater when tasting calories compared to no calories. In both the left and right inferior frontal gyrus (triangular part), tasting calories during satiety resulted in more activation than tasting calories during hunger. For the non-caloric condition, however, this was the other way around (Figure 2.6). In the right insula, tasting the non-caloric stimulus induced more activation during hunger than during satiety (Figure 2.7). Taste activation by calories was not significantly different between hunger and satiety in the right insula. Finally, taste activation in the right thalamus showed a different pattern. Here, tasting calories resulted in more activation during satiety compared to hunger (Figure 2.8). For the non-caloric condition there was no difference between hunger and satiety.

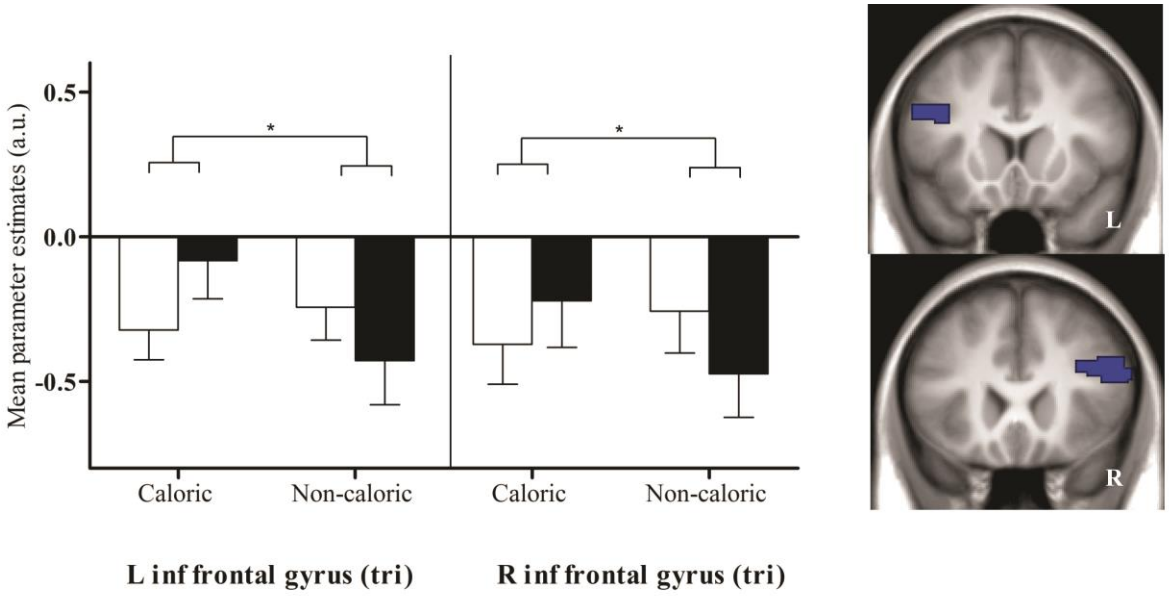

Figure 2.6 Mean ± SEM parameter estimates (arbitrary units) in comparison to the control condition after tasting caloric and non-caloric solutions during hunger (☐) and satiety (**■**), in the left and right inferior frontal gyrus (triangular part).

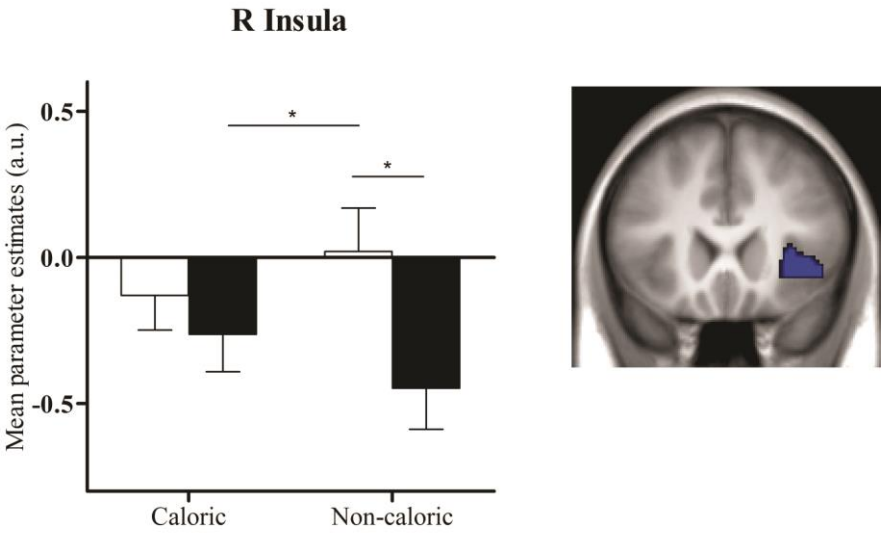

Figure 2.7 Mean ± SEM parameter estimates (arbitrary units) in comparison to the control condition after tasting caloric and non-caloric solutions during hunger (☐) and satiety (**■**) in the right insula.

Stimulus characteristics

The ratings for sweetness, wanting, liking and viscosity measured inside (during scanning) and outside the scanner can be found in Table 2.2. In line with the sensory panel ratings (see section 'Stimuli'), sweetness ratings were lowest for water, followed by maltodextrin and finally by sucralose

and maltodextrin + sucralose. The latter two did not differ significantly in sweetness. Liking ratings, measured outside the scanner, were not significantly different for maltodextrin, sucralose and maltodextrin + sucralose. During scanning, maltodextrin was liked significantly more in comparison to the other stimuli (except for water). Finally, viscosity of the maltodextrin, sucralose and maltodextrin + sucralose solutions did not differ significantly. Water was rated significantly thinner than maltodextrin + sucralose.

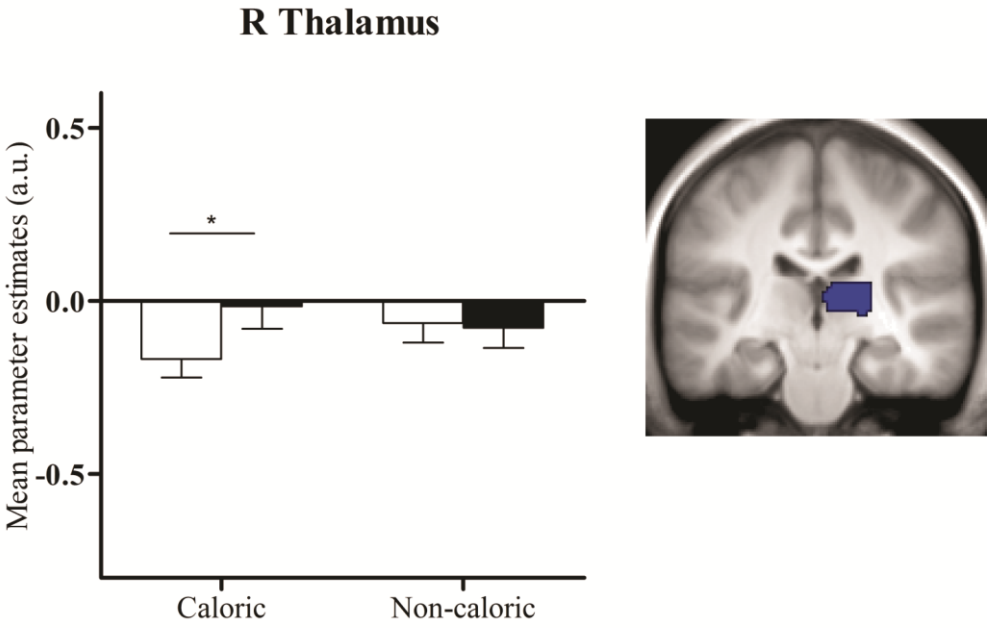

Figure 2.8 Mean ± SEM parameter estimates (arbitrary units) in comparison to the control condition after tasting caloric and non-caloric solutions during hunger (□) and satiety (**■**) in the right thalamus.

Food intake and hunger

During the ad libitum lunch, participants ate on average mean±SD 585.0±160.5 kcal. The average appetite ratings for the hunger and satiety session can be found in Table 2.3. Average hunger ratings before the hunger scan were significantly greater than those before the satiety scan ($P<0.001$). This demonstrates that the hunger-satiety manipulation worked.

Table 2.2 Mean (SEM) sweetness, wanting, liking and viscosity ratings of water, maltodextrin, sucralose and maltodextrin + sucralose, obtained outside and inside (liking only) the scanner.

	Water	Maltodextrin	Sucralose	Malt + Suc
Sweetness	1.5 (0.1) [(a)]	3.2 (0.3) [(b)]	6.8 (0.3) [(c)]	6.5 (0.3) [(c)]
Wanting	5.4 (0.4) [(a)]	3.3 (0.4) [(b)]	3.0 (0.3) [(b)]	2.7 (0.3) [(b)]
Liking	6.1 (0.4) [(a)]	3.6 (0.4) [(b)]	3.4 (0.4) [(b)]	2.9 (0.3) [(b)]
Liking during scan	5.8 (0.3) [(a)]	5.7 (0.3) [(a)]	4.1 (0.3) [(b)]	4.3 (0.3) [(b)]
Viscosity	1.7 (0.2) [(a)]	1.9 (0.2) [(a b)]	2.5 (0.3) [(a b)]	2.7 (0.3) [(b)]

Entries within a row with different superscript letters differ significantly from each other (one-way-anova, Tukey's HSD test, $P<0.05$).

Discussion

We investigated whether oral exposure to caloric and non-caloric stimuli elicits discriminable responses in the brain, and in how far these responses are modulated by hunger state and sweetness.

In general (averaged over hunger and satiety), taste activation did not differ between caloric and non-caloric stimuli. Furthermore, in several areas like the left inferior parietal gyrus, left superior frontal gyrus (medial part), left medial frontal gyrus (orbital part) and right inferior frontal gyrus (opercular part) maltodextrin induced significantly different taste activation than sucralose + maltodextrin did. Furthermore, taste activation by the sucralose solution (sweetness, no energy), did not differ from that by the maltodextrin + sucralose solution in which energy and sweetness were combined. Thus, there was no interaction between energy content and sweetness. However, we did observe an interaction between hunger and energy content, in which tasting energy during satiety resulted in more activation than tasting energy during hunger in, among other regions, the median cingulate, inferior frontal gyrus (triangular part), anterior insula and thalamus.

Energy sensing and the effect of sweetness

Overall (averaged over hunger and satiety), we found no difference in activation between tasting caloric and non-caloric stimuli in any of the fROIs. In hypothesized brain regions associated with reward and salience, such as the striatum and amygdala, tasting of caloric and non-caloric stimuli elicited similar responses as the control condition (water). A possible explanation for the lack of difference in activation in these regions could be that the stimuli were not reinforcing enough due to their relatively low pleasantness. Nevertheless, in only a few areas activation by tasting sucralose + maltodextrin was significantly different from that by maltodextrin. This difference was no longer significant when sweetness was added as a covariate, indicating that the difference between these two stimuli is due to their dissimilar sweetness.

The above results are in contrast with other studies, which did find differences in taste activation between caloric and non-caloric stimuli [12,17–19]. These findings, however, are heterogeneous with regard to the identified brain regions responding differently to caloric and non-caloric tastants. Our study used a block-design, which has high statistical power and large BOLD signal change relative to baseline [34], with more participants than other above mentioned studies, and, in most cases, a comparable number of repetitions per stimulus. Inconsistencies among studies could be caused by the low reproducibility of fMRI research. Between-study variations such as the use of different (artificial) sweeteners, differences in study design and participant instructions, imaging data processing and analysis, and personality traits of participants can cause discrepancies between results [35–37]. Our results suggest that generally speaking, energy sensing does not take place in the oral cavity. However, more research is needed to substantiate this finding.

Table 2.3 Mean (SEM) appetite and thirst ratings obtained before and after the scan during the hunger and satiety session.

Measure	Hunger session	Satiety session
Before the scan		
Hunger	6.6 (0.2)[a]	1.3 (0.1)[b]
Fullness	2.5 (0.2)[a]	7.3 (0.2)[b]
Prospective consumption	6.8 (0.2)[a]	2.3 (0.2)[b]
Desire to eat	7.1 (0.2)[a]	1.8 (0.1)[b]
Thirst	4.1 (0.4)[a]	2.1 (0.3)[b]
After the scan		
Hunger	5.6 (0.3)[a]	1.4 (0.1)[b]
Fullness	4.5 (0.4)[a]	7.5 (0.3)[b]
Prospective consumption	6.1 (0.3)[a]	2.0 (0.2)[b]
Desire to eat	6.2 (0.4)[a]	1.8 (0.2)[b]
Thirst	2.9 (0.4)[a]	2.1 (0.3)[b]

Entries within a row with different superscript letters differ significantly from each other (paired T-test, $P<0.05$).

Interaction between energy content and hunger state

In the median cingulate, we found differential taste activation by energy content during hunger compared to satiety. This area has previously been associated with processing of pain [38,39] but more recently also with energy sensing [19]. In addition, Small et al. (2001) [40] found greater taste activation in the median cingulate (MNI: 8, -33, 47) when participants rated chocolate as highly pleasant or unpleasant compared to neutral. Therefore, the median cingulate is also thought to be involved in the processing of emotionally salient stimuli, regardless of their valence [40,41]. Thus, the median cingulate

seems to be involved in processing of pain and emotion, which, like ingestive behavior, require approach or avoidance behaviors, to ensure survival [39,40]. In the current study, the median cingulate responded more strongly to energy during satiety compared to during hunger. During satiety, energy is not needed and should be avoided, whereas during hunger, energy is necessary, and needs to be approached. Additionally, we found that taste activation in the median cingulate did not differ between hunger and satiety for the non-caloric stimulus, suggesting that in this case there is no inclination to either approach or avoid. Above findings indicate that the median cingulate is involved in energy sensing, and regulating approach and avoidance behaviors appropriate for hunger state.

Furthermore, we found a similar hunger effect (taste activation by calories is greater during satiety compared to hunger) in the inferior frontal gyrus (triangular part). In previous research, this areas was already found to be associated with energy sensing [17,18]. Furthermore, increasing satiety has been found to increase blood flow in the inferior frontal gyri [40]. Interestingly, the triangular part of the inferior frontal gyrus (BA 45, part of the ventrolateral prefrontal cortex [42]), is also thought to be involved in inhibition of no longer required or inappropriate responses [43–47]. Consequently, a possible explanation of our results could be that inferior frontal gyrus (triangular part) is activated more during satiety than during hunger in response to tasting energy, because the consumption of calories (the approach towards calorie rich products) is inhibited.

In addition, in the right anterior insula and right thalamus, which are important areas in the gustatory network [48], taste activation was also modulated by hunger state. Previous research already showed that hunger state modulates taste activation in the insula and thalamus [21,49]. Furthermore, both these areas have been implicated in energy sensing [17–19]. Here we show that both hunger state and caloric content are integrated in these areas.

In summary, it appears that energy content can be sensed in the oral cavity, but that this effect can only be measured when hunger state is taken into account, by comparing taste activation during hunger to taste activation during satiety.

Stimulus characteristics

A strength of this study was that sweetness was matched well for the sweet stimuli, as reflected in the sweetness ratings from both the sensory panel and the participants. Furthermore, participants liked and wanted the stimuli equally, as measured outside the scanner. On the other hand, liking for the stimuli was not equal during scanning (maltodextrin was liked more than the others). This could have influenced the differences in activation between stimuli. However, to prevent this, liking was added as a covariate to the analyses.

Next to this, we observed deactivation rather than activation relative to the control condition (water). This might be due to the fact that water was liked more than the other stimuli in this study. Furthermore, tasting water has also been found to activate portions of the insula and operculum [50,51]. Nevertheless, in the current study, using water as a control was still the best alternative (in comparison to, for example, artificial saliva), because it was also the vehicle substance for the other stimuli. In addition, our main interest was in the relative differences between the stimuli, which were independent of (de) activation.

Conclusion

To summarize, tasting caloric and non-caloric stimuli did not elicit discriminable responses in the brain, as averaged over hunger and satiety. Furthermore, tasting sweetness without energy resulted in similar responses compared to tasting sweetness with energy in all fROIs, which indicates that there is no interaction between energy content and sweetness.

At the same time, we found that tasting an energy containing liquid differentially affects brain activation during hunger and satiety in the median cingulate, ventrolateral prefrontal cortex (inferior frontal gyrus), anterior insula and thalamus. Thus, in principle, energy can be detected in the oral cavity, but we were only sensitive enough to detect this when hunger state was taken into account.

Our results show that the anterior insula and thalamus, areas in which hunger state and taste of a stimulus are integrated, also integrate hunger state with caloric content of a taste stimulus. Furthermore, our results suggest that the median cingulate and ventrolateral prefrontal cortex, areas involved in processes that require approach and avoidance such as emotion, pain and inhibition of inappropriate responses, are involved in guiding ingestive behavior by determining when to approach and avoid energy containing foods. In conclusion, energy sensing is a hunger state dependent process, in which the median cingulate, ventrolateral prefrontal cortex, anterior insula and thalamus play a central role by integrating hunger state with stimulus relevance.

Acknowledgements

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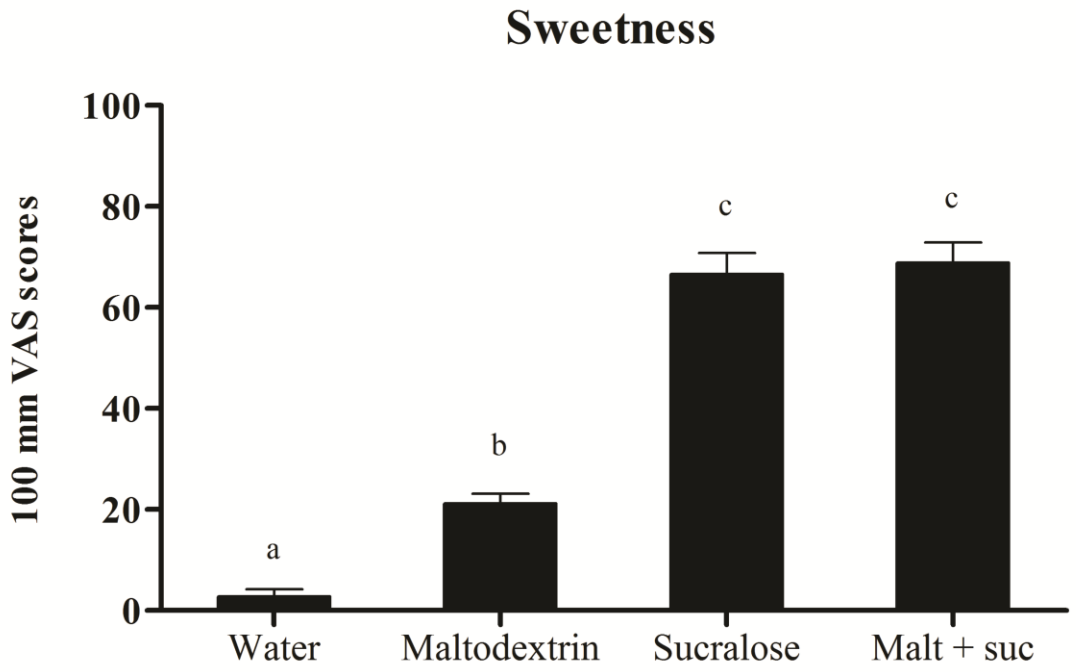
Appendix



Supplementary Figure 2.1 Mean ± SEM sweetness of the stimuli, rated by a trained sensory panel on a 100 mm VAS scale.

Supplementary Table 2.1 Identified fROIs and results of MANOVA with liking, sweetness and viscosity as covariates of mean parameter estimates in each fROI for tasting caloric and non-caloric stimuli during hunger and satiety.

fROI	Peak voxel coordinate of fROI			Main effect stimulus
	x	y	z	p-value
Frontal cortex				
L superior frontal gyrus, medial	-9	56	4	0.14
R superior frontal gyrus, medial	15	68	13	0.12
L superior frontal gyrus, dorsolateral	-15	-1	70	0.31
L inferior frontal gyrus (triangular part)	-39	11	25	0.97
R inferior frontal gyrus (triangular part)	36	29	28	0.64
R inferior frontal gyrus (opercular part)	42	14	28	0.80
L middle frontal gyrus	-33	29	31	0.92
R middle frontal gyrus	36	5	55	0.61
Orbitofrontal cortex				
L medial frontal gyrus, orbital part	-15	65	-2	0.09
L inferior frontal gyrus, orbital part	-42	44	-2	0.55
R inferior frontal gyrus, orbital part [a]	48	20	-5	0.74
R inferior frontal gyrus, orbital part[a]	51	44	-11	0.84
R middle frontal gyrus, orbital part	39	53	-8	0.88
Thalamus				
L thalamus	-15	-22	7	0.38
R thalamus	15	-25	7	0.79
Cingulate gyrus				
L anterior cingulate	-3	35	7	0.40
L median cingulate	-9	-37	55	0.89
R median cingulate	6	-13	46	0.73
Insula				
R insula[a]	39	23	-2	0.22
R insula[a]	42	5	-11	0.59
Pre and postcentral gyrus				
L postcentral gyrus[a]	-33	-34	61	0.33
L postcentral gyrus[a]	-63	-7	25	0.84
R postcentral gyrus	42	-25	52	0.56
L precentral gyrus[a]	-33	-1	64	0.23
L precentral gyrus[a]	-21	-25	61	0.40
Fusiform gyrus				
L fusiform gyrus	-48	-58	-17	0.99
R fusiform gyrus	36	-76	-17	0.62
Parietal gyrus				
L inferior parietal gyrus	-30	-55	46	0.72
R inferior parietal gyrus	36	-49	49	0.36
L superior parietal gyrus	-24	-40	64	0.64
Temporal gyrus				
L inferior temporal gyrus	-54	-52	-8	0.45
L superior temporal gyrus	-57	-1	-2	0.53
R middle temporal gyrus [a]	57	-46	13	0.38
R middle temporal gyrus [a]	60	-43	-2	0.93

[a]Two identified fROIs in one anatomical ROI.

Supplementary Table 2.2 Mean (SEM) parameter estimates in comparison to the control condition after tasting caloric and non-caloric stimuli during hunger and satiety in fROIs that showed an interaction between hunger state and energy content ($P<0.05$).

	Caloric		Non-caloric	
fROI	**Hunger**	**Satiety**	**Hunger**	**Satiety**
L fusiform gyrus	-0.34 (0.08)	0.03 (0.08)	-0.14 (0.06)	-0.10 (0.07)
R fusiform gyrus	-0.40 (0.13)	0.14 (0.17)	-0.08 (0.15)	-0.11 (0.16)
R postcentral gyrus	-0.25 (0.08)	0.10 (0.08)	-0.07 (0.09)	-0.12 (0.09)
L postcentral gyrus	-0.23 (0.08)	0.18 (0.08)	-0.07 (0.08)	0.09 (0.10)
L precentral gyrus[a]	-0.33 (0.12)	0.18 (0.11)	-0.13 (0.11)	-0.16 (0.16)
L precentral gyrus[b]	-0.29 (0.09)	0.15 (0.08)	-0.15 (0.08)	0.00 (0.10)
L superior frontal gyrus, dorsolateral	-0.45 (0.17)	0.14 (0.13)	-0.19 (0.14)	-0.16 (0.17)
L inferior frontal gyrus (triangular part)	-0.32 (0.10)	-0.08 (0.13)	-0.24 (0.11)	-0.43 (0.15)
R inferior frontal gyrus (triangular part)	-0.37 (0.14)	-0.22 (0.16)	-0.26 (0.14)	-0.47 (0.15)
R middle frontal gyrus	-0.35 (0.12)	-0.11 (0.15)	-0.18 (0.16)	-0.37 (0.14)
R inferior frontal gyrus (orbital part)	-0.28 (0.19)	-0.28 (0.18)	-0.05 (0.21)	-0.65 (0.22)
R thalamus	-0.17 (0.05)	-0.02 (0.06)	-0.06 (0.06)	-0.08 (0.06)
R insula	-0.13 (0.12)	-0.26 (0.13)	0.02 (0.15)	-0.45 (0.14)
R median cingulate	-0.21 (0.09)	-0.01 (0.10)	-0.02 (0.08)	-0.17 (0.11)
L median cingulate	-0.25 (0.10)	0.11 (0.11)	-0.10 (0.09)	-0.09 (0.12)
L inferior parietal gyrus	-0.22 (0.08)	0.01 (0.10)	-0.19 (0.08)	-0.21 (0.11)
L inferior temporal gyrus	-0.27 (0.07)	-0.02 (0.08)	-0.19 (0.06)	-0.17 (0.07)
L superior temporal gyrus	-0.21 (0.11)	-0.11 (0.12)	0.16 (0.11)	-0.27 (0.17)
R middle temporal gyrus	-0.21 (0.11)	-0.08 (0.13)	-0.09 (0.08)	-0.33 (0.15)

[a]MNI coordinates -33, -1, 64.
[b]MNI coordinates -21, -25, 61.

Supplementary Table 2.3 Results of MANOVA with liking, viscosity (and sweetness) as covariates of mean parameter estimates in each fROI for tasting caloric and non-caloric stimuli during hunger and satiety.

fROI	Peak voxel coordinate of fROI			Main effect hunger[a]	Main effect hunger[b]
	x	y	z	p-value	p-value
Frontal cortex					
L superior frontal gyrus, medial	-9	56	4	0.90	0.55
R superior frontal gyrus, medial	15	68	13	0.20	0.11
L superior frontal gyrus, dorsolateral	-15	-1	70	**0.01**	**<0.001**
L inferior frontal gyrus (triangular part)	-39	11	25	0.77	0.27
R inferior frontal gyrus (triangular part)	36	29	28	0.80	0.78
R inferior frontal gyrus (opercular part)	42	14	28	0.89	0.78
L middle frontal gyrus	-33	29	31	0.19	0.06
R middle frontal gyrus	36	5	55	0.74	0.33
Orbitofrontal cortex					
L medial frontal gyrus, orbital part	-15	65	-2	0.91	0.65
L inferior frontal gyrus, orbital part	-42	44	-2	0.10	**0.02**
R inferior frontal gyrus, orbital part[c]	48	20	-5	0.06	0.18
R inferior frontal gyrus, orbital part[c]	51	44	-11	**<0.01**	**<0.01**
R middle frontal gyrus, orbital part	39	53	-8	**0.01**	**<0.01**
Thalamus					
L thalamus	-15	-22	7	0.08	**0.02**
R thalamus	15	-25	7	0.19	**0.04**
Cingulate gyrus					
L anterior cingulate	-3	35	7	0.15	0.38
L median cingulate	-9	-37	55	**0.04**	**<0.01**
R median cingulate	6	-13	46	0.76	0.27
Insula					
R insula[c]	39	23	-2	**<0.01**	**0.01**
R insula[c]	42	5	-11	0.28	0.31
Pre and postcentral gyrus					
L postcentral gyrus[c]	-33	-34	61	**<0.001**	**<0.001**
L postcentral gyrus[c]	-63	-7	25	0.32	0.20
R postcentral gyrus	42	-25	52	**0.01**	**<0.001**
L precentral gyrus[c]	-33	-1	64	**0.01**	**<0.001**
L precentral gyrus[c]	-21	-25	61	**<0.001**	**<0.001**
Fusiform gyrus					
L fusiform gyrus	-48	-58	-17	**<0.01**	**<0.001**
R fusiform gyrus	36	-76	-17	**<0.05**	**<0.01**
Parietal gyrus					
L inferior parietal gyrus	-30	-55	46	0.09	**0.02**
R inferior parietal gyrus	36	-49	49	0.15	**<0.05**
L superior parietal gyrus	-24	-40	64	**0.04**	**<0.01**
Temporal gyrus					
L inferior temporal gyrus	-54	-52	-8	**0.02**	**<0.01**
L superior temporal gyrus	-57	-1	-2	0.07	0.37
R middle temporal gyrus[c]	57	-46	13	0.42	0.99
R middle temporal gyrus[c]	60	-43	-2	0.44	0.20

[a]Results from MANOVA with energy and hunger state as independent variables, and liking, viscosity and sweetness as covariates.

[b]Results from MANOVA with stimulus and hunger state as independent variables, and liking and viscosity as covariates.

[c]Two identified fROIs in one anatomical ROI.

Chapter 3

Glucose versus fructose: Differences in taste activation during hunger and satiety

Based on: van Rijn, I., de Graaf, C. & Smeets, P. A. M. Glucose versus fructose: Differences in taste activation during hunger and satiety (*in preparation for submission).*

Abstract

Caloric sweeteners that contain fructose are frequently used in processed food products. This may have unfavorable consequences as the ingestion of fructose relative to glucose is associated with potentially appetite enhancing effects, including decreased secretion of appetite suppressing hormones and increased brain reward responses to food cues. In contrast to these post-ingestive responses, it is unknown how brain responses differ upon direct oral exposure to these two sugars. Moreover, such taste response may be highly dependent on hunger state, which is known to modulate food induced brain response. Therefore, we aimed to investigate the differences in brain activation by oral exposure to fructose and glucose during hunger and satiety. In a randomized crossover design, brain responses of thirty female participants were measured using functional magnetic resonance imaging while they tasted a glucose and a fructose solution on two days, once during hunger and once during satiety. Oral glucose relative to fructose evoked greater activation in a food reward region (right anterior cingulate cortex) during hunger and in a region associated with food motivation (left precentral gyrus) during hunger and satiety. On the contrary, tasting fructose versus glucose resulted in increased responses only during satiety in the left superior frontal gyrus, an area involved in inhibitory control. In conclusion, these findings suggest that oral glucose is more rewarding than oral fructose and may elicit a stronger approach tendency during both hunger and satiety, possibly driven by its biological relevance. Furthermore, our findings suggest that the appetite enhancing properties of fructose compared with glucose ingestion are not apparent during oral exposure.

Introduction

The amount of fructose in our diet has increased extensively over the past decades due to the frequent use of caloric sweeteners such as sucrose (a disaccharide made up of glucose and fructose) and high fructose corn syrup [1]. Recently, the usage of fructose has become debated because of its potentially appetite enhancing and adverse properties relative to glucose (for a detailed overview see Page & Melrose (2016) [2]). First, glucose and fructose ingestion trigger different secretion patterns of satiety hormones. For example, consumption of fructose relative to glucose leads to less secretion of insulin and other appetite suppressing hormones such as GLP-1 and leptin [3–6]. Second, the majority of fructose is extracted by the liver where it stimulates lipogenesis to a greater extent than glucose and possibly contributes to an increase in circulating lipid levels in the bloodstream [4,6,7]. Finally, cerebral blood flow in regions important for energy homeostasis (hypothalamus) and reward (striatum) differs following ingestion of fructose compared to glucose [8,9]. For example, consumption of glucose, but not fructose, reduced cerebral blood flow in the hypothalamus and striatum [9]. Differences in circulating levels of insulin following fructose compared to glucose consumption may account for this by acting on neurons in the hypothalamus [8–10]. Moreover, after consumption of fructose compared to glucose, brain responses induced by food viewing were greater in, among other regions, the orbitofrontal cortex, which is known to be involved in coding food pleasantness [11,12].

Oro-sensory as well as gastric and intestinal signals are needed to achieve optimal appetite control [13,14]. While there is an abundance of literature on differential metabolic and neural effects related to the ingestion of glucose and fructose, physiological and neural responses to direct oral exposure to these two sugars have not been investigated. This latter is of particular interest because recent literature suggests that carbohydrates might be sensed in the oral cavity independent of sweet taste [15–20]. In the current study, we aimed to investigate the differences in brain activation by oral exposure to fructose compared to glucose. Furthermore, previous research indicated that food induced brain responses to taste can vary as a function of hunger state [21,22]. Therefore, we compare brain responses to oral fructose and glucose during hunger and satiety.

Materials and Methods

Data discussed in this paper were selected from a larger data-set. Full experimental details regarding the collecting of these data are described in van Rijn et al. (2015) [23]. Relevant details are outlined below.

Study design

In a randomized crossover design, brain responses of thirty young, healthy, normal-weight female participants with a mean ± SD age of 22 ± 3 y and a BMI of 22.6 ± 1.4 kg/m^2 were measured using functional magnetic resonance imaging while they tasted 2 mL sips of six solutions (water, sucralose, maltodextrin, maltodextrin + sucralose, glucose and fructose) on two days, once during hunger and once during satiety. Here, we focus on the responses to fructose and glucose. Prior to the hunger session, participants had fasted for at least 3 hours (no food, only water) after consumption of a small self-chosen breakfast. Prior the satiety session, participants were offered an ad libitum lunch. During a scan session, every solution was tasted 12 times. Furthermore, liking ratings for every solution were given 3 times per scan session on a 9-point scale. Sweetness ratings were given before and after the scan.

Stimuli

Stimuli were an isocaloric fructose and glucose solution made by dissolving respectively fructose (152.3 g Natufood fructose - Natudis B.V., Harderwijk, The Netherlands - per liter, 608 kcal per liter) and glucose (153.8 g Dextrose anhydrous - AVEBE FOOD, Veendam, The Netherlands - per liter, 608 kcal per liter) in demineralized water. Because the solutions were matched on caloric content, they were not equisweet. At the same concentration, fructose is sweeter than glucose [24].

Analysis

In the subject level analyses, nine conditions were modeled: delivery of sucralose, maltodextrin, maltodextrin + sucralose, glucose, fructose and water, swallowing, rinsing and stimulus rating. Only responses to glucose and fructose are under study and included in the current analyses. Contrast images were calculated for every participant by subtracting activation by glucose from activation by fructose for both the hunger and satiety condition. On the group level, these contrast images were entered into one sample and paired sample t-tests with the difference in liking and sweetness between fructose and glucose added as covariates, to allow a clean comparison between the stimuli. Resulting T-maps were thresholded at $P<0.001$ (uncorrected for multiple comparisons) and a cluster size of $k>5$.

Results

Liking and sweetness

Mean liking and sweetness ratings for glucose and fructose can be found in Figure 3.1 and 3.2. During both hunger and satiety, liking for glucose was higher than for fructose (significant during hunger). Furthermore, both glucose and fructose were liked significantly more during hunger compared to satiety. As expected, sweetness for fructose was significantly higher compared to glucose during both conditions.

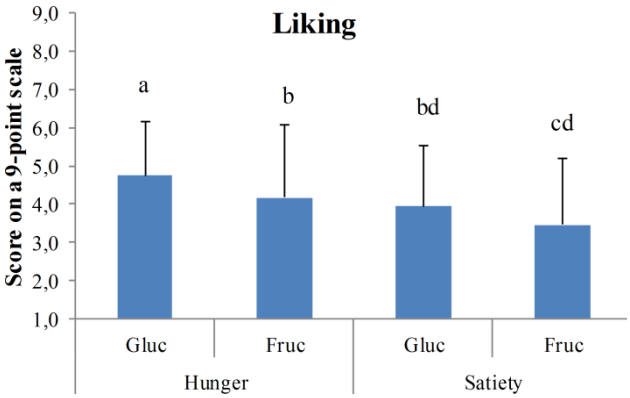


Figure 3.1 Mean (SD) liking scores for the glucose and fructose solution during hunger and satiety, obtained during scanning. Bars having a different letter differ significantly (paired sample t-tests, P<0.05).

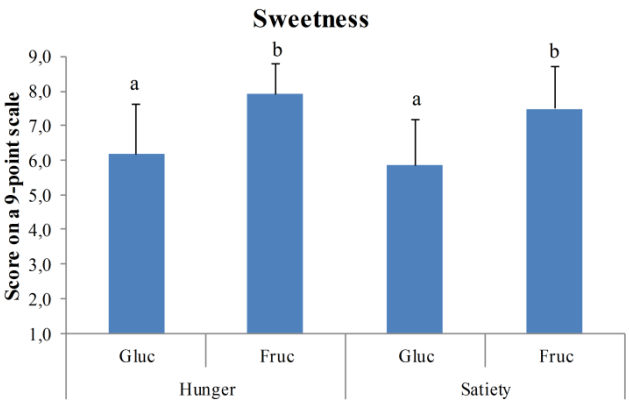


Figure 3.2 Mean (SD) sweetness scores for the glucose and fructose solution during hunger and satiety (averaged over before and after the scan). Bars having a different letter differ significantly (paired sample t-tests, P<0.05).

Taste activation

Differences in taste activation between glucose and fructose during hunger and during satiety can be found in Table 3.1. During hunger, tasting glucose compared to fructose evoked more activation in the left precentral gyrus and right anterior cingulate cortex (ACC) (Figure 3.3). During satiety, taste activation differences in the left precentral gyrus remained, but those in the ACC disappeared (Figure 3.4). Tasting fructose resulted in greater activation than glucose during satiety only in the left superior temporal pole and left superior frontal gyrus (Figure 3.4).

Table 3.1 Differences in taste activation for tasting a fructose and glucose solution during hunger and satiety.

Contrast	*Brain region*	*Cluster size*	*Z-score*	*Peak coordinate*		
				x	***y***	***z***
Hunger						
Fruc > Gluc	-					
Gluc > Fruc	L precentral gyrus	23	3.9	-60	5	22
	R ant cingulate cortex	7	3.5	9	47	28
Satiety						
Fruc > Gluc	L sup temporal pole	9	3.8	-48	14	-11
	L sup frontal gyrus	14	3.7	-27	65	7
	R cerebellum	10	3.6	15	-49	-17
	Vermis	8	3.4	3	-37	-8
	R cerebellum		3.3	12	-37	-11
Gluc > Fruc	L precentral gyrus	8	3.5	-54	2	43

Activations were thresholded at p<0.001 and a cluster extent threshold of k>5 contiguous voxels. Ant = anterior, sup = superior, = left and R = right.

The comparison of taste activation for [fructose – glucose] during hunger to [fructose – glucose] during satiety can be found in Supplementary Table 3.1 (Appendix).Taste activation for [fructose – glucose] was greater during satiety than during hunger in among others, the left superior frontal gyrus, left middle frontal gyrus and left caudate. In the left caudate and left superior frontal gyrus, brain activation evoked by oral glucose was greater than that evoked by oral fructose during hunger. However, during satiety, this was the other way around (Supplementary Figure 3.1, Appendix).

Discussion

We investigated the difference in brain activation by oral exposure to fructose compared to glucose during both hunger and satiety. We found that oral glucose relative to fructose evoked greater activation in the right ACC during hunger and the left precentral gyrus during hunger and satiety. Moreover, tasting fructose resulted in greater left superior temporal pole and left superior frontal gyrus activation than tasting glucose only during satiety.

Glucose versus fructose taste activation

As mentioned above, tasting glucose induced greater taste activation in the left precentral gyrus during both hunger and satiety than tasting fructose. The precentral gyrus is part of the primary motor cortex [25,26] and its activation is associated with preparation and readiness for voluntary movement [27]. Previously, precentral gyrus activation was found to be greater in responses to food images in obese relative to normal-weight individuals and successful weight-loss maintainers [28]. Moreover, the degree

of acute caloric deprivation (hours since last ate) correlated positively with precentral gyrus activation to palatable food images [29]. Thus, the precentral gyrus seems to be involved in coding approach tendencies (motivation) regarding food. Therefore, in the current study, the motivation to consume glucose relative to fructose may have been higher.

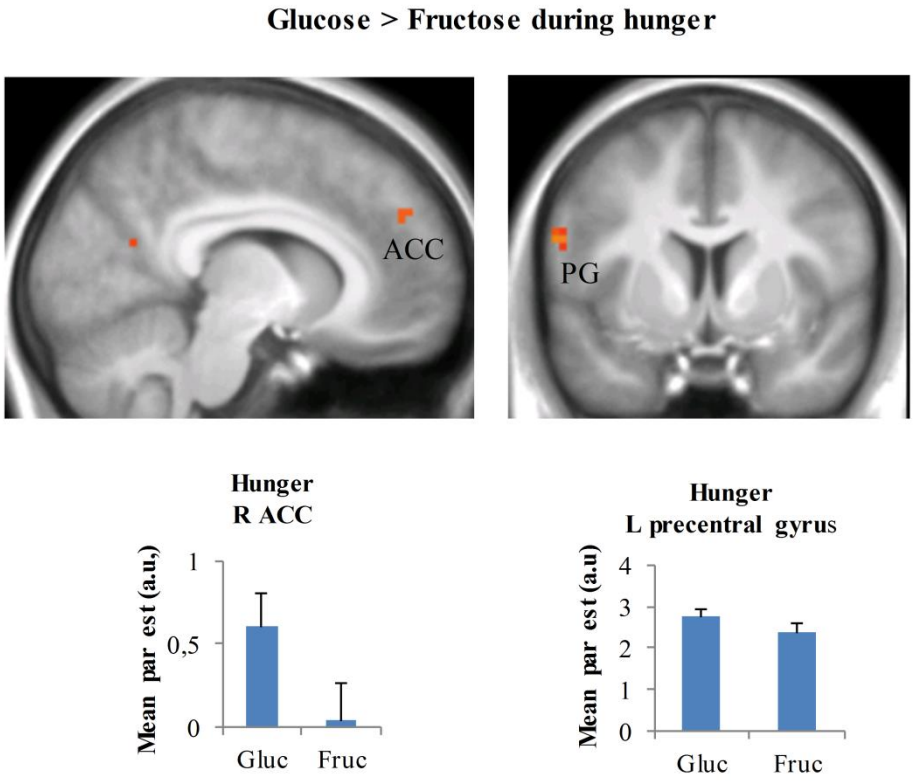


Figure 3.3 Significant brain activation during hunger for the subtraction [glucose – fructose] in the right anterior cingulate cortex (ACC, MNI (9, 47, 28)) and left precentral gyrus (PG, MNI (-60, 5, 22)).

In addition, tasting glucose relative to fructose evoked greater right ACC activation during hunger but not satiety. Previous research associated ACC activation with reward responses related to food tasting [30] and the presence of calories in the mouth [19]. In the current study, however, ACC activation does not represent pleasantness or calorie differences between glucose and fructose, since the two sugars were isocaloric and liking differences were covaried out. Instead, activation in this reward-related area may results from the fact that oral glucose is more biologically relevant than fructose. The increased approach tendency to glucose versus fructose, as suggested by the increased precentral gyrus activation, is in line with this hypothesis.

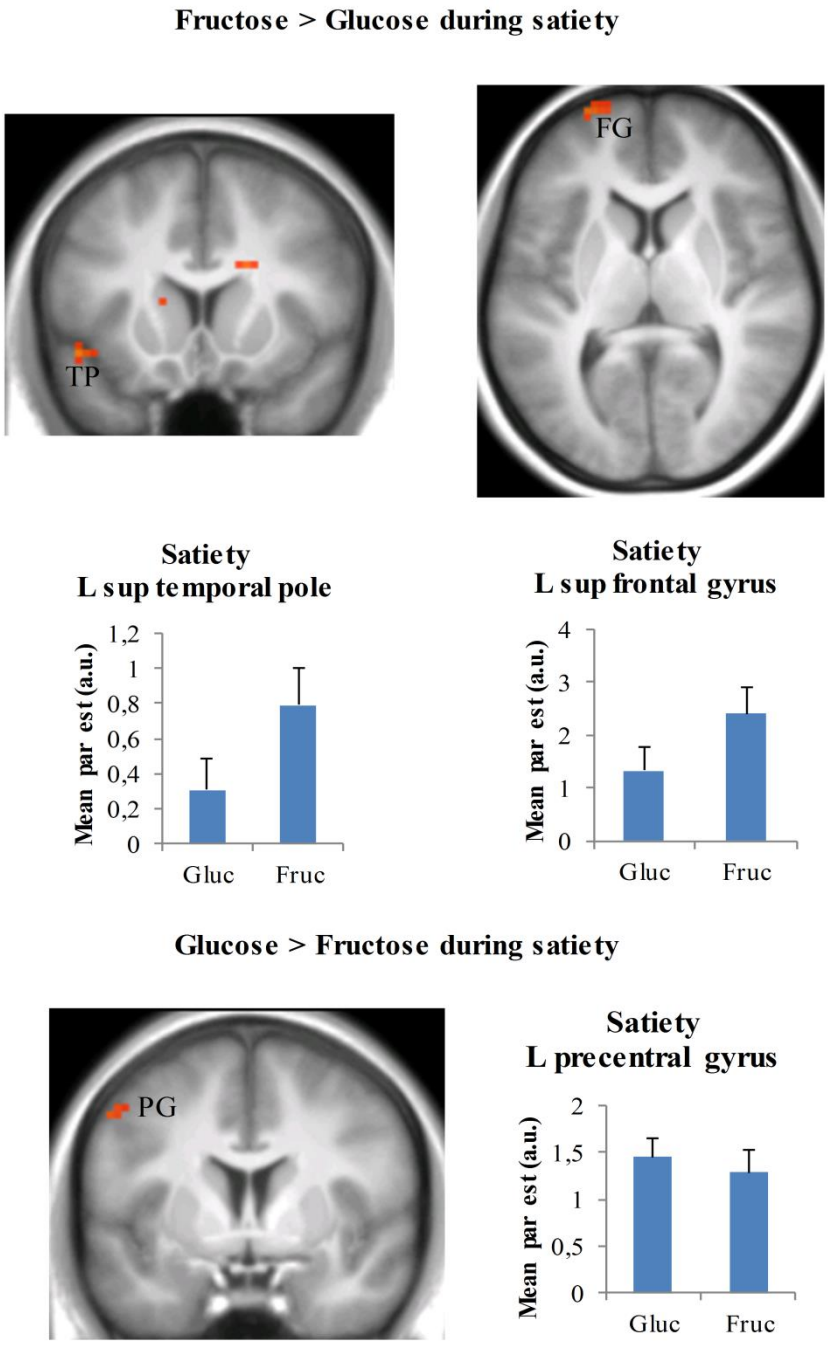


Figure 3.4 Significant brain activation during satiety for the subtraction [fructose – glucose] in the left superior temporal pole (TP, MNI (-48, 14, -11)) and left superior frontal gyrus (FG, MNI (-27, 65, 7)) and for the subtraction [glucose – fructose] in the left precentral gyrus (PG, MNI (-54, 2, 43)).

Taste activation for fructose versus glucose

During satiety, oral exposure to fructose compared to glucose induced more activation in the left superior temporal pole. The temporal pole is commonly known for its function in mapping meaning to sound [31] and its responsiveness to complex visual stimuli [32]. In contrast, little is known about the

involvement of the temporal pole in taste processing. In the macaque monkey, the temporal pole and the orbitofrontal cortex (OFC), which contains the putative secondary taste cortex, are connected [33]. Neuronal firing in the OFC in response to food cues reflects pleasantness and is modulated by hunger state [34–36]. Whether the temporal pole activation in this study is related to its connection with the OFC and what the exact meaning is of this activation needs to be established in future research.

Additionally, we observed greater left superior frontal gyrus (prefrontal cortex, Brodmann area (BA) 10) activation during satiety in response to tasting fructose compared to glucose. In previous research, the superior frontal gyrus (BA 10) was found to be more responsive to oral sucrose compared to sucralose [19]. This indicates the capability of the superior frontal gyrus to respond differently to different oral sweeteners. In other studies, satiation was associated with increased [37] as well as decreased [38] prefrontal cortex activation (respectively middle and superior frontal gyrus, BA 8), indicating its responsiveness to internal hunger state. Furthermore, greater superior frontal gyrus activation is associated with greater dietary inhibition [39–41]. For example, when trying to inhibit responses to appetizing foods in a go/no-go task, BMI correlated inversely with brain activation in the superior frontal gyrus (BA 8) [39]. Furthermore, in individuals who underwent gastric bypass surgery, a postoperative reduction in desire to eat for high- versus low-calorie food cues was associated with a postoperative reduction in neural responsivity to high- versus low-calorie food cues in the superior frontal gyrus (BA 8) [40]. Finally, successful dieters compared to non-dieters showed greater activation in the superior frontal gyrus (BA 10) in response to consumption of a satiating liquid meal relative to mere oral exposure [41]. This superior frontal gyrus activation was also found to correlate positively with participants dietary restraint score. Thus, in the current study, greater superior frontal gyrus activation during satiety may reflect a greater inhibitory control response to oral fructose compared to glucose. Possibly, this activation arises from the integration of internal hunger and oro-sensory signals such as sugar type.

Stimuli

Both a strength and a limitation of the current study are the stimuli used, i.e. simple sugar solutions. To our knowledge, we are the first to investigate the differences in taste activation between glucose and fructose in humans. Simple sugar solutions thus lend themselves well to provide a proof of principle. Furthermore, many other studies that compared the metabolic effects of fructose to glucose after ingestion, also used simple solutions (e.g. Kong et al. (1999) [3] and Page et al. (2013) [9]). The usage of similar stimuli, therefore, added the most scientific value, since all these studies together contribute to a more complete picture regarding the effect of fructose versus glucose on the human body. Nevertheless, a limitation of our stimulus choice is the lack of extrapolation to more complex

products or meals. Whether the results found in the current study also hold true for more complex products containing fructose and glucose needs to be addresses in follow-up research.

Conclusion

Oral glucose versus fructose induced greater brain responses in regions associated with food reward (right ACC) during hunger and motivation (left precentral gyrus) during both hunger and satiety. Oral fructose versus glucose responses were greater only during satiety in, among other regions, the left superior frontal gyrus, a region associated with inhibitory control. In conclusion, these findings suggest that oral glucose is more rewarding than oral fructose and may elicit a stronger approach tendency during both hunger and satiety, possibly driven by its biological relevance. Furthermore, our findings suggest that the appetite enhancing properties of fructose compared with glucose ingestion are not apparent during oral exposure.

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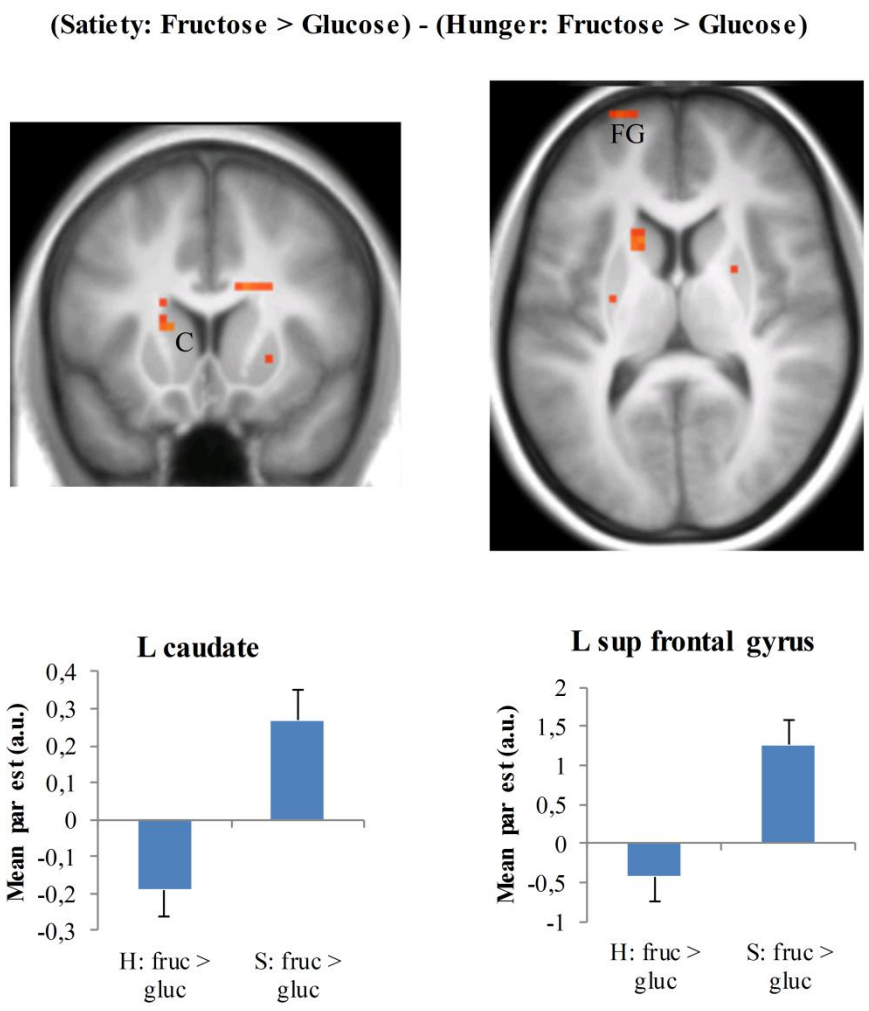
Appendix

Supplementary Table 3.1 Differences in taste activation for the delta between fructose and glucose during hunger and satiety.

Contrast	***Brain region***	***Cluster size***	***Z-score***	*Peak coordinate*		
				x	***y***	***z***
(Hunger: Fruc > Gluc) - (Satiety: Fruc > Gluc)	-					
(Satiety: Fruc > Gluc) - (Hunger: Fruc > Gluc)	White matter	17	3.9	18	17	28
	L caudate	11	3.7	-15	14	10
	R cerebellum	8	3.6	12	-37	-11
	White matter	30	3.6	-21	35	19
	L mid frontal gyrus		3.1	-30	41	22
	R lingual gyrus	7	3.5	12	-73	-11
	L sup frontal gyrus	7	3.5	-24	65	10
	L cerebellum	7	3.4	-15	-40	-14

Activations were thresholded at $p<0.001$ and a cluster extent threshold of $k>5$ contiguous voxels. Sup = superior, mid = middle, L = left and R = right.



Supplementary Figure 3.1 Significant brain activation for the subtraction [fructose – glucose] during hunger versus satiety in the left caudate (C, MNI (-15, 14, 10)) and left superior frontal gyrus (FG, MNI (-24, 65, 10)).

Chapter 4

Neural processing of calories in brain reward areas can be modulated by reward sensitivity

Republished (with minor changes/adaptations) from: van Rijn, I., Griffioen-Roose, S., de Graaf, C. & Smeets, P. A. M. Neural processing of calories in brain reward areas can be modulated by reward sensitivity. *Frontiers in Behavioral Neuroscience* **9**, 1–10 (2016).

Abstract

A food's reward value is dependent on its caloric content. Furthermore, a food's acute reward value also depends on hunger state. The drive to obtain rewards (reward sensitivity), however, differs between individuals. Here, we assessed the association between brain responses to calories in the mouth and trait reward sensitivity in different hunger states. Firstly, we assessed this in data from a functional neuroimaging study [1], in which participants (n=30) tasted simple solutions of a non-caloric sweetener with or without a non-sweet carbohydrate (maltodextrin) during hunger and satiety. Secondly, we expanded these analyses to regular drinks by assessing the same relationship in data from a study in which soft drinks sweetened with either sucrose or a non-caloric sweetener were administered during hunger (n=18) [2]. First, taste activation by the non-caloric solution/soft drink was subtracted from that by the caloric solution/soft drink to eliminate sweetness effects and retain activation induced by calories. Subsequently, this difference in taste activation was correlated with reward sensitivity as measured with the BAS drive subscale of the Behavioral Activation System (BAS) questionnaire. When participants were hungry and tasted calories from the simple solution, brain activation in the right ventral striatum (caudate), right amygdala and anterior cingulate cortex (bilaterally) correlated negatively with BAS drive scores. In contrast, when participants were satiated, taste responses correlated positively with BAS drive scores in the left caudate. These results were not replicated for soft drinks. Thus, neural responses to oral calories from maltodextrin were modulated by reward sensitivity in reward-related brain areas. This was not the case for sucrose. This may be due to the direct detection of maltodextrin, but not sucrose in the oral cavity. Also, in a familiar beverage, detection of calories per se may be overruled by a conditioned response to its flavor. In conclusion, the brain reward response to calories from a long chain starch sugar (maltodextrin) varies with trait reward sensitivity. The absence of this effect in a familiar beverage warrants further research into its relevance for real life ingestive behavior.

Introduction

In our Western society, there is an abundance of food cues and an enormous supply of different kinds of appetizing and calorie-rich foods. Therefore, many of us easily engage in overeating. Consequently, it is no surprise that obesity rates are high and still increasing [3]. However, it is still unclear why some of us are more inclined to engage in overeating than others.

The answer may lie in how sensitive we are to the food rewards surrounding us. Reward sensitivity is a personality trait that can be described as "the ability to derive pleasure or reward from natural reinforcers like food, and from pharmacological rewards like addictive drugs" [4]. Reward sensitivity can be measured with the Behavioral Inhibition System and Behavioral Activation System (BIS/BAS) questionnaire [5]. This questionnaire is based on the theory of Gray [5–7], which describes two neurobiological systems that both respond to environmental cues: the Behavioral Inhibition System (BIS) and the Behavioral Approach System (BAS). The BIS is sensitive to signals of punishment, and activation of this system inhibits behavior and induces negative feelings. The BAS is sensitive to signals of reward and activation of this system promotes behavior and positive feelings. Food reward is reflected by the BAS [5]. More specifically, the BAS is activated by cues that indicate the possibility of attaining food rewards rather than by food consumption [8]. Sensory signals like taste and sight of food can be seen as such cues, because they signal the presence of nutrients.

High reward sensitivity has been associated with food cravings, overeating, overweight, obesity and eating disorders [4,9–13]. Beaver et al. (2006) [14] showed that trait reward sensitivity as measured with the BAS scale, is associated with differential processing of food cues in the brain. In their study, reward sensitivity scores of healthy participants correlated strongly with brain activation by pictures of appetizing foods in reward areas such as the ventral striatum, amygdala, midbrain and orbitofrontal cortex.

Foods are not only rewarding because of their palatability, but also because of their caloric value. Several recent studies found that oral exposure to calories, independent of sweet taste, induced responses in classical reward areas such as the striatum, anterior cingulate cortex (ACC) and amygdala [15–17]. The presence of calories in the oral cavity may directly signal the imminent arrival of a rewarding (caloric) food. Therefore, it is plausible that neural processing of oral calories may be modulated by reward sensitivity in a similar way as was found for food pictures by Beaver et al. (2006) [14]. In addition, several studies found that hunger state interacts with brain activation in response to oral calories [1,15]. Currently, though, it is still unknown in how far reward sensitivity differentially affects brain responses to calories during hunger and satiety.

Based on the above, we hypothesized that 1) brain activation in reward areas in response to oral calories depends on trait reward sensitivity, in particular in the striatum, amygdala and ACC, and 2) that this association will be most prominent during hunger. Thus, we aimed to assess the correlation between reward sensitivity and the brain responses to calories in the mouth in different hunger states. Firstly, we assessed this in data from a functional neuroimaging study [1], in which simple solutions of a non-caloric sweetener with or without a non-sweet carbohydrate (maltodextrin) were administered during hunger and satiety. Secondly, we sought to extrapolate these findings to regular drinks by assessing the same relationship in data from a study in which soft drinks sweetened with either sucrose or a non-caloric sweetener were administered during hunger [2]. BAS drive and BAS reward, two subscales of the BIS/BAS questionnaire that respectively reflect the tendency to take action in response to a food reward and the amount of positive feelings experienced in response to this reward [5,18], were used as measures of reward sensitivity.

Materials and Methods

Data from two separate studies were used. Relevant details are described below. For full experimental details see van Rijn et al. (2015) and Griffioen-Roose et al. (2013) [1,2].

Participants

For both studies we recruited healthy, normal-weight (BMI between 18.5-25 kg/m^2) participants (age between 18-35 y). Exclusion criteria were among others: a restrained eating score higher than 2.80 (women) or 2.25 (men) (Dutch Eating Behavior Questionnaire [19], an energy restricted diet during the past two months, change in body weight of more than five kg during the past two months, lack of appetite, stomach or bowel diseases, diabetes, thyroid disease or any other endocrine disorder, use of daily medication other than oral contraceptives, having difficulties with swallowing and/or eating, having taste or smell disorders, being allergic and/or intolerant for products under study, smoking more than one cigarette/cigar a day, exclusive consumption or avoidance of light versions of beverages, being pregnant or lactating or having any contra-indication for MRI scanning. Thirty female participants completed Study 1 and eighteen participants completed the fMRI part of Study 2 (15 men, three women, see Table 4.1). Before enrollment, participants were screened on inclusion and exclusion criteria via a questionnaire including a medical history questionnaire and completed an fMRI training session in which they were familiarized with the fMRI procedure. All participants gave written informed consent. Both studies were conducted according to the principles of the Declaration of Helsinki, approved by the Medical Ethical Committee of Wageningen University and registered in the Dutch Trial Register (Study 1: NTR 3749, Study 2: NTR: 3289).

Table 4.1 Participant characteristics.

Characteristics	Study 1	Study 2
N	30	18
Gender	Female	Male (15) and Female (3)
BMI (kg/m^2)[1]	22.6 ± 1.4	22.1 ± 1.6
Age (y)[1]	22 ± 3	22 ± 2
BAS drive score[1]	11 ± 2	12 ± 2
BAS drive range	8-16	9-15
BAS reward score[1]	17 ± 1	18 ± 1
BAS reward range	15-20	15-20

[1]Mean±SD.

Study design

Study 1 had a randomized crossover design in which participants were scanned on two occasions, once during hunger and once during satiety. During the two scan sessions participants tasted fixed amounts of a control stimulus (water) and five stimuli containing carbohydrates, artificial sweeteners or both (sucralose, maltodextrin, maltodextrin + sucralose, glucose and fructose solutions), while their brain responses were measured using functional MRI. Here, we focus on the responses to two of these stimuli, the sweet caloric (maltodextrin + sucralose) and the sweet non-caloric (sucralose) solution.

Study 2 had a randomized crossover design consisting of two periods, which consisted of three parts: a pre-measurement, a conditioning period, and a post-measurement. In the conditioning period, subjects were offered a non-caloric sweetened and sugar sweetened version of a soft drink or a yoghurt drink for breakfast (10 times per drink). During scan sessions in the pre-measurement and post-measurement periods, participants tasted fixed amounts of the non-caloric sweetened and sugar sweetened drinks and a control stimulus (water) while their brain responses were measured using functional MRI. Here, we further analyze the brain responses to tasting the non-caloric sweetened and sugar sweetened soft drinks in the pre-measurement period.

Stimuli

The sweet non-caloric solution and the sweet caloric solution, used in Study 1, were made by dissolving, sucralose (Brenntag specialties, 0.254 g SPLENDA® Sucralose per liter, 0 kJ / 0 kcal per liter) and maltodextrin + sucralose (158.2 g Nutricia Fantomalt (90% polysaccharides - DE 19, 6% mono/disaccharides) + 0.140 g SPLENDA® Sucralose per liter, 2541 kJ / 607 kcal per liter) in demineralized water. The solutions were equisweet. Sweetness was matched in a pilot study using the

method of constant stimuli (n = 10). Furthermore, prior to the study, stimuli were rated on sweetness by a trained sensory panel and during the study by the participants. In both cases, no significant differences in sweetness were found between the two solutions (for more details see: van Rijn et al. (2015) [1]).

The non-caloric sweetened and sugar sweetened soft drinks used in Study 2 were developed and prepared by Royal FrieslandCampina (Amersfoort, The Netherlands) and contained 0 kJ / 0 kcal per liter (0.11 g sucralose per liter) and 1673 kJ / 400 kcal per liter (68.6 g sucrose per liter).The soft drinks were grape/lemon flavored and matched on sensory characteristics, including sweetness.

BAS scores

Reward sensitivity was measured with the Dutch version of the BIS/BAS questionnaire developed by Carver & White (1994) [5] . The Dutch BIS/BAS questionnaire was validated by Franken et al. (2005) [20], and is considered a reliable and valid measure. The BAS scale consist of three subscales: BAS drive, BAS reward and BAS fun. BAS drive and BAS reward are most relevant for appetitive motivation and discussed in this paper. "BAS fun reflects the tendency to seek out and impulsively engage in potentially rewarding activities" (Gomez et al., 2005) [18]. This scale is not discussed because the food-context of this paper concerns primary reward rather than 'activities'. Moreover, we investigate a classic well-known reward (food/calories) rather than a potential reward. In addition, BIS scores are also outside the scope of this paper.

The BIS/BAS questionnaire consists of 20 questions. The BAS drive scale is comprised of four of those questions (min-max score: 4-16) and the BAS reward scale of five (min-max score: 5-20). BAS scores for Study 1 were acquired during the fMRI training session and BAS scores for Study 2 were acquired on the last scan day (after scanning). Scores and ranges of BAS drive and BAS reward for Study 1 and Study 2 can be found in Table 4.1.

Experimental procedures

Study 1

Participants arrived between 10:25 h and 14:00 h at the test location (Hospital Gelderse Vallei, Ede, The Netherlands) after a fast of at least 3 h (no food, only water). Participants were instructed to eat a small self-chosen breakfast, prior to the 3 h fast. Hereafter participants were placed in the MRI scanner and scanned while tasting the solutions several times. During the satiety session participants started with an ad libitum lunch consisting of bread rolls (1063 kJ / 254 kcal per 100 g), full fat cheese (1570

kJ / 375 kcal per 100 g), boiled eggs (645 kJ / 154 kcal per 100 g), butter (1549 / 370 kcal per 100 g), sandwichspread (984 kJ / 235 kcal per 100 g), cucumber, tomato, orange juice (167 kJ / 40 kcal per 100 g) and skimmed milk (197 kJ / 47 kcal per 100 g). Participants were instructed to eat until comfortably full. After lunch, the same procedures were followed as during the hunger session.

Study 2

Participants arrived between 7.00 h and 11.00 h at the study location (Hospital Gelderse Vallei, Ede, The Netherlands) after a fast of at least 3 h (no food, only water) and were scanned while tasting the soft drinks several times. Note that in this study there was no satiety session.

Scanning procedure

In study 1, a scan session consisted of a high-resolution T_1-weighted anatomical scan and 3 functional runs during which 300 functional volumes were acquired using a T_2*-weighted gradient echoplanar imaging sequence on a 3-T Siemens Magnetom Verio (Siemens, Erlangen, Germany). During each functional run all solutions were tasted 4 times, resulting in a total of 12 taste trials per solution per scan session. Solutions were offered in 2 mL sips in a semi-random order. Each taste event (11 s) was followed by a 3-s swallow, a 4-s rinse with water, a 3-s swallow and a 3 to 5-s rest.

In study 2, a scan session consisted of a high-resolution T_1-weighted anatomical scan and 3 functional runs during which 262 functional volumes were acquired using a T_2*-weighted gradient echo imaging sequence on a 3-Tesla Siemens Magnetom Verio (Siemens, Erlangen, Germany). Each functional run consisted of 5 taste trials for every drink, leading to a total of 15 taste trials per drink. Drinks were offered in 2 mL sips in a semi-random order. Participants tasted every drink for 11 s while a picture of the drink was shown, followed by a 3-s swallow, a 4-s rinse with water, a 3-s swallow and a 3 to 5-s rest.

For both Study 1 and 2, participants rated liking once for every stimulus on a 9-point scale during each functional run. Instructions to either taste, swallow, rate, rinse, or rest were given to participants via visual cues on a screen placed in the bore at the back end of the scanner. Stimuli were administered with the use of programmable syringe pumps (New Era Pump Systems Inc,Wantagh, NY) at 50 mL/min.

Analysis

In both Study 1 and 2, functional volumes of every participant were preprocessed and analyzed with the SPM8 software package (Wellcome Department of Imaging Neuroscience, London, UK) in conjunction with the MarsBar toolbox (http://marsbar.sourceforge.net/) run with MATLAB 7.12 (The Mathworks Inc, Natick, MA). Details about the preprocessing steps can be found in van Rijn et al. (2015) and Griffioen-Roose et al. (2013) [1,2].

In the subject level analyses of Study 1, nine conditions were modeled: delivery of sucralose, maltodextrin, maltodextrin + sucralose, glucose, fructose and water, and swallowing, rinsing and stimulus rating. In the subject level analyses of Study 2, seven conditions were modeled: delivery of the non-caloric sweetened soft drink, sugar sweetened soft drink, tomato juice and water, and swallowing, rinsing and stimulus rating. Responses to swallowing, rinsing, stimulus rating, maltodextrin, glucose, fructose, tomato juice and water are not of interest for answering our current research question and are therefore disregarded. After modelling of the conditions, a so-called contrast image was calculated for every participant by subtracting activation by sucralose from activation by maltodextrin + sucralose (Study 1) or activation by the non-caloric sweetened soft drink from that by the sugar sweetened soft drink (Study 2). For Study 1, this was done for both the hunger and satiety condition. Subsequently, these contrast images were entered into separate one-sample t-tests with liking, BAS reward and BAS drive as covariates (for Study 1 this was done separate for the hunger and satiety condition). Liking was added as a covariate of no interest to regress out possible effects of differences in liking between the stimuli. Using the other two covariates we tested for correlations between BAS drive/BAS reward scores and taste activation across the whole brain. The resulting correlation T-maps were thresholded at $P<0.001$ (uncorrected for multiple comparisons) and a cluster size of $k>9$ contiguous voxels. A priori regions of interest (ROIs) were the amygdala, striatum and ACC. A mask of these regions was created with the WFU Pickatlas tool [21] and was used to do a ROI-analysis in with small volume correction over the mask volume. Whole brain results are reported in Supplementary Table 4.1, 4.2 and 4.3 (Appendix).

Results

Main effects

Main effects for study 1 have been reported in van Rijn et al. (2015) [1]. There were no differences in taste activation between the maltodextrin + sucrose and sucralose solution. Main effects for study 2 have been reported in Griffioen-Roose et al. (2013) [2]. More activation was found for the sugar

sweetened soft drink than for the non-caloric sweetened soft drink in the middle cingulum, precentral gyrus and rolandic operculum.

Correlations between covariates

Pearson correlation coefficients for correlations between the covariates used in the analyses (liking, BAS drive and BAS reward) for Study 1 and 2 can be found in Table 4.2. BAS drive and BAS reward scores obtained during Study 1 correlated significantly (r = 0.38, P<0.05).

Table 4.2 Pearson correlation coefficients (r) for the correlations between the difference in liking between the caloric and non-caloric stimulus, BAS drive and BAS reward scores.

Study	Liking and BAS drive	Liking and BAS reward	BAS drive and BAS reward
1	H: 0.28 S: -0.08	H: 0.05 S: -0.13	0.38*
2	-0.03	-0.04	0.28

* = Significant at the 0.05 level, H = hunger, S = satiety.

Study 1: Sugar solution and reward sensitivity

The ROIs in which correlations between BAS drive scores and brain activation in response to calories (maltodextrin and sucralose minus sucralose) during hunger and satiety were found, are shown in Table 4.3. BAS drive scores correlated with taste activation in the amygdala, ACC and striatum. BAS reward scores did not correlate with taste activation in any of the ROIs.

Taste activation in the right caudate (ventral striatum) correlated negatively with BAS drive scores during hunger (r = -0.62) (Figure 4.1). During satiety, however, BAS drive scores were positively correlated with activation in the left caudate (r = 0.60) (Figure 4.2). Taste activation in the ACC (bilaterally) and the right amygdala correlated negatively with BAS drive scores during hunger (left ACC: r = -0.63, right ACC: r= -0.59, right amygdala: r=-0.48), but not during satiety (Figure 4.3 and Figure 4.4).

Study 2: Soft drink and reward sensitivity

Brain activation during tasting of soft drinks with versus without calories did not correlate with BAS drive and BAS reward scores in any of the ROIs.

Table 4.3 ROIs in which brain activation by oral calories (maltodextrin and sucralose minus sucralose) correlated significantly with reward sensitivity (BAS drive score) during hunger and satiety.

Contrast	*Brain region*	*Cluster size*	*Z-score*	Peak coordinate		
				x	**y**	**z**
Hunger						
Positive correlation	No regions were found					
Negative correlation	R caudate	54	4.15	12	17	-8
	R putamen		4.09	21	17	-8
	R amygdala		3.44	18	11	-14
	R amygdala	21	3.85	18	-1	-17
	R anterior cingulate	74	3.72	3	32	16
	L anterior cingulate		3.33	0	23	22
Satiety						
Positive correlation	L caudate	15	3.76	-12	26	4
Negative correlation	No regions were found					

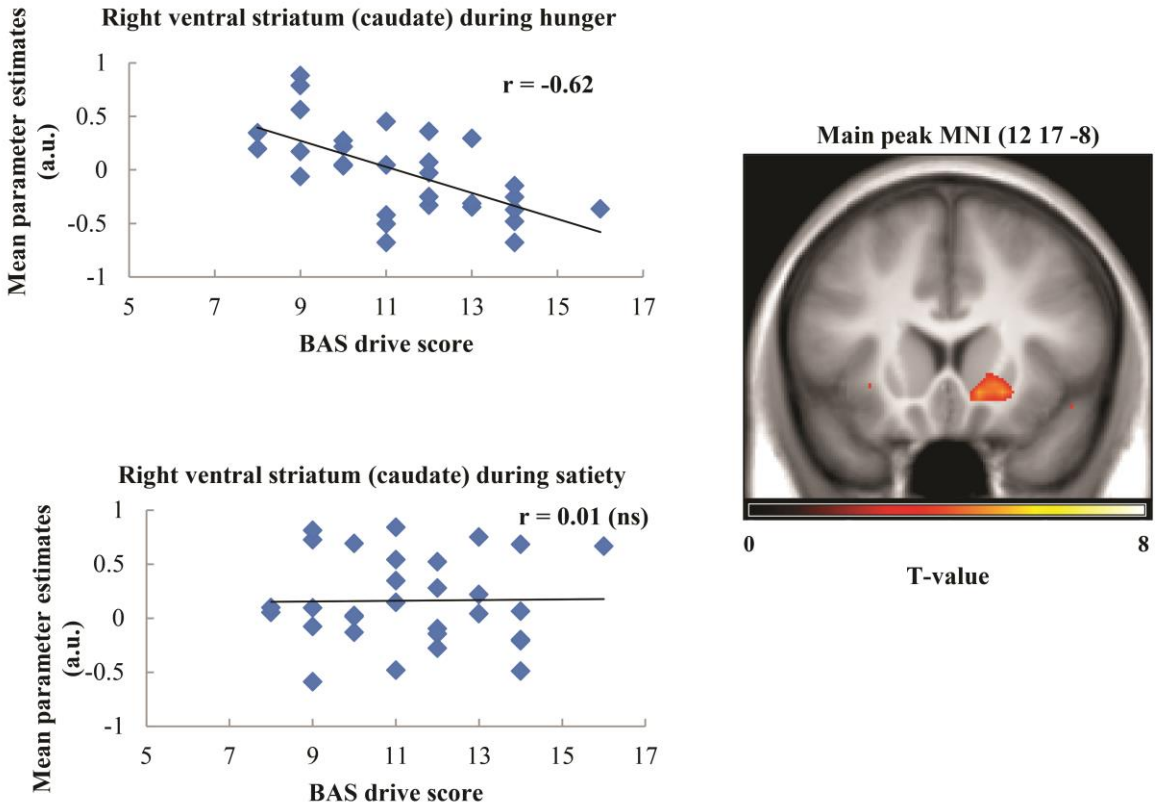


Figure 4.1 Scatterplot of brain activation in response to oral calories (maltodextrin and sucralose minus sucralose) and reward sensitivity (BAS drive score) during hunger (significant) and satiety (not significant) in the right ventral striatum (caudate).

Discussion

We assessed the correlation between reward sensitivity and brain responses to calories in the mouth in different hunger states. Firstly, we assessed this in data from a functional neuroimaging study, in which simple solutions of a non-caloric sweetener with or without maltodextrin were administered during hunger and satiety [1]. We found that when participants were hungry and tasted calories, brain activation in the right ventral striatum (caudate), amygdala and ACC (bilaterally) correlated negatively with BAS drive scores. In contrast, when participants were satiated, brain responses correlated positively with BAS drive scores in the left caudate. BAS reward scores did not correlated with taste activation in reward related areas.

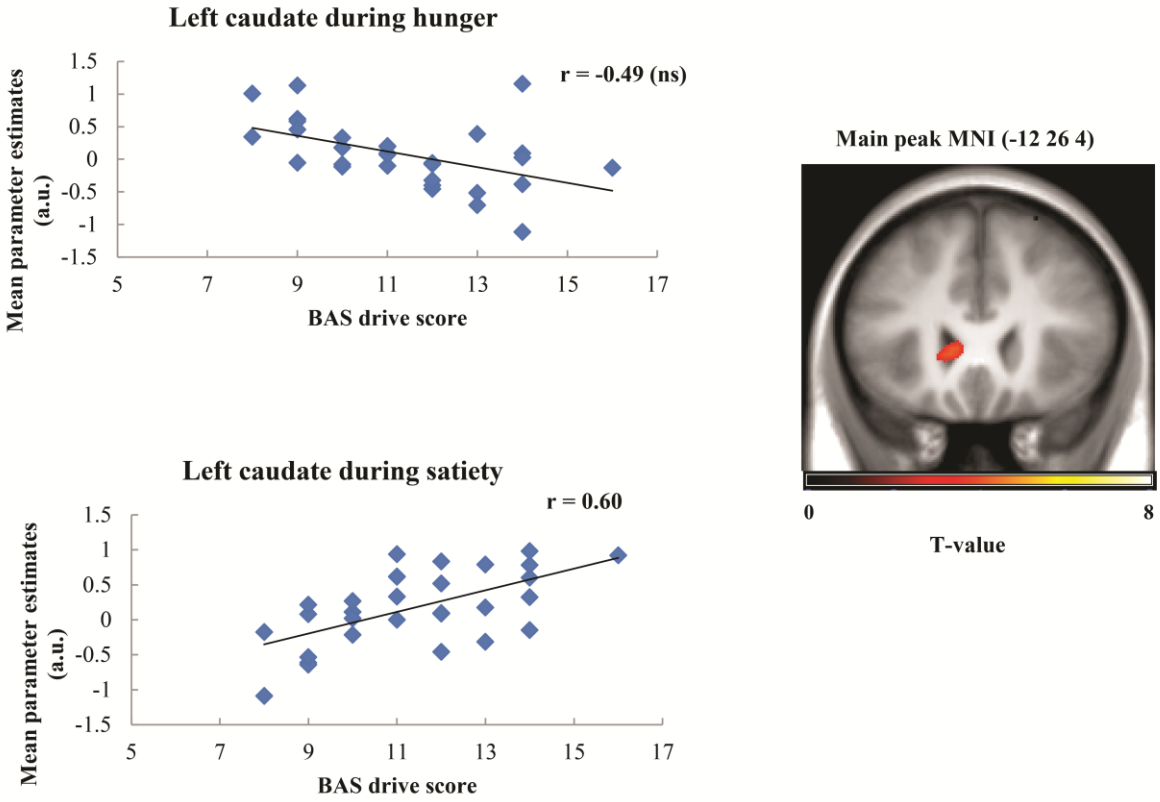


Figure 4.2 Scatterplot of brain activation in response to oral calories (maltodextrin and sucralose minus sucralose) and reward sensitivity (BAS drive score) during hunger (not significant) and satiety (significant) in the left caudate.

Secondly, we sought to extrapolate these findings to regular drinks by assessing the relationship between brain responses to calories in the mouth and reward sensitivity in data from a study in which soft drinks sweetened with either sucrose or a non-caloric sweetener were administered during hunger [2]. Here, we found no correlations between reward sensitivity and brain responses to calories in any reward related area.

For simple solutions, correlations with taste activation were found for BAS drive but not for BAS reward. The lack of findings for BAS reward may be explained by the valence of the solutions. BAS reward is related to the degree of positive feelings people experience in response to a reward. Solutions were, on average, disliked by the participants (mean liking scores on 9-point scale: 2.9 (maltodextrin + sucralose), 3.4 (sucralose), see van Rijn et al. (2015) [1]) and probably did not elicit positive feelings. BAS drive scores, which are related to the tendency to take action in response to a food reward, did correlate with the response to calories in the brain reward system. Thus, the response to oral calories is associated with BAS drive, independent of stimulus valence.

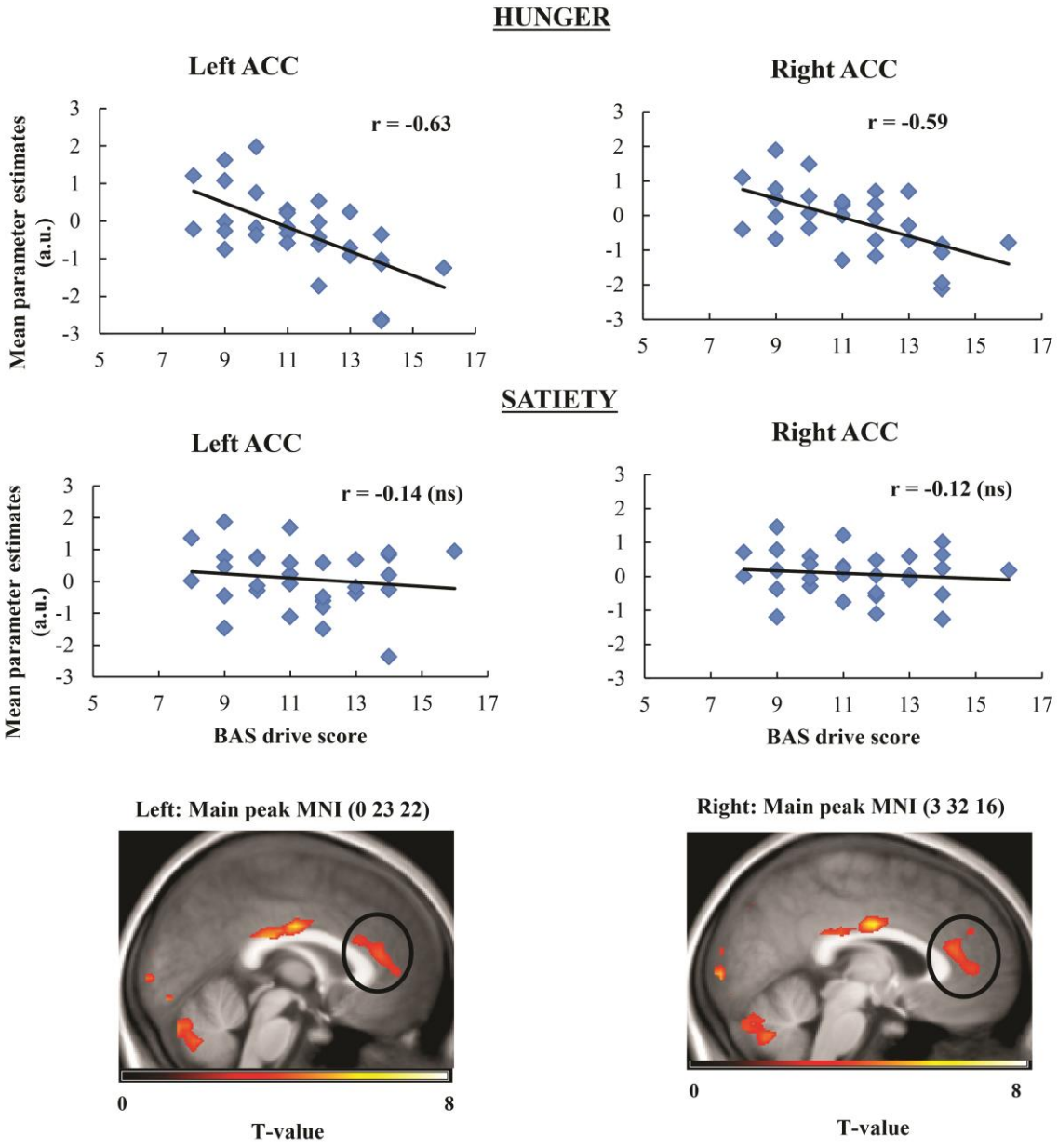


Figure 4.3 Scatterplot of brain activation in response to oral calories (maltodextrin and sucralose minus sucralose) and reward sensitivity (BAS drive score) during hunger (significant) and satiety (not significant) in the left and right ACC.

Brain responses during food-viewing have been found to correlate with reward sensitivity in the caudate, amygdala and ACC [14,22]. We focused on brain responses during exposure to another food-cue: the presence of calories in the oral cavity. In line with the food-viewing studies, we found that taste activation in the striatum, amygdala and ACC is correlated with reward sensitivity. Both the striatum and ACC are important in encoding food reward. They were found to be consistently activated in a meta-analysis of 28 studies in response to a pleasant tastant [23]. The amygdala has also been implicated in food reward [24–26]. In addition, several other studies found that the striatum, ACC and amygdala are also involved in the neural encoding of oral calories [2,15,17]. Our results extend this by showing that activation in response to oral calories in the ACC, caudate and amygdala varies with the degree to which individuals are sensitive to reward.

We found an inverse relationship between reward sensitivity and the brain response to oral calories in the amygdala, ACC and caudate. The amygdala plays a central role in the emotional processing of sensory stimuli [27–29]. Aversive stimuli have been found to activate the amygdala [30,31]. However, positive stimuli may also deactivate it [27]. This might explain our inverse relationship in the amygdala, because calories can been seen as positive stimuli. In line with this, previous research also showed that tasting a caloric soft drink deactivates the amygdala [15].

Concerning the caudate, Smeets et al. (2011) [15] showed an opposite effect, namely that tasting a caloric soft drink resulted in more activation than tasting a non-caloric one. Few studies with a fMRI-taste paradigm have reported deactivation in the striatum. At this moment, it is known that omission of an expected reward can produce deactivations in the ventral striatum [32,33]. However, in the current study there was no negative prediction error, thus this cannot explain the negative correlation in the striatum. We speculate that an alternative explanation for caudate deactivation might be the firing of GABA-neurons. The basal ganglia exert inhibitory control over several motor areas via GABAergic output [34]. The presence of calories in the mouth compared to a non-caloric liquid, might induce such firing to inhibit motor movements such as searching for other foods. GABA-neurons of individuals with a higher reward-sensitivity level might respond stronger to calories.

Another explanation for the deactivation in the striatum, ACC and amygdala might be that the response to calories in individuals with lower sensitivity to reward is more adapted to internal hunger. If so, they may experience calories as more rewarding during hunger, when calories are necessary for survival, and as less rewarding during satiety, when calories are not necessary. In line with this explanation, we found a positive correlation between reward sensitivity and caudate activation in response to oral calories during satiety.

For soft drinks, we found no correlations with reward sensitivity in any reward related area during hunger. The discrepancy between this finding and the associations found for simple solutions can be explained in a number of ways. Firstly, the source of calories was different: maltodextrin versus sucrose. Sucrose activates the sweet taste receptor, i.e. calories from sucrose are signaled by sweetness. On the contrary, maltodextrin, a tasteless substance for humans [35], is most likely directly detected by an oral maltodextrin receptor, independent of sweet taste [36]. In line with this, brain activation is different for a simple sugar compared to maltodextrin [16]. Thus, the different calorie sources may trigger different signaling mechanisms, which could have led to different results. In addition, it must be noted that we did not explicitly test for and excluded maltodextrin-tasters in the study with simple solutions. Previous research showed that a small percentage of the population can taste maltodextrin [37]. This could have amplified the results. Secondly, one study used unfamiliar solutions whereas the other study used familiar products (soft drinks that were very similar to commercially available variants). In both studies, we only included participants that consumed more sugar sweetened than artificially sweetened beverages in daily life. Therefore, we assume that participants were conditioned to link the flavor of the soft drinks to calories [38–40]. This conditioning

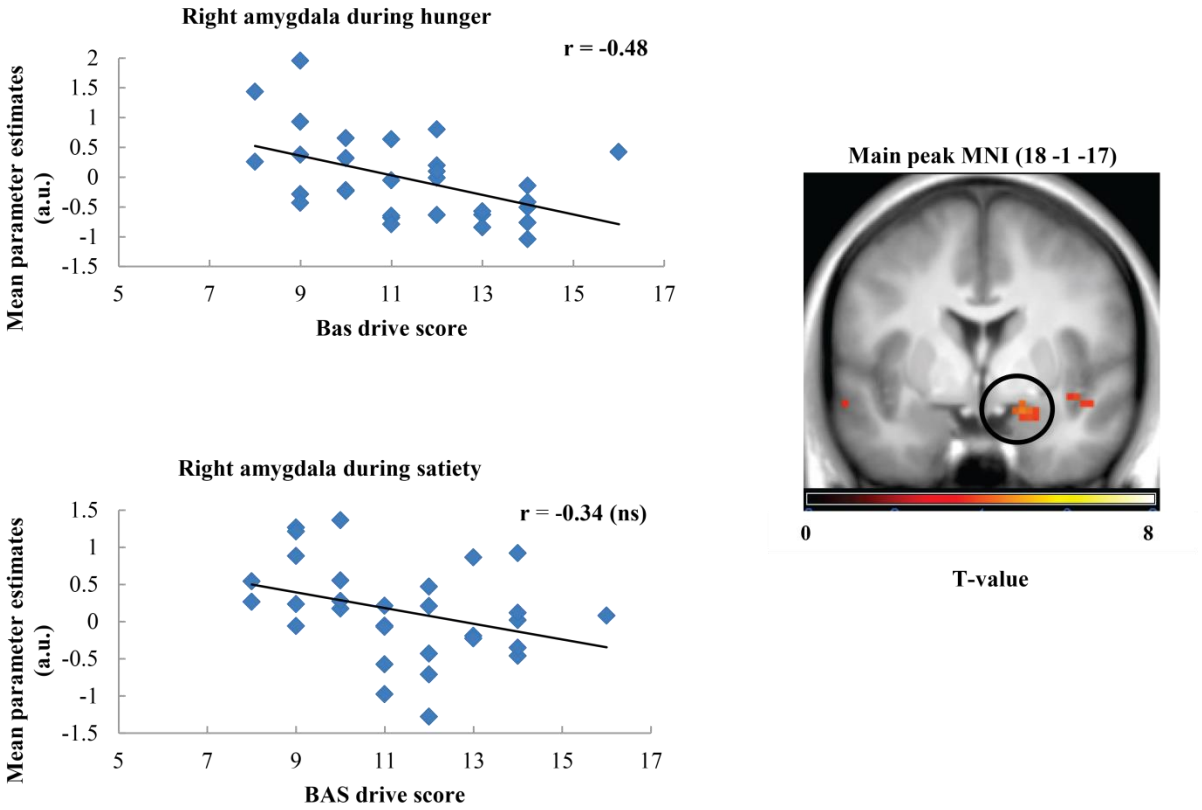


Figure 4.4 Scatterplot of brain activation in response to oral calories (maltodextrin and sucralose minus sucralose) and reward sensitivity (BAS drive score) during hunger (significant) and satiety (not significant) in the right amygdala.

might have overruled the effect of actual caloric content. Thirdly, the studies used participants of different genders. This may have led to dissimilar results because male and female brain responses to

food can differ [26,41–44]. In particular, women may respond stronger to external food-related stimuli than men [41,44], which could explain why we find effects for women, but not men. Finally, the study with mainly men included fewer participants (n=18). Many fMRI papers with a tasting-paradigm have used a comparable sample size and have shown significant results, for example: Bender et al. (2009) (n=19), Frank et al. (2008) (n=12), Haase et al. (2009) (n=18), O'Doherty et al. (2001) (n=7) and Spetter et al. (2010) (n=15) [17,30,45–47]. Nevertheless, it is possible that the relatively low sample size has prevented detection of small effects.

Conclusion

We found that neural responses to oral calories from a maltodextrin solution are modulated by reward sensitivity in reward-related areas such as the caudate, amygdala and ACC. This was not the case for a sucrose sweetened soft drink. This discrepancy may be due to the direct detection of maltodextrin, but not sucrose in the oral cavity. Also, in a familiar drink (soft drink), detection of calories per se may be overruled by a conditioned response to the familiar flavor. In conclusion, the brain reward response to calories from a long chain starch sugar (maltodextrin) varies with reward sensitivity. The absence of this effect in a familiar soft drink warrants further research into its relevance for real life ingestive behavior.

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Appendix

Supplementary Table 4.1 Brain regions in which brain activation by oral calories (maltodextrin and sucralose minus sucralose) correlated significantly with reward sensitivity (BAS drive score) during hunger and satiety.

Contrast	*Brain region*	*Cluster size*	*Z-score*	Peak coordinate		
				x	**y**	**z**
Hunger						
Positive correlation	No regions were found					
Negative correlation	R median cingulate	66	4.79	3	-10	31
	R posterior cingulate		3.46	6	-40	22
	L cerebellum	409	4.51	-33	-58	-26
	L calcarine sulcus		3.92	-3	-85	-11
	Vermis		3.83	3	-67	-35
	L fusiform gyrus		3.64	-27	-82	-17
	R cerebellum		3.24	6	-70	-29
	R cerebellum	102	4.42	24	-67	-26
	L superior frontal gyrus	169	4.39	-18	47	-2
	L middle frontal gyrus		3.76	-36	53	10
	L superior medial frontal gyrus		3.37	-15	65	4
	L thalamus	27	4.35	-12	-4	-2
	L calcarine sulcus	13	4.29	3	-94	1
	R caudate	55	4.15	12	17	-8
	R putamen		4.09	21	17	-8
	R rectus		3.80	12	23	-11
	R amygdala		3.44	18	11	-14
	R middle frontal gyrus	37	3.91	24	41	4
	R superior frontal gyrus		3.22	15	56	4
	R amygdala	21	3.85	18	-1	-17
	R inferior temporal gyrus	34	3.83	48	-52	-11
	R anterior cingulate	80	3.72	3	32	16
	R medial frontal gyrus (orb)		3.36	9	41	-5
	L anterior cingulate		3.33	0	23	22
	L precuneus	14	3.44	-9	-70	37
	L superior parietal gyrus		3.14	-12	-73	43
	R hippocampus	14	3.35	42	-10	-17
	R insula		3.27	42	-1	-11
	R middle frontal gyrus (orb)	13	3.21	39	50	-2
	R middle frontal gyrus		3.20	39	53	7
Satiety						
Positive correlation	L caudate	20	3.76	-12	26	4
Negative correlation	R cerebellum	15	3.81	27	-82	-38
	L mid temporal gyrus	10	3.62	-51	-34	10

Supplementary Table 4.2 Brain regions in which brain activation by oral calories (maltodextrin and sucralose minus sucralose) correlated significantly with reward sensitivity (BAS reward score) during hunger and satiety.

Contrast	*Brain region*	*Cluster size*	*Z-score*	Peak coordinate		
				x	**y**	**z**
Hunger						
Positive correlation	R cerebellum	21	3.93	9	-73	-29
Negative correlation	L lingual gyrus	25	3.61	-27	-61	-2
Satiety						
Positive correlation	No regions were found					
Negative correlation	R inferior frontal gyrus (tri)	22	4.08	45	35	1
	R insula	19	3.86	39	5	1

Supplementary Table 4.3 Brain regions in which brain activation by oral calories (sugar sweetened soft drink minus non-caloric sweetened soft drink) correlated significantly with reward sensitivity (BAS reward score) during hunger.

Contrast	*Brain region*	*Cluster size*	*Z-score*	Peak coordinate		
				x	**y**	**z**
Hunger						
Negative correlation	L Precentral gyrus	20	3.87	-42	2	58

Chapter 5

Health interest modulates brain reward responses to a perceived low-caloric beverage in females

Republished (with minor changes/adaptations) from: van Rijn, I., Wegman, J., Aarts, E., de Graaf, C. & Smeets, P. A. M. Health interest modulates brain reward responses to a perceived low-caloric beverage in females. *Health Psychology* (2016) (*advance online publication*).

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Abstract

Health labels are omnipresent in the supermarket. Such labels give rise to expectations about the product experience and may change flavor perception and perceived reward value. Consumers vary in their degree of health interest and may be differentially affected by such labels. However, how health interest influences neural reward responses to anticipation and receipt of heath-labeled foods is not known. This study assessed to what extent brain responses induced by anticipation and receipt of a beverage with different levels of perceived caloric content are associated with health interest. Twenty-five females completed an fMRI motivational taste-task in which they were presented with a low-caloric cue or a high-caloric cue and subsequently worked for sips of lemonade by moving a joystick. If they responded correctly and in time, they received the lemonade as a reward. Because of the two cue types, participants believed they were receiving two different lemonades, a high-caloric (HC-receipt) and a low-caloric (LC-receipt) one. Health interest was assessed with the General health interest subscale of the Health and Taste Attitude Scales. Health interest scores correlated significantly ($r = 0.65$) with LC- versus HC-receipt activation in the dorsal striatum (putamen), a region involved in encoding food reward. These findings suggest that the reward value of a healthy product compared to its unhealthy counterpart increases with health interest. This provides more insight into the working mechanism of government campaigns that focus on increasing health interest to encourage the formation of healthy eating habits.

Introduction

Food intake comprises an anticipatory phase, in which the food is smelled or seen, and a consummatory phase, in which the food is tasted and ingested. Both these phases can entail feelings of reward or pleasure [1,2]. Reward associated with consumption helps us ingest biologically relevant (nutritious) foods, because pleasant tastes signal for the presence of nutrients. After we have learned the reward value of a food during consumption, reward related to anticipation helps us to approach these foods (faster) the next time. Furthermore, anticipation also prepares the body for consumption by inducing cephalic phase responses [3]. Thus, reward related to anticipation and reward related to receipt have separate functions.

Accordingly, there may be different reward-related patterns in the brain for reward anticipation and receipt. Several neuroimaging studies are in line with this hypothesis [4,5]. For instance, one study compared the two reward phases using visual cues as reward anticipation and a sweet taste as reward receipt [4]. Expectation of the pleasant taste produced activation in the dopaminergic midbrain, amygdala, striatum and orbitofrontal cortex. Apart from the orbitofrontal cortex, these areas did not activate during reward receipt.

Expectations are provoked by product labels such as ‘healthy choice’, ‘diet’ or ‘light’, which are ubiquitous in the supermarket these days [6–8]. By means of such labels, foods are categorized in ‘healthy’ and ‘less healthy’ options. Consumers’ health interest is an important factor in the choice between healthy and unhealthy foods [9,10]. For example, individuals scoring high on the HTAS-General health interest scale more often choose healthy snacks or report higher consumption of healthy snacks compared to individuals with a lower score [9,10]. Previous research has shown that expectations created by a product label can change flavor pleasantness and intensity and attitude towards a product [11–14]. Furthermore, several neuroimaging studies demonstrate that brain responses can be modulated by product labels [14–20]. For example, labels that promote the tastiness of foods increase the neural encoding of taste pleasantness in the amygdala [19] and labels that promote the healthiness increase the neural encoding of reward in the ventral striatum [20].

In daily life, consumers vary in their degree of health interest and may thus be differentially affected by health-related product labels. Often, government campaigns try to increase health interest by means of education regarding a healthy eating pattern to induce the formation of healthy eating habits. Unravelling the association between increasing health interest and neural reward responses related to anticipation and receipt of health-labeled foods may give more insight into how these campaigns work. The current study aimed to assess to what extent brain responses induced by anticipation and

receipt of a beverage with different levels of perceived (but not actual) caloric content are associated with health interest. It was hypothesized that health interest affects brain activation in areas that have been implicated in food reward such as the basal ganglia, orbitofrontal cortex (OFC), anterior cingulate cortex (ACC) and amygdala [21–25].

Materials and Methods

Participants

Twenty-five young (mean ±SD age of 21±2 y), healthy, right handed, female participants with a normal weight (mean±SD BMI of 22.1±2 kg/m^2) completed the study. Participants were included if they consumed artificially sweetened beverages at least two times per month. Restrained eaters (score greater than 2.80 on the Dutch Eating Behavior Questionnaire [26] were excluded, since they are known to respond differently to food cues [27,28] and have a different brain anatomy [29]. Additional exclusion criteria were: an energy restricted diet during the past two months, change in body weight of more than five kg during the past two months, lack of appetite, stomach or bowel diseases, diabetes, thyroid- or kidney disease or any other endocrine disorder, having a history of neurological disorders, having a mental illness, use of daily medication other than oral contraceptives or paracetamol, having difficulties with swallowing and/or eating, having taste or smell disorders, being allergic and/or intolerant for products under study, smoking more than one cigarette/cigar a day, having a history of or current alcohol consumption of more than 28 units per week, exclusive consumption or avoidance of light versions of beverages, being pregnant or lactating, having any contra-indication for MRI scanning or disliking the product under study (liking < 40mm on a 100mm VAS-scale). Before enrollment, participants were screened on inclusion and exclusion criteria via a questionnaire and a taste test. After screening, included participants completed an functional magnetic resonance imaging (fMRI) practice session, which took place on a day prior to the study day. During this session they practiced with tasting in a dummy fMRI scanner. In total 106 participants were screened, of which 34 participants were included in the study. Nine participants dropped out because of various reasons, among other things technical issues regarding the setup of the study and feelings of discomfort in the scanner. All participants gave written informed consent. This study was conducted in accordance with the Declaration of Helsinki, approved by the Medical Ethical Committee of Wageningen University and registered in the Dutch Trial Register (NTR4249).

Study design

The study had a within-subjects design during which participants were scanned on one occasion by means of fMRI. During this scan session participants completed an fMRI motivational taste-task in which they were first exposed to a low-caloric cue (LC-cue) or a high caloric cue (HC-cue) and subsequently tasted a sweet beverage. Not disclosed to the participants was that this sweet beverage was always the same. Participants therefore believed that they were tasting a low-caloric beverage (LC-receipt) and a high-caloric beverage (HC-receipt).

The task was designed in such a way that participants did not receive the beverage passively, but had to work for it by means of joystick approach and avoidance movements. This paradigm was used in order to be optimally sensitive to brain responses during the anticipation and reward outcome phases. By having participants work for the beverages we achieved two goals. First, by having to work to obtain rewards participants had to remain highly motivated throughout the task. As a result, participants were put in a more ecologically valid mind-set when it comes to obtaining food; in daily life, acquiring food also requires effort and motivation. Secondly, using an approach-avoidance measure allowed us to investigate implicit approach biases towards the 'low'- and 'high'-caloric beverage. Such an approach-avoidance measure has been used successfully before for measuring approach tendencies to food cues [30–34]. Reaction times for the approach and avoidance movements were obtained and discussed in the paper as secondary measures.

Health and Taste Attitude Scales

The Dutch version of the Health and Taste Attitude Scales (HTAS) was filled in by participants during the fMRI practice session. The HTAS is a validated questionnaire which consists of 44 items ranging from 'strongly disagree' to 'strongly' agree (7-point scale) [10]. The HTAS can be divided into 7 subscales: General health interest, Light product interest, Natural product interest, Craving for sweet foods, Using food as a reward, Feeling guilty (Dutch HTAS only) and Pleasure. In the current study, scores from the General health interest subscale were used as measure for health interest. Light product interest and Natural product interest subscales were also included because of their relatedness to health interest. Reliabilities (Cronbach's α) of these subscales vary between 0.76 to 0.89 [9,10,35]. The General health interest subscale consists of 8 items and the maximum score is 7; the Light product interest and Natural product interest subscales consist of 6 items and the maximum score is 7.

Experimental procedures

On the study day participants arrived at the test location after a 3 h fast. The session started with a training in the scanner (~10 min), followed by an fMRI motivational taste-task (~1 h). After this, participants were presented with a real-life choice between two 250-mL bottles of the beverage, one labeled 'low-caloric' and the other 'high-caloric'. The bottle of choice was taken home.

Training

Participants first read a manual that explained the fMRI motivational taste-task. They were informed that they could earn sips of a low-caloric, high-caloric and neutral beverage by means of joystick movements. After this, they were placed in the scanner. There, they were familiarized with the different cue-beverage combinations by repeatedly showing a cue and directly afterwards administering the corresponding beverage. Hereafter, participants practiced the task for a short period of time.

fMRI motivational taste-task

During the fMRI motivational taste-task participants were presented with either a LC-cue or HC-cue and subsequently worked for a sip of beverage. Depending on the cue, the beverage was either perceived as low-caloric or high-caloric. In addition there was a neutral control condition, in which participants were presented with a neutral cue (N-cue) and worked for a sip of tap water (N-receipt).

Figure 5.1 shows a schematic overview of the trial structure of the task. The cue was depicted on the screen for 850 ms. After a jittered interval (2-6 s), participants were shown a symbol to which they had to response as fast as possible by moving a joystick in the right direction; when a diamond or a square was shown participants had to pull the joystick towards them, and when a triangle or circle was shown they had to push the joystick away. After another jittered interval (2-6 s), feedback (correct, incorrect or too late) was shown. In case of an incorrect or too slow response, the feedback event took 1.2 s, and participants received no liquid. In case of a correct response, 1 mL of beverage was administered during the feedback. A receipt event was jittered and lasted between 3.2 - 6.2 s after which a 2 s swallow took place. The swallow was either the last event of the trial or was followed by a liking VAS. When a liking VAS appeared, participants had to answer the following question on a 100-unit VAS anchored with 'not at all' to 'extremely': "How pleasant do you find the taste of this beverage?" Liking VASs for tasting the 'low-caloric' beverage, 'high-caloric' beverage and water occurred once per block from the 8th trial onwards, in a correct trial. A trial ended with a jittered interval (2-6 s) that occurred after the swallow or liking VAS in a correct trial, and after the feedback in an incorrect trial.

Wanting VASs occurred halfway the first, fourth, and last block of the task. The wanting question was: "How much do you want to consume this beverage right now?" In total, the task consisted of 7 blocks, and each block consisted of 24 trials (8 trials per cue type).

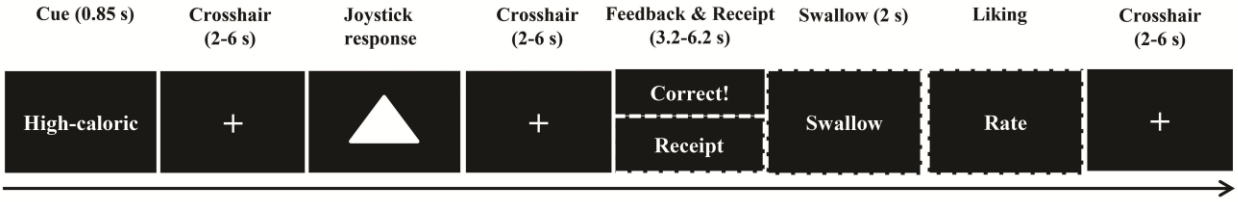

Figure 5.1 Schematic overview of the trial structure of the fMRI motivational taste-task.

Stimuli

Visual cues were the words 'neutral', 'low-caloric' and 'high-caloric', which were presented on a screen placed in the bore at the back end of the scanner (font: Tahoma, font size: 28, text color: white, background color: gray). Beverages were tap water and Grenadine lemonade (380 kcal/L, 1600 kJ/L). Grenadine lemonade was made by mixing 120 gram grenadine syrup (Karvan Cevitam) with 700 gram tap water. Beverages were administered in sips of 1 mL with the use of programmable syringe pumps (New Era Pump Systems Inc,Wantagh, NY) at 50 mL/min.

Scan settings

A scan session consisted of a functional run during which functional volumes were acquired using a multi-echo echo-planar imaging (EPI) sequence [36] (TR = 2080 ms, TE = 9.00 ms, 19.25 ms, 29.50 ms and 39.75 ms, 90◦ flip angle, FOV = 192 mm × 192 mm, 34 axial slices, ascending order, voxel size 3.5 mm × 3.5 mm × 3.5 mm) on a 3-T Siemens Magnetom Verio (Siemens, Erlangen, Germany). Additionally, a high-resolution T1- weighted anatomical scan was acquired (MPRAGE, TR = 1900 ms, TE = 2.26 ms, 9◦ flip angle, FOV = 256 mm × 256 mm, 192 sagittal slices, voxel size = 1 mm × 1 mm × 1 mm).

Analysis

fMRI data

fMRI data were preprocessed and analysed with the SPM8 software package (Wellcome Department of Imaging Neuro- science, London, UK) in conjunction with the MarsBar toolbox (http://marsbar.sourceforge.net/) run with MATLAB 7.12 (The Mathworks Inc., Natick, MA). The

volumes for each echo time were realigned to correct for motion artifacts (estimation of the realignment parameters is done for the first echo and then copied to the other echoes). The 4 echo images were combined into a single MR volume based on 60 volumes acquired before the actual experiment started using an optimised echo weighting method [36]. Combined functional images were slice time corrected, coregistered to the anatomical image, globally normalized to the Montreal Neurological Institute space (MNI space), and spatially smoothed with a Gaussian kernel of 7 mm full-width at half-maximum. A statistical parametric map was generated for every participant by fitting a delta function to each time series, convolved with the canonical hemodynamic response function. Data were high-pass filtered with a cut-off of 128 s.

The following conditions were modelled: viewing of the N-cue, LC-cue, HC-cue and response picture, joystick movement, feedback, delivery of the N-receipt, LC-receipt and HC-receipt, swallowing and stimulus and appetite rating. Responses to swallowing and rating were not included in further analyses and responses to the response picture, joystick movement and feedback are not under study in the current analyses. To account for motion-related variance, 6 realignment parameters were added to the model as regressors of no interest. For every participant, parameters were estimated for the contrasts [LC-cue – N-cue], [HC-cue – N-cue], [LC-receipt – N-receipt], [HC-receipt – N-receipt], [HC-cue – LC-cue] and [LC-receipt – HC-receipt] by means of t-tests.

For the group level analysis, the contrast images [LC-cue – N-cue] , [HC-cue – N-cue], [LC-receipt - N-receipt], [HC-receipt – N-receipt], [HC-cue – LC-cue] and [LC-receipt – HC-receipt] were entered into separate one-sample t-tests, with General health interest scores, Light product interest scores and Natural product interest scores as covariate. There was tested for correlations between the subscale scores and brain activation with a multiple regression model. In addition, differences in activation between the HC- and LC-cue and the HC- and LC-receipt were assessed by means of one-sample t-tests. All resulting T-maps were thresholded at $P<0.001$ (uncorrected for multiple comparisons) and a cluster size of $k>5$ contiguous voxels. A priori regions of interest were the OFC, ACC, amygdala, caudate, putamen, pallidum and substantia nigra. A mask of these regions was created with the WFU Pickatlas tool [37] and was used in a ROI-analysis with a small volume correction over the mask volume.

Reaction times

Reaction times consisted of the difference between the moment the response picture was visible on the screen and the moment the movement was initiated. Only reaction times of trials in which participants had been correct were analysed. Furthermore, reaction time scores lower than 150 ms were not included in the analyses. Mean reaction times were calculated for the approach and avoid movements during the low, high and neutral trials. Differences in reaction times were assessed by means of paired

samples t-tests. One participant had no correct responses during all the low-caloric avoidance trials. Therefore, comparisons involving this condition were performed without this participant.

Results

Behavioral data

HTAS subscale scores

The mean±SD scores for the General health interest, Light product interest and Natural product interest scales were respectively 4.6±0.6, 3.2±0.9 and 3.6±1.2 and the ranges 3.1-5.6, 1.5-4.8 and 1.3-5.5. General health interest scores correlated positively with Light product interest scores ($r = 0.46$, $P<0.05$). Natural product interest scores did not correlate with General health interest and Light product interest scores.

Subjective ratings and choice

Liking and wanting scores did not differ significantly between the N-, LC- and HC-receipt (Table 5.1). When presented with a choice between the low or high-caloric version of the beverage, 64% of the participants chose the low-caloric version.

Table 5.1 Mean (SD) liking and wanting ratings (cm) during the scan on a 100mm VAS-scale.

Beverage	Liking	Wanting
N-receipt	5.5 (2.1)	5.3 (2.6)
LC-receipt	6.1 (2.1)	5.0 (2.3)
HC-receipt	6.1 (2.4)	4.6 (2.9)

Liking and wanting scores did not differ significantly between the beverages (repeated measures ANOVA, $P>0.05$).

Correct responses

A correct joystick response was given and, in turn, beverage was administered in mean±SD 67±17 % of the low-caloric trials, 67±17 % of the high-caloric trials and 68±16 % of the neutral trials.

Reaction times

Mean±SD reaction times for the approach movement in the neutral, low-caloric and high-caloric condition were respectively 608.6±85.1, 604.0±83.1 and 608.2±74.6, and for the avoid movement 615.1±70.1, 607.4±74.1 and 588.2±127.6. Reaction times did not differ significantly between the approach and avoid joystick movements in any of the conditions (paired samples t-tests, P>0.05). Furthermore, comparison of reaction times for the avoid and approach movements over beverage type also yielded no significant differences (paired samples t-tests, P>0.05).

fMRI data

The effect of perceived caloric content on reward activation associated with cue and receipt

During the ROI-analysis no differences were found between the HC-cue and LC-cue. However, more activation was observed for LC-receipt compared to HC-receipt in the left putamen (cluster size: 9, Z-score: 3.4, MNI peak coordinates: -29, 7, -8) (Figure 5.2). For HC-receipt minus LC-receipt no differences were found. In addition, (low minus high-caloric) liking ratings did not correlate with (low minus high-caloric) neural cue or receipt activation in any of the ROIs.

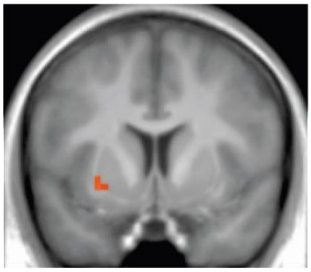

Figure 5.2 Taste activation for [LC-receipt – HC-receipt] in the left putamen, MNI (-29, 7, -8).

Correlation between reward activation associated with cue and receipt and HTAS subscales scores

The scores of General health interest, Light product interest and Natural product interest subscales did not correlate with brain activation in the ROIs for these contrasts: [LC-cue – N-cue], [HC-cue – N-cue], [LC-receipt – N-receipt], [HC-receipt – N-receipt] and [HC-cue – LC-cue]. However, General health interest score did correlate positively with brain activation during LC- compared to N-receipt in the right putamen (cluster size: 19, Z-score: 3.6, MNI peak coordinates: 27, 7, 6). Furthermore, a positive correlation was found for General health interest score and LC- compared to HC-receipt in the right putamen (cluster size: 11, Z-score: 3.6, MNI peak coordinates: 31, 7, 6) (Figure 5.3).

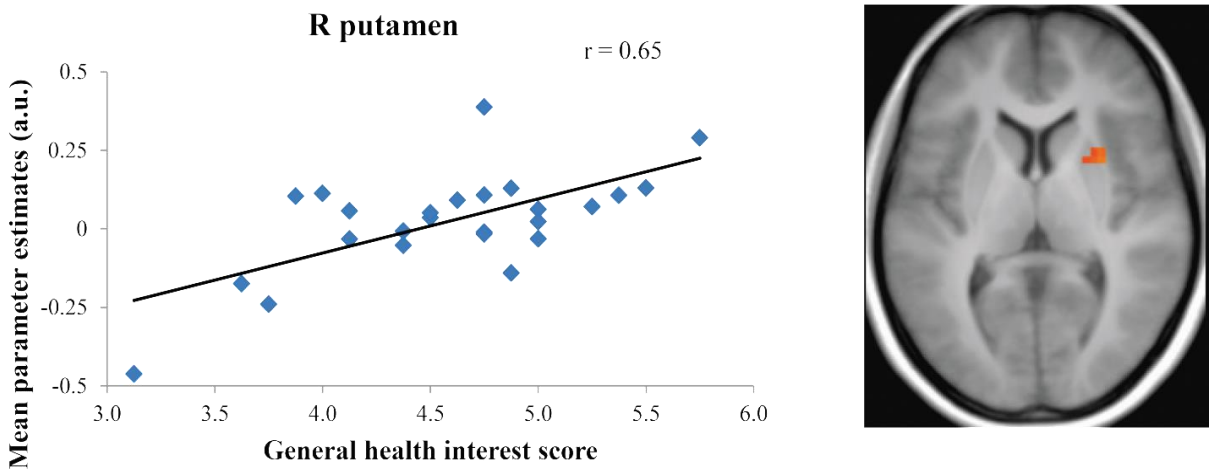

Figure 5.3 Scatterplot showing the correlation between brain activation during [LC-receipt – HC-receipt] and General health interest score in the right putamen, MNI (31, 7, 6).

Discussion

In this study, LC- compared to HC-receipt was associated with greater activation in the left ventral putamen. The putamen is part of the (dorsal) striatum, a region known for integrating affective, motor and cognitive information and for influencing goal-directed behavior by generating feelings of pleasure [1,38]. The ventral putamen in particular is involved in reward [39]. The greater putamen activation in the current study therefore suggests that LC-receipt was experienced as more rewarding than HC-receipt. This may be attributable to a cognitively driven preference for the low-caloric over the high-caloric beverage. The behavioral choice data reflect this as well since 64% of the participants chose the low-caloric beverage. However, motivation to obtain the low-caloric or high-caloric beverage as measured with approach and avoidance reaction times did not differ. Possibly, this measure was not sensitive enough.

In addition, for the cues, no differences in activation were observed in the putamen. The ventral putamen may therefore be more involved in reward associated with receipt than with anticipation. This has also been observed in a study that measured brain activation in drug-addicts in response to cocaine injections [38,40]. In these subjects, drug rush ratings, i.e. drug receipt, correlated stronger with activation in the dorsal striatum than drug craving ratings, i.e. drug anticipation. Additionally, Delgado et al (2007) [38] hypothesized, after comparison of several experiments, that the dorsal striatum is involved in learning and updating actions that lead to reward, rather that representing and identifying rewards. Therefore the lack of difference in activation between the LC- and HC-cue in this area may be plausible.

One can speculate that the greater putamen activation for LC-receipt compared to HC-receipt in the current study is population-specific. The population consisted of highly educated women (predominantly college students). Literature shows that this group has a more positive attitude towards healthy eating compared to men and individuals with a lower education level [41–46]. The attitude towards a product can be reflected in brain activation and may even alter flavor experience. This was for instance shown in the famous Coca-Cola versus Pepsi experiment [47]. When the two cokes were delivered without a brand, participants showed no behavioral preference for either Pepsi or Coca-Cola and taste activation was not different between the two, other than in the ventromedial prefrontal cortex where activation correlated with subjects' behavioral preference. In contrast, when the brand was revealed, participants showed a behavioral preference for Coca-Cola, but not for Pepsi, over an unlabeled coke. Taste activation corresponded with the behavioral findings and was greater for branded over unbranded Coca-Cola, but not Pepsi, in regions involved in emotion and affect, the dorsolateral prefrontal cortex, hippocampus and midbrain. In line, another study showed that both liking ratings and brain reward activation increased when a wine was presented as more expensive [14]. Many other behavioral and neuroimaging studies have reported this assimilation effect, where flavor experience is modified towards the expectation [13,15,48–51]. In the current study, personal preferences of the sample may explain why LC-receipt elicits more reward-related brain activation than HC-receipt. Highly educated women may have a better understanding of what a balanced food pattern should and shouldn't contain compared to the average population [52]. For this subgroup 'health labeling' might be a good strategy to improve healthy choices. Nevertheless, more research is needed to establish in how far these findings generalize to other groups.

Another finding was the positive correlation between LC- compared to HC-receipt activation and health interest scores in the dorsal striatum, namely in the dorsal putamen. Interestingly, dorsal putamen activation was found to be modulated by willingness to pay in a functional connectivity analysis during passive viewing of food images [53]. This indicates that cognitive factors can affect dorsal putamen activation. Futhermore, this region of the putamen is known to be involved in food reward [54]. This suggest that in the current population the reward value of receipt of the low-caloric relative to the high-caloric beverage increased with health interest. Several other studies have shown differences in brain responses when focusing attention on foods' healthiness instead of pleasantness [17], taste [19] or regular foods [20]. In general, promoting healthiness decreases the reward value of a product [17,19,55]. However, in line with results of the current study, Linder et al. (2010) [20] found that the reward value of food products increased when individuals were more health-minded, i.e. when they bought organic foods more often. Thus, health labels may appeal to the health-minded consumer but may deter consumers that highly value pleasantness [55].

In the present study, a positive correlation was found between LC-receipt activation and General health interest, but not Natural and Light product interest scores. Products with a label emphasizing their low caloric content therefore seem to categorize as healthy, rather than as natural or light products. Health interest (as measured with the General health interest subscale) is a good predictor of healthy food choices [9,10,56]. Therefore, increasing health interest through government campaigns might be a fruitful way to promote healthier food choices.

To summarize, receipt of a beverage perceived as low- compared to high-caloric induced more activation in the dorsal striatum during a fMRI motivational taste-task. Furthermore, health interest scores correlated positively with dorsal striatal activation during the receipt of a perceived low- compared to high-caloric beverage.

In conclusion, above findings suggest that emphasizing a product's health benefits compared to its health risks makes a product more rewarding for young, highly educated females. Furthermore, these results indicate that the reward value of a healthy product increases with health interest. Government campaigns that focus on increasing health interest may therefore be successful in inducing the formation of healthy eating habits.

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Chapter 6

It’s in the eye of the beholder: Selective attention to food properties influences taste activation in gustatory and reward regions

Based on: van Rijn, I., de Graaf, C. & Smeets, P. A. M. It’s in the eye of the beholder: Selective attention to food properties influences taste activation in gustatory and reward regions (*submitted*).

Abstract

Statements regarding pleasantness, taste intensity or caloric content on a food label may influence the attention consumers pay to such characteristics during consumption. There is little research on the effects of such statements on taste perception and associated brain activation. The aim of this study was to investigate the effect of selective attention to hedonics, intensity and caloric content on brain responses during tasting. Using functional MRI brain responses of 27 women were measured while they payed attention to the intensity, pleasantness or caloric content of fruit juice, tomato juice and water. Taste activation for the three selective attention conditions largely overlapped and was found in the rolandic operculum, insula and overlying frontal operculum, striatum, amygdala, thalamus, anterior cingulate cortex and middle orbitofrontal cortex (OFC). Taste activation was higher during selective attention to intensity compared to calories in the right middle OFC and during selective attention to pleasantness compared to intensity in the right putamen, right ACC and bilateral middle insula. Intensity ratings correlated with taste activation during selective attention to intensity in the anterior insula and lateral OFC. Our data suggests that not only the anterior insula but also the middle and lateral OFC are involved in evaluating taste intensity. Furthermore, selective attention to pleasantness engages regions associated with food reward. Overall, our results indicate that statements regarding food properties can alter the consumption experience through attention-driven effects on the activation of gustatory and reward regions.

Introduction

Selective attention to one specific food property over another may alter the taste perception of a food [1,2]. In daily life, attention of consumers is often directed towards a specific property by product labels that emphasize either the hedonics, sensory characteristics or caloric content [3]. Such product labels may, in turn, influence consumers' buying and eating behaviour [4–6]. Better understanding of the association between selective attention and brain responses during consumption may give us more insight into how product labels can affect the consumption experience.

Previously, selective attention on brain activation induced by food viewing and tasting has been studied via complex cognitive manipulations such as words, symbols or labels emphasising either the taste, caloric value or health aspects of a food cue [7–11]. These manipulations were shown to modulate brain activation in reward-related regions such as the OFC, ACC, amygdala and ventral striatum. These cognitive manipulations all represent specific aspects of taste such as intensity, affect or health/caloric value. Nevertheless, only one study explicitly investigated and compared the effect of selective attention on two of these dimensions, namely intensity and pleasantness [12]. They found that when participants focussed their attention on intensity, taste activation was greater in the insular cortex, but when they focussed on pleasantness, the medial orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) were more responsive during tasting a monosodium glutamate solution. In line with this, the anterior insula and overlying operculum, but not the OFC, show greater activation when participants are instructed to detect a taste in a tasteless solution, in comparison to passive tasting [13]. These studies show that taste activation in the insular cortex, OFC and ACC can be altered by selective attention. However, more research is needed to further elucidate how selective attention to specific taste aspects influences the consumption experience of real foods rather than simple solutions.

Neural processing of taste intensity and valence have been linked to specific brain regions. The insula and overlying frontal operculum (which contain the primary taste cortex [14]), are believed to represent taste intensity [15–18]. Beside intensity, the primary taste cortex also represents taste quality and valence [15,16]. Food valence is believed to be represented in the OFC, an area that receives neural signals directly from the primary taste cortex and has been designated as secondary taste cortex [14,18–21]. The OFC projects to the striatum and ACC [14], which are involved in processing affective value and taste intensity [17,18,21–23]. In addition, the primary taste cortex and OFC project to the amygdala, a region possibly involved in integrating affect and intensity [17,18,24–26]. Recently, the presence of calories in the mouth has also been associated with activation in several brain regions including the amygdala, striatum, ACC and insula and overlying frontal operculum [27–32]. Selective attention to intensity, valence and caloric content may affect taste activation in the above listed regions that had earlier been

associated with these properties. The aim of the current study was to investigate the effect of selective attention to hedonics, intensity and caloric content on brain responses during tasting. Secondary, we assessed the association between taste activation during selective attention and subjective pleasantness, intensity and caloric content ratings.

Materials and methods

Participants

Thirty young, healthy, right-handed females with a normal weight were included in the study. One participant dropped out because of feelings of discomfort in the scanner. Furthermore, due to technical issues with the gustometer, data was not reliable for two of the subjects. Therefore, twenty-seven participants with a mean (± SD) age of 22 (± 3) y and a mean (± SD) BMI of 21.5 (± 1.7) kg/m^2 were included in the analyses. Exclusion criteria were: a restrained eating score higher than 3.40 (Dutch Eating Behavior Questionnaire [33]), an energy restricted diet during the past two months, change in body weight of more than five kg during the past two months, lack of appetite, stomach of bowel diseases, chronic diseases such as diabetes, thyroid- or kidney disease, having a history of neurological disorders, having a mental illness, use of daily medication other than oral contraceptives or paracetamol, having difficulties with swallowing and/or eating, having taste or smell disorders, being allergic and/or intolerant for products under study, smoking more than one cigarette/cigar a day, having a history of or current alcohol consumption of more than 21 units per week, being pregnant or lactating, having any contra-indication for MRI scanning or disliking the product under study (liking < 5 on a 9-point scale). Before enrollment, participants were screened on inclusion and exclusion criteria via a questionnaire and a taste test. After screening, included participants completed a training session in which they practiced the fMRI procedure. All participants gave written informed consent. This study was conducted in accordance with the Declaration of Helsinki (amendment of Fortaleza, 2013), approved by the Medical Ethical Committee of Wageningen University and registered in the Dutch Trial Register (NTR5253).

Stimuli

Stimuli consisted of a commercially available fruit juice (Dubbel Drank orange and peach, Appelsientje, 48 kcal/100 mL, Royal FrieslandCampina, Amersfoort, The Netherlands) and tomato juice (Zontomaat, Appelsientje, 18 kcal/100 mL, Royal FrieslandCampina, Amersfoort, The Netherlands) and tap water.

Experimental procedure

Participants arrived between 08:00 and 10:00 h at the test location (Hospital Gelderse Vallei, Ede, The Netherlands) after a fast of at least 3h (no food, only water) and were placed into the MRI scanner to engage in an fMRI taste-task. During this task, participants tasted small sips (2 mL) of the fruit juice, tomato juice and water while they had been instructed to pay attention to either the pleasantness, taste intensity or amount of calories of the stimulus. Participants were led to believe that they were tasting two types of fruit juice and two types of tomato juice. They were told that the two fruit juices and the two tomato juices were very similar tasting, but that there were slight differences in ingredients. The task consisted of three runs and one run consisted of three blocks: a pleasantness block, an intensity block and a calorie block. Figure 6.1 shows a schematic overview of the trial structures during a block. At the beginning of each block, a screen was show that indicated to which characteristic participants had to pay attention. This was indicated in words (pay attention to the pleasantness, calories or taste intensity), as well as with the color of a square that was depicted on the top of the screen. This colored square was present during the whole task and changed color at the start of a new block. Moreover, beforehand, participants also had been asked to memorize the three color-instruction combinations. The order of the blocks varied during the runs and the order of the runs varied between participants. During each block, every stimulus was tasted 4 times. This resulted in 12 trials per characteristic per stimulus in total. A trial consisted of a 11-s taste-event, followed by a 3-s swallow, a 4-s rinse with water, a 3-s swallow and a 3-5-s rest. During each block, participants rated either the pleasantness, taste intensity or amount of calories one time for each stimulus on a 5-point scale, anchored with ‘not at all’ till ‘very’, or for calories, ‘none’ till ‘very much’. Ratings were given directly after swallowing the taste stimulus. Instructions to either taste, swallow, rate, rinse or rest were given to participants via visual cues on a screen placed in the bore at the back end of the scanner. Stimuli were administered with the use of programmable syringe pumps (New Era Pump Systems Inc,Wantagh, NY) at 50 mL/min.

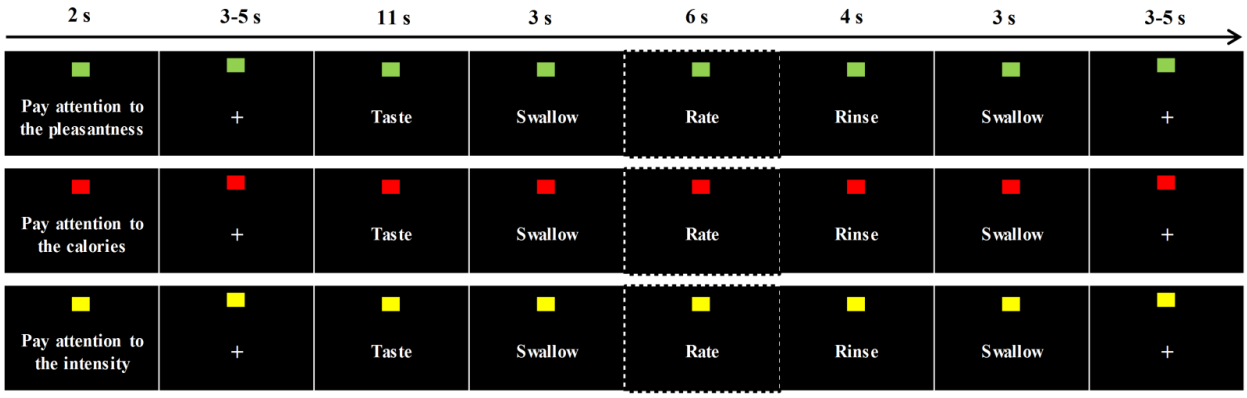

Figure 6.1 Schematic overview of trial structures during a block of the taste-task.

MRI data acquisition

A scan session consisted of 3 functional runs during which 460 functional volumes were acquired using a T_2^*-weighted gradient echoplanar imaging sequence (TR = 2140 ms, TE = 25 ms, 90◦ flip angle, FOV = 192 × 192 mm, 43 axial slices, descending order, voxel size 3 × 3 × 3 mm) on a 3T Siemens Magnetom Verio (Siemens, Erlangen, Germany). The stack was tilted at an angle of 30◦ to the anterior-posterior commissure line to reduce signal dropout in orbitofrontal cortex and ventral temporal lobe [34]. Additionally, a high-resolution T_1-weighted anatomical scan was acquired (MPRAGE, TR = 2300 ms, TE = 2.98 ms, 9◦ flip angle, FOV = 256 × 256 mm, 192 sagittal slices, voxel size = 1 × 1 × 1 mm).

Data Analysis

fMRI data were preprocessed and analyzed with the SPM8 software package (Wellcome Department of Imaging Neuro-science, London, UK) in conjunction with the MarsBar toolbox (http://marsbar.sourceforge.net/) run with MATLAB 7.12 (The Mathworks Inc., Natick, MA).The functional volumes of every participant were slice time corrected, realigned to the first volume of the first run, coregistered to the anatomical image, globally normalized to the Montreal Neurological Institute space (MNI space), and spatially smoothed with a Gaussian kernel of 6 mm full-width at half-maximum. A statistical parametric map was generated for every participant by fitting a boxcar function to each time series, convolved with the canonical hemodynamic response function. Data were high-pass filtered with a cutoff of 128 s. For each taste stimulus, 3 conditions of interest were modelled: paying attention to intensity, caloric content and pleasantness. Furthermore, 4 conditions of no interest were modelled: rinsing, swallowing, task instructions and rating. To account for motion-related variance, realignment parameters were added to the model as regressors of no interest. For every participant, parameters were estimated for the intensity, calorie and pleasantness conditions by averaging over fruit juice, tomato juice and water (versus baseline) in a T-contrast. Brain responses were averaged over the stimuli to increase power and to be able to generalize over taste quality and pleasantness level (see e.g. [35]). Furthermore, selective attention conditions were also contrasted against each other using T-contrasts.

On the group level, region of interest (ROI) analyses were performed. A priori ROIs were areas associated with taste processing: OFC, insula, frontal and rolandic operculum, ACC, amygdala, caudate, putamen, pallidum and thalamus [14,22,26,36]. A combined mask of these regions was created with the WFU Pickatlas tool [37] and used in ROI analyses with small volume correction over the mask volume.

First, common activation for the selective attention conditions in the ROIs was examined by means of a conjunction analysis. An one-way within-subject ANOVA was performed using the subject-level contrasts for each selective attention condition versus baseline to create a model with the three selective attention conditions as levels. Hereafter, separate T-maps were created for the selective attention conditions and these were combined into a conjunction T-map (conjunction null). The resulting conjunction T-map was thresholded at T=8 (uncorrected for multiple comparisons) and a cluster size of k>4 contiguous voxels.

Second, differences in activation within the ROIs were examined with multiple one-sample t-tests in which the subject-level contrast images were entered. In addition, we tested for correlations between taste activation during selective attention (versus baseline) and subjective ratings within the ROI mask by means of multiple regression. Resulting T-maps were thresholded at P<0.001 (uncorrected for multiple comparisons) and a cluster size of k>4 contiguous voxels. This threshold is based on Lieberman and Cunningham (2009) [38], who argue for less conservative thresholding and even advise a less stringent threshold of P<0.005 with a 10 voxel cluster extent. Too conservative thresholding in an attempt to decrease false positive effect (type I errors), may increase the possibility for missing true effects (Type II errors), and may introduce biases toward studying large rather than small effects and observing sensory and motor processes rather than complex cognitive and affective processes [38]. For visualization of the correlations average parameter estimates for each cluster were extracted with the use of the MarsBar toolbox.

Results

Subjective ratings

Subjective ratings for intensity, caloric content and pleasantness of the fruit juice, tomato juice and water, obtained during scanning can be found in Figure 6.2. Water was significantly less intense than the juices. Furthermore, fruit juice was perceived as most calorie dense, followed by tomato juice and water. Finally, fruit juice was perceived as most pleasant.

Common taste activation

Figure 6.3 shows the brain responses during tasting when participants payed attention to the intensity, caloric content or pleasantness and the conjunction for these selective attention conditions (also see Supplementary Table 6.1, 6.2, 6.3 and 6.4, Appendix). Common taste activation was observed in the

rolandic operculum, insula and overlying frontal operculum, striatum, amygdala, thalamus, anterior cingulate cortex and middle OFC.

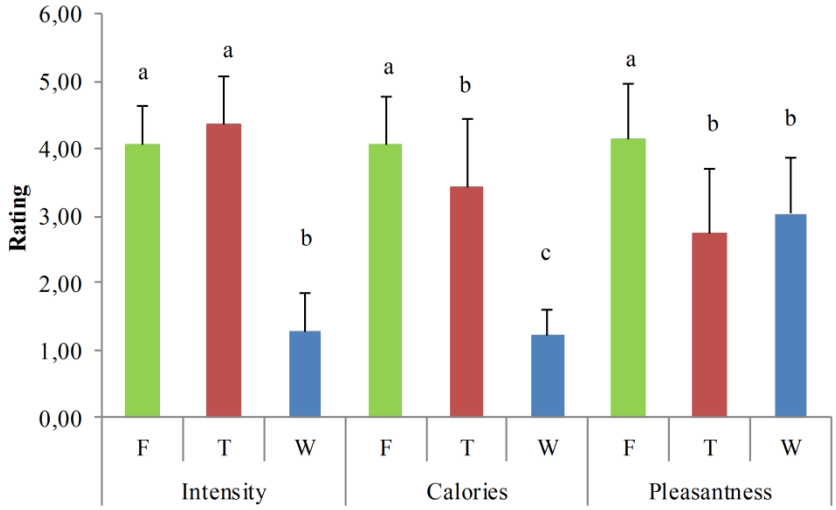

Figure 6.2 Subjective ratings (mean + SD) on a 5-point scale for intensity during selective attention to intensity, caloric content during selective attention to caloric content and pleasantness during selective attention to pleasantness of a fruit juice (F), tomato juice (T) and water (W), obtained during scanning. Repeated measures ANOVA, post-hoc t-tests, $P<0.05$, LSD-corrected for multiple comparisons. Bars within each condition that have a different letter differ significantly.

Attention-driven differences in taste activation

Table 6.1 shows the differences in taste activation between the three selective attention conditions (also see Figure 6.4). Attentional focus on intensity resulted in more taste activation in the right middle OFC compared to when attention was directed to caloric content. Paying attention to the pleasantness compared with intensity, induced more activation in the right and left middle insula, the left frontal operculum, right ACC and right putamen.

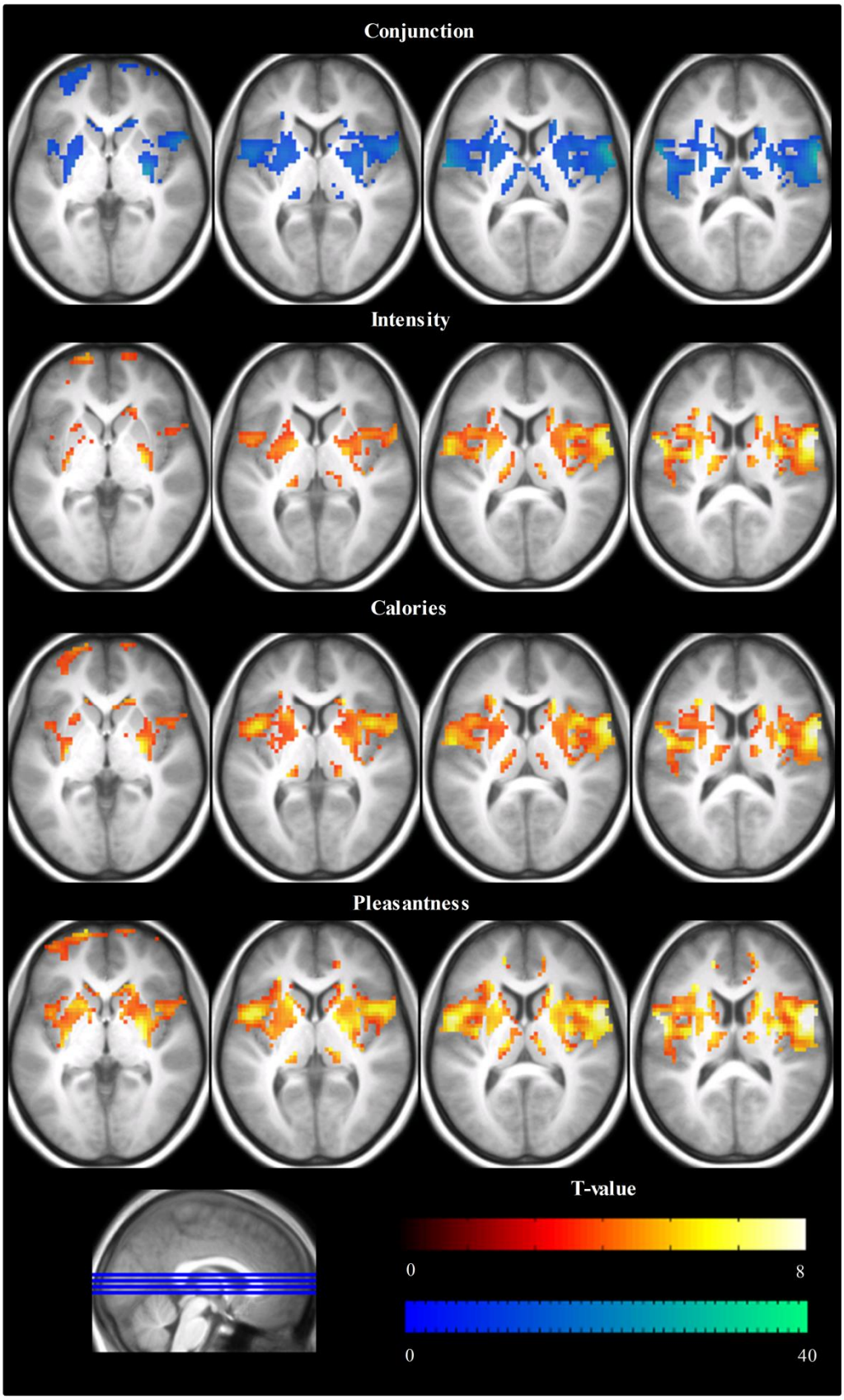

Figure 6.3 Taste activation during selective attention to intensity, caloric content and pleasantness (MNI z-coordinates: -1, 4, 9 and 14).

Table 6.1 Brain activation during tasting while paying attention to intensity, calories or pleasantness.

Comparison	*Brain region*	*Cluster size*	*Z-score*	*Peak coordinate*		
				x	***y***	***z***
Intensity - Calories	R sup frontal gyrus (mid OFC)	7	3.4	15	62	-5
Calories - Intensity	No regions					
Intensity - Pleasantness	No regions					
Pleasantness - Intensity	R putamen	5	3.5	30	-4	-2
	R ant cingulate cortex	5	3.4	15	44	19
	R mid insula	5	3.2	45	2	-2
			3.2	42	-1	-5
	L inf frontal gyrus (frontal operculum)/extending into L mid insula	8	3.2	-45	11	4
Calories - Pleasantness	No regions					
Pleasantness - Calories	No regions					

Activations were thresholded at $p<0.001$, with small volume correction over the ROI volume and a cluster extent threshold of $k>4$ contiguous voxels. Ant = anterior, sup = superior, inf = inferior, mid = middle, L = left and R = right.

Relationship between taste activation during attentional focus on intensity, calories or pleasantness and subjective ratings

Table 6.2 shows brain regions in which an association was found between subjective ratings and taste activation in the three selective attention conditions. There was a positive correlation between intensity ratings and taste activation during attentional focus on intensity in the right anterior insula and right lateral OFC (Figure 6.5). No correlations were found between calorie and pleasantness ratings and taste activation during attentional focus on respectively calories and pleasantness.

Discussion

We investigated the effect of selective attention to hedonics, intensity and caloric content on brain responses during tasting. Taste activation for these selective attention conditions largely overlapped; common activation was found in regions associated with taste processing and food reward such as the rolandic operculum, insula and overlying frontal operculum, striatum, amygdala, thalamus, ACC and middle OFC. Taste activation during selective attention to intensity compared to calories was higher in the right middle OFC. Furthermore, taste activation during selective attention to pleasantness compared to intensity was greater in the right putamen, right ACC and bilateral middle insula, areas associated with food reward. In addition, there was a positive association between taste activation

during selective attention to intensity and intensity ratings in the right anterior insula and right lateral OFC.

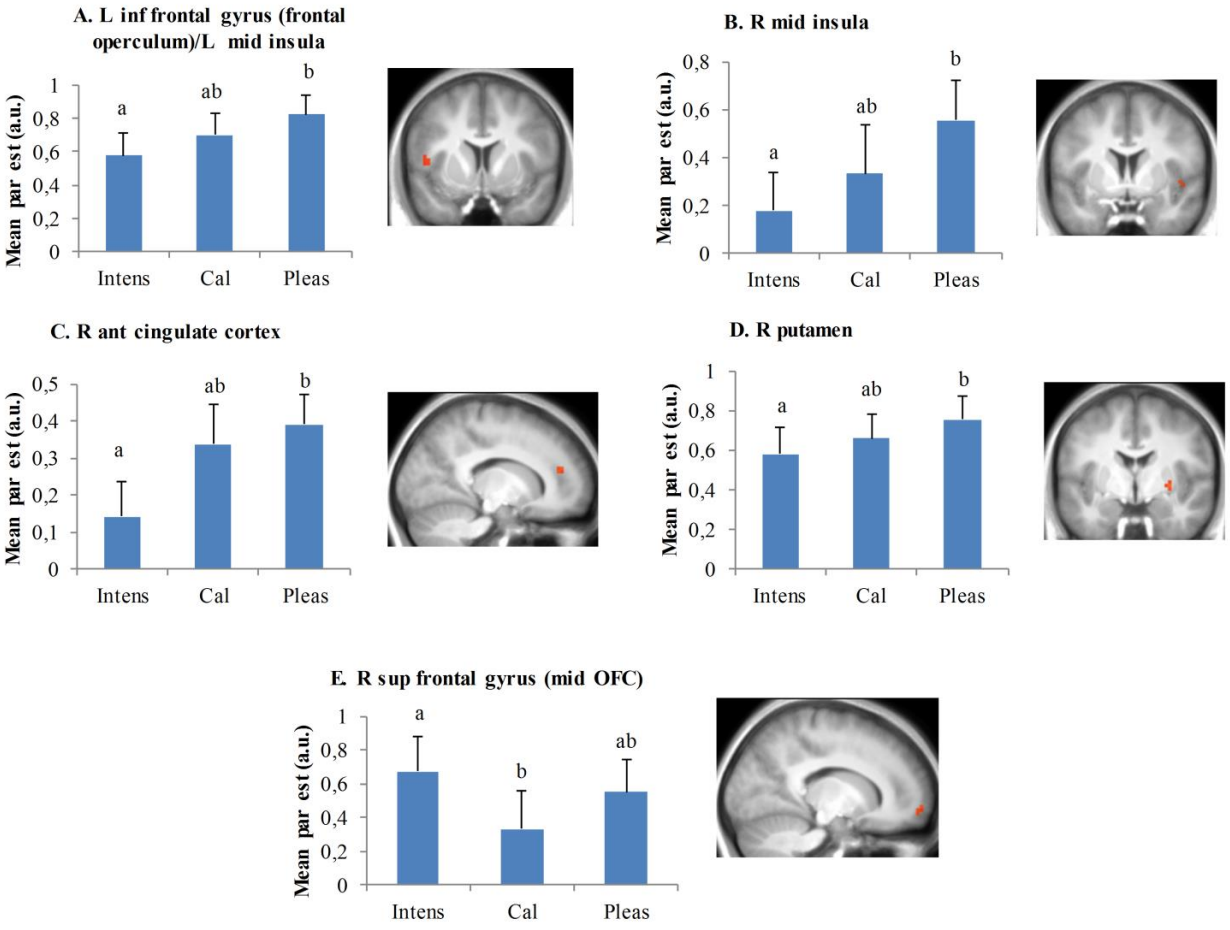

Figure 6.4 Differences in taste activation during selective attention to intensity (intens), caloric content (cal) and pleasantness (pleas) in the (A) left inferior frontal gyrus (frontal operculum)/left middle insula (peak at MNI: -45, 11, 4), (B) right mid insula (peak at MNI: 45, 2, -2), (C) right anterior cingulate cortex (peak at MNI: 15, 44, 19), (D) right putamen (peak at MNI: 30, -4, -2), and (E) right superior frontal gyrus (mid OFC) (peak at MNI: 15, 62, -5). Bars having a different letter differ significantly. Ant = anterior, sup = superior, inf = inferior, mid = middle, L = left and R = right.

Common taste activation

There was overlap in taste activation during selective attention to intensity, caloric value and pleasantness in regions involved in the neural processing of food stimuli. This may be expected because 'bottom-up' effects are equal for all selective attention conditions. This is in line with a study with a similar paradigm, in which taste activation overlapped greatly in the anterior insula and overlying frontal operculum irrespective of whether participants had to indicate the quality, the presence or the pleasantness of a taste, or just tasted passively [35]. Cognitive effects, such as selective attention, are often more difficult to detect with neuroimaging than motor or sensory effects due to

greater trial-to-trial and person-to-person variation [38,39]. This could be the reason why we found relatively few effects of selective attention.

Table 6.2 Brain regions in which there was a significant positive correlation between subjective ratings and taste activation in the three selective attention conditions.

Correlation	*Brain region*	*Cluster size*	*Z-score*	*Peak coordinate*		
				x	***y***	***z***
Intensity ratings (in intensity condition)	R ant insula	18	3.5	33	17	-14
	R inf frontal gyrus (lat OFC)		3.4	33	29	-14
Calorie ratings (in calorie condition)	No regions					
Pleasantness ratings (in pleasantness condition)	No regions					

Activations were thresholded at $p<0.001$, with small volume correction over the ROI volume and a cluster extent threshold of $k>4$ contiguous voxels. Ant = anterior, inf = inferior, lat = lateral and R = right.

Attention-driven differences in taste activation

Insula

Selective attention to pleasantness versus intensity but not caloric content, induced more activation in the bilateral middle insula and left overlying frontal operculum. The middle insula has been implicated in the detection of actual intensity differences [17,18]. However, recently Dalenberg et al. (2015) [16] examined the functional specialization of the insula regarding taste processing in more detail and found that activation in the middle insula is related to the presence of a taste and it's corresponding pleasantness. Several other studies are consistent with a role for the middle insula in pleasantness coding [35,40,41]. For example, Bender et al. (2009) [35] reported that attending to the pleasantness of a taste produced large responses in the middle insula. De Araujo et al. (2003) [40] found that water in the mouth activated the middle insula, but only when participants were thirsty, and thus perceived the water as more pleasant. Finally, Pelchat et al. (2004) [41] found that imagining to eat a pleasant food in comparison to a bland food resulted in increased middle insula activation for participants who had been consuming a monotonous diet for 1.5 days. This latter study agrees with ours that an attentional focus on pleasantness increases middle insula activation. Furthermore, we found that intensity ratings correlated with taste activation during selective attention to intensity in the right anterior insula. This is in concurrence with Dalenberg and colleagues' [16] finding that right anterior insula activation is associated with concentration rather than pleasantness. Overall, our results strengthen the idea that the middle insula is involved in pleasantness coding and the anterior insula in intensity coding.

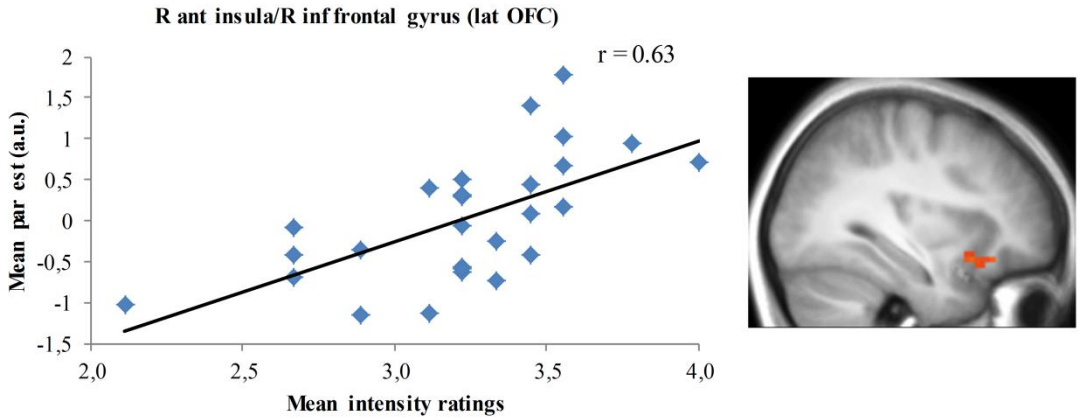

Figure 6.5 Scatterplot of taste activation during selective attention to intensity and subjective intensity ratings in the right anterior insula extending into the right inferior frontal gyrus (lateral OFC) (peaks at MNI: 33 17 -14 and 33 29 -14). Ant = anterior, inf = inferior, lat = lateral and R = right.

OFC

Compared to caloric content, both focussing on intensity and pleasantness during tasting activated the middle OFC more (statistically significant for intensity, trend for pleasantness). Tang et al. (2014) [42] showed that humans are poor in estimating the caloric content of food on pictures. Estimating caloric content may be a largely unconscious process and thus less accessible to conscious evaluations. For example, actual, rather than estimated caloric density of food pictures, predicts the willingness to pay for a food item [42]. Furthermore, estimated expected satiety of food images is not in line with their actual energy content [43]. Evaluating calories based on oral sensations rather than on food pictures, as was done in the current study, may even be more difficult. It is likely that our participants were focussing on sensory cues associated with caloric content such as sweetness, viscosity and creaminess to try and detect caloric content. This may have introduced more variability in taste activation than in other attention conditions. Consequently, this could explain why consciously focussing on calories did not elicit greater activation compared to focussing on the other product properties in any of the ROIs.

No differences between attentional focus to intensity versus pleasantness were found in the middle OFC. The medial parts of the OFC are involved in decoding and monitoring reward, whereas the lateral parts are involved in evaluating punishment [44,45]. Therefore, evaluating the pleasantness of a stimulus was expected to result in the most prominent activation in this area. However, taste intensity and pleasantness are not independent: in general their relationship can be captured in an inverted U-shaped curve [46]. For salty and sour stimuli, intensity and pleasantness are positively correlated up to the peak, whereupon pleasantness declines. Sweetness is almost increasingly pleasant with increasing

intensity [47]. It is therefore difficult to disentangle brain regions involved in encoding pleasantness and intensity [16,17]. Thus, food induced activation in the middle OFC may not only dependent on pleasantness, but also on intensity. Based on our results, this also holds true for the lateral OFC. In this area, we observed a positive association between taste activation when focussing on intensity and intensity ratings.

Putamen

Attentional focus to pleasantness compared to intensity resulted in more taste activation in the putamen, a part of the dorsal striatum [23]. Taste activation in the putamen was found to be modulated by sweetness, saltiness and bitterness irrespective of valence, [17,18], implying its involvement in intensity processing. However, others found that the dorsal striatum is involved in coding food reward [41,48–50]. Especially reward receipt, rather that reward anticipation, is processed by the dorsal striatum [26]. Our findings are consistent with a role for the dorsal striatum in reward receipt, and additionally suggest that selective attention to hedonics enhances taste activation in this region.

ACC

The ACC is implicated in reward receipt [26]. We found that selective attention to taste pleasantness in comparison to intensity was associated with increased ACC activation. In agreement with this, Grabenhorst and Rolls (2008) [12] also observed increased responses in the ACC for paying attention to pleasantness compared to intensity of a monosodium glutamate (umami) solution. We show that this generalizes over taste qualities (sweet, savory and neutral liquids). The exact location of our finding is in the dorsal (also referred to as posterior) part of the ACC (Brodmann area 32). This specific part has been labeled as the 'cognitive division' and is activated by cognitive rather than by emotionally demanding tasks [51]. According to one of the response selection theories, the dorsal ACC controls several motor control systems and decides upon their activation via input received from the dopaminergic midbrain about reward prediction errors [52,53]. Valence, rather than intensity, of a taste may serve as a reward predictor as it signals the value of a reward or punishment, i.e. the degree of nutritiousness or poisonousness of a food. Interestingly, ACC activation evoked by tasting correlated with subsequent ad libitum intake of both a fruit and tomato juice [54]. It must, however, be noted that this location was slightly more anterior than ours. Overall, our results indicate that attentional focus on valence may influence eating behavior by affecting activation in the posterior ACC.

Selective attention in functional neuroimaging taste paradigms

Across functional neuroimaging studies, differences in selective attention are introduced by the different participant instructions used during the delivery of taste stimuli, such as the words: 'taste', 'test' or 'hold the solution in your mouth', a colored field or just a crosshair on the screen [17,55–60]. As a result, participant attention is directed in many different ways, which could lead to variability in taste activation within and between studies. Accordingly, we observed that selective attention to different food properties can indeed result in differences in taste activation. During neuroimaging taste research, selective attention may therefore act as a confounding factor. Reproducibility of (food-related) neuroimaging findings is often difficult [61–63]. Our results indicate that selective attention biases, introduced by a variety of participant instructions, may play a part in the rather low concurrence of neuroimaging findings.

Conclusion

Paying attention to the hedonics, caloric content or taste intensity of a liquid predominantly resulted in common brain activation in regions involved in the neural processing of food stimuli. This likely resulted from 'bottom-up' sensory effects, which are more prominent than 'top-down' attentional effects. Nevertheless, differences were observed between selective attention to intensity versus calories in the right middle OFC, and between selective attention to pleasantness versus intensity in the right putamen, right ACC and bilateral middle insula. Furthermore, intensity ratings correlated with taste activation during selective attention to intensity in the anterior insula and lateral OFC.

Our data suggest a role for the middle and lateral OFC and anterior insula in evaluating intensity of a stimulus rather than caloric content or pleasantness. Moreover, selective attention to pleasantness enhances activation in regions associated with food reward, such as the putamen, ACC and middle insula. Attentional focus on caloric content did not increase taste activation in any region. This implies that explicitly evaluating caloric content is difficult for humans and that this is probably done in a more implicit manner.

In conclusion, statements regarding pleasantness, taste intensity or caloric content can alter the consumption experience through attention-driven effects on the activation of gustatory and reward regions.

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Appendix

Supplementary Table 6.1 Overlapping brain activation during tasting while paying attention to the intensity, calories or pleasantness.

Conjunction	*Brain region*	*Cluster size*	*Z-score*	*Peak coordinate*		
				x	***y***	***z***
Intensity, calories and pleasantness	R rolandic operculum	1072	Infinity	60	-4	16
			Infinity	54	-7	19
			Infinity	60	2	16
			Infinity	57	-16	16
			Infinity	39	-31	16
			Infinity	54	11	-2
	R caudate		Infinity	21	5	22
			Infinity	21	17	16
			Infinity	15	26	-5
			Infinity	21	26	10
	R insula		Infinity	33	-10	16
			Infinity	42	8	7
	R putamen		Infinity	30	-10	-2
			Infinity	30	-7	-8
			Infinity	27	-1	-8
	R amygdala		Infinity	24	2	-14
	L inf frontal gyrus (frontal operculum)	807	Infinity	-60	5	16
			Infinity	-60	11	22
	L rolandic operculum		Infinity	-60	-4	10
			Infinity	-48	-13	22
			Infinity	-51	-10	13
			Infinity	-51	2	4
			Infinity	-45	-28	13
			Infinity	-51	-22	13
	L caudate		Infinity	-21	2	22
			Infinity	-18	-10	19
	L thalamus		Infinity	-12	-19	7
			Infinity	-15	-13	16
			Infinity	-3	-10	10
	L putamen		Infinity	-24	5	7
			Infinity	-24	-4	13
	L insula		Infinity	-36	-10	16
	L ant cingulate cortex	76	Infinity	-9	14	28
			Infinity	-6	5	28
			Infinity	0	2	28
	R ant cingulate cortex		Infinity	9	17	28
	L sup frontal gyrus (mid OFC)	87	Infinity	-21	62	-5
			7.8	-21	47	-11
			7.6	-18	44	-14
	L mid frontal gyrus (mid OFC)		7.4	-30	47	-2
			7.4	-21	38	-14
	R mid frontal gyrus (mid OFC)	36	Infinity	33	59	-8
	R sup frontal gyrus (mid OFC)		Infinity	30	62	-5
	R med frontal gyrus (mid OFC)		7.5	12	68	-2
	R thalamus	9	Infinity	15	-31	1
			7.3	18	-31	7
	L caudate	8	7.7	-12	23	-5
			7.7	-15	26	-2
			7.2	-6	20	-2
	R sup frontal gyrus (mid OFC)	5	7.3	24	41	-14
	L inf frontal gyrus (frontal operculum)	9	6.6	-45	20	34

Activations were thresholded at t = 8, with small volume correction over the ROI volume and a cluster extent threshold of k>4 contiguous voxels. Ant = anterior, sup = superior, inf = inferior, mid = middle, med = median, L = left and R = right.

Supplementary Table 6.2 Average brain activation during tasting compared to rest, while paying attention to the intensity.

Contrast	*Brain region*	*Cluster size*	*Z-score*	*Peak coordinate*		
				x	***y***	***z***
Intensity	R rolandic operculum	913	6.1	60	2	13
	R rolandic operculum		5.7	54	-10	19
	R insula		5.6	33	-4	16
	R rolandic operculum		5.4	39	-34	22
	R pallidum		4.9	27	-7	-5
	R pallidum		4.8	27	-13	-2
	R caudate		4.6	21	2	22
	R putamen		4.5	21	17	13
	R inf frontal gyrus (frontal operculum)		4.5	42	11	10
	R insula		4.5	36	8	10
	R thalamus		4.5	12	-19	7
	R amygdala		4.4	27	2	-11
	R amygdala		4.3	33	-1	-23
	R pallidum		4.3	24	-4	4
	R insula		4.3	36	-22	10
	R putamen		4.2	27	8	13
	L rolandic operculum	596	5.6	-48	-13	22
	L rolandic operculum		5.3	-57	2	13
	L insula		5.2	-33	-7	16
	L insula		5.0	-36	11	16
	L rolandic operculum		4.9	-51	-1	16
	L rolandic operculum		4.7	-51	-7	13
	L rolandic operculum		4.7	-57	-4	10
	L caudate		4.6	-21	-1	19
	L putamen		4.6	-27	-10	13
	L thalamus		4.5	-15	-10	13
	L thalamus		4.4	-15	-19	10
	L pallidum		4.4	-15	-1	4
	L caudate		4.3	-18	17	16
	L rolandic operculum		4.2	-48	2	7
	L caudate		4.2	-18	8	19
	L putamen		4.2	-30	-13	-2
	L inf frontal gyrus (lat OFC)	20	5.5	-39	44	-14
	L inf frontal gyrus (lat OFC)		4.1	-30	38	-17
	R mid frontal gyrus (mid OFC)	85	4.8	30	59	-8
	R sup frontal gyrus (mid OFC)		4.1	21	47	-14
	R mid frontal gyrus (mid OFC)		3.8	36	53	-11
	R sup frontal gyrus (mid OFC)		3.7	24	65	-5
	R sup frontal gyrus (mid OFC)		3.3	15	59	-14
	L sup frontal gyrus (mid OFC)	79	4.7	-18	65	-5
	L sup frontal gyrus (mid OFC)		4.3	-18	56	-11
	L mid frontal gyrus (mid OFC)		3.6	-33	50	-8
	L insula	13	4.5	-27	23	13
	R sup frontal gyrus (mid OFC)	5	4.2	15	23	-17
	R inf frontal gyrus (lat OFC)	5	4.1	30	29	-20
	R mid cingulate cortex	16	4.0	12	17	28
	R mid frontal gyrus (mid OFC)	5	4.0	33	41	-14
	R mid frontal gyrus (mid OFC)		3.7	39	44	-14
	R mid frontal gyrus (mid OFC)		3.4	45	47	-14
	L ant cingulate cortex	8	3.8	-9	14	28
	L insula	5	3.2	-36	5	-8

Activations were thresholded at $p<0.001$, with small volume correction over the ROI volume and a cluster extent threshold of $k>4$ contiguous voxels. Ant = anterior, sup = superior, inf = inferior, mid = middle, lat = lateral, L = left and R = right.

Supplementary Table 6.3 Average brain activation during tasting compared to rest, while paying attention to the calories.

Contrast	*Brain region*	*Cluster size*	*Z-score*	*Peak coordinate*		
				x	***y***	***z***
Calories	R rolandic operculum	1062	6.0	54	-7	16
	R rolandic operculum		5.8	60	2	13
	R insula		5.7	33	-4	16
	R caudate		5.1	21	26	7
	R pallidum		5.1	27	-7	-5
	R insula		5.0	42	8	7
	R pallidum		4.9	27	-13	-2
	R insula		4.9	36	-31	19
	R caudate		4.8	21	8	19
	R putamen		4.8	27	-1	-8
	R caudate		4.5	18	-7	22
	R thalamus		4.4	18	-13	16
	R caudate		4.3	15	26	-5
	R insula		4.3	33	-22	10
	R putamen		4.2	33	-1	4
	R thalamus		4.1	18	-31	4
	L rolandic operculum	747	5.6	-48	-10	19
	L rolandic operculum		5.2	-51	-7	13
	L insula		4.8	-27	23	13
	L rolandic operculum		4.7	-57	2	13
	L insula		4.7	-45	5	7
	L insula		4.7	-36	-10	16
	L rolandic operculum		4.6	-60	5	16
	L putamen		4.5	-30	-13	-2
	L putamen		4.5	-21	14	7
	L inf frontal gyrus (frontal operculum)		4.5	-36	5	22
	L thalamus		4.4	-21	-31	4
	L caudate		4.4	-21	-1	22
	L thalamus		4.4	-12	-19	7
	L putamen		4.3	-33	-19	-5
	L insula		4.2	-33	11	16
	L caudate		4.1	-18	11	19
	R ant cingulate cortex	25	4.6	12	17	28
	R ant cingulate cortex		3.5	15	29	25
	L ant cingulate cortex	21	4.4	-9	14	25
	L ant cingulate cortex		3.5	-6	5	28
	R mid frontal gyrus (mid OFC)	52	4.3	33	59	-8
	R sup frontal gyrus (mid OFC)		3.6	15	68	-2
	R mid frontal gyrus (mid OFC)		3.3	45	53	-11
	L inf frontal gyrus (lat OFC)	7	4.1	-42	47	-14
	L med frontal gyrus (mid OFC)	54	4.1	-15	68	-2
	L sup frontal gyrus (mid OFC)		4.0	-18	65	-5
	L sup frontal gyrus (mid OFC)		3.9	-24	65	-2
	L mid frontal gyrus (mid OFC)		3.6	-33	44	-5
	L mid frontal gyrus (mid OFC)		3.5	-30	47	-2
	L mid frontal gyrus (mid OFC)		3.4	-36	53	-5
	L caudate	6	4.0	-15	26	-2
	L caudate		3.7	-12	23	-5
	L rolandic operculum	6	3.7	-39	-31	22

Activations were thresholded at $p<0.001$, with small volume correction over the ROI volume and a cluster extent threshold of $k>4$ contiguous voxels. Ant = anterior, sup = superior, inf = inferior, mid = middle, med = median, L = left and R = right.

Supplementary Table 6.4 Average brain activation during tasting compared to rest, while paying attention to the pleasantness.

Contrast	*Brain region*	*Cluster size*	*Z-score*	*Peak coordinate*		
				x	***y***	***z***
Pleasantness	R rolandic operculum	1294	6.5	57	2	13
	R rolandic operculum		6.3	54	-7	16
	R rolandic operculum		5.8	39	-34	22
	R pallidum		5.7	27	-7	-5
	R pallidum		5.4	27	-13	-2
	R caudate		5.4	21	8	16
	R putamen		5.3	33	-7	13
	R caudate		5.3	21	17	10
	R caudate		5.2	21	26	10
	R insula		5.0	45	8	7
	R rolandic operculum		4.9	45	2	22
	R insula		4.9	39	5	-11
	R putamen		4.9	30	-19	1
	R insula		4.8	33	-22	10
	R thalamus		4.8	12	-19	7
	R caudate		4.8	21	26	1
	L rolandic operculum	1008	5.7	-57	2	13
	L rolandic operculum		5.7	-48	-10	19
	L insula		5.5	-45	8	7
	L rolandic operculum		5.5	-51	-7	13
	L insula		5.2	-27	26	13
	L putamen		5.1	-30	-13	-2
	L thalamus		5.0	-12	-19	7
	L insula		5.0	-33	-10	16
	L putamen		4.9	-18	14	4
	L putamen		4.9	-18	14	16
	L insula		4.9	-27	32	4
	L insula		4.8	-30	14	16
	L putamen		4.8	-21	8	10
	L putamen		4.8	-21	14	10
	L thalamus		4.7	-15	-10	13
	L thalamus		4.6	-18	-28	1
	L ant cingulate cortex	7	5.0	-15	44	13
	L med frontal gyrus (mid OFC)	89	4.8	-15	68	-2
	L sup frontal gyrus (mid OFC)		4.6	-18	65	-5
	L mid frontal gyrus (mid OFC)		3.8	-39	56	-2
	L sup frontal gyrus (mid OFC)		3.5	-12	59	-14
	L mid frontal gyrus (mid OFC)		3.2	-33	44	-5
	R mid frontal gyrus (mid OFC)	62	4.6	33	59	-8
	R sup frontal gyrus (mid OFC)		3.8	15	65	-5
	R mid frontal gyrus (mid OFC)		3.5	45	47	-14
	R inf frontal gyrus (lat OFC)		3.4	54	44	-11
	R mid frontal gyrus (mid OFC)		3.4	45	53	-11
	R inf frontal gyrus (lat OFC)		3.3	51	50	-5
	R ant cingulate cortex	71	4.6	15	44	19
	R ant cingulate cortex		4.5	12	17	28
	R ant cingulate cortex		4.3	18	44	7
	R ant cingulate cortex		4.3	18	44	13
	R ant cingulate cortex		4.3	15	50	13
	R ant cingulate cortex		4.1	15	29	25
	R ant cingulate cortex		3.9	12	47	22
	R ant cingulate cortex		3.8	15	38	10
	R ant cingulate cortex		3.7	12	29	16

R ant cingulate cortex		3.6	6	5	28
L inf frontal gyrus (lat OFC)	9	4.5	-45	47	-14
L ant cingulate cortex	13	4.1	-9	14	28
L caudate	10	3.9	-6	20	-2
L caudate		3.9	-12	23	-5
L caudate		3.8	-15	26	-2
L sup frontal gyrus (mid OFC)	8	3.8	-18	41	-14
R sup frontal gyrus (mid OFC)	9	3.7	15	23	-17
R sup frontal gyrus (mid OFC)		3.6	15	32	-17
R sup frontal gyrus (mid OFC)	8	3.5	21	44	-14

Activations were thresholded at $p<0.001$, with small volume correction over the ROI volume and a cluster extent threshold of $k>4$ contiguous voxels. Ant = anterior, sup = superior, inf = inferior, mid = middle, lat = lateral, med = median, L = left and R = right.

Chapter 7

General discussion

Table 7.1 Overview of the main findings.

Study design	Main findings
Randomized crossover study in which brain responses evoked by oral exposure to glucose, fructose, maltodextrin, sucralose and maltodextrin + sucralose were obtained on two occasions, once during hunger and once during satiety.	**Content** *Chapter 2* • Stimulus energy content interacted with hunger state in among others the anterior insula, thalamus and middle cingulate cortex. *Chapter 3* • Brain responses to oral glucose versus fructose were greater in the ACC during hunger and the precentral gyrus during hunger and satiety. • Brain responses to oral fructose versus glucose were greater only during satiety in, among other regions, the superior frontal gyrus. **Character** *Chapter 4* • During hunger, negative correlations were found between brain activation induced by oral exposure to calories from a simple maltodextrin solution and trait reward sensitivity in the caudate, amygdala and ACC. In contrast, during satiety, taste responses correlated positively with trait reward sensitivity in the caudate. • These results were not replicated when using a sucrose sweetened soft drink.
Within-subject study in which brain responses evoked by a low- and high-caloric lemonade cue and oral exposure to a perceived low- and high-caloric lemonade receipt were obtained.	**Character/Cognitive effects** *Chapter 5* • No differences in brain activation were found for visual exposure to a low-caloric versus high-caloric anticipatory cue. • However, tasting a lemonade that was perceived as low-caloric compared to high-caloric did result in more brain activation in the putamen. • Taste activation for the perceived low-caloric compared to high-caloric lemonade correlated positively with general health interest in the putamen.
Within-subject study in which brain responses evoked by oral exposure to a tomato juice, fruit juice and water were obtained while paying attention to the taste intensity, pleasantness or caloric content.	**Cognitive effects** *Chapter 6* • In general, taste-related responses greatly overlapped during selective attention to calories, intensity and pleasantness. Relatively small differences were found: • Paying attention to pleasantness compared to intensity induced greater taste activation in the putamen, ACC and middle insula. • Paying attention to intensity compared to calories induced greater taste activation in the middle OFC. • Paying attention to calories compared to intensity or pleasantness yielded no significant differences in taste activation.

In this thesis we sought to gain more insight into how the experience of food consumption varies from person-to-person and situation-to-situation. The overall aim was to assess the effect of food nutrient content, cognitive effects and character on brain activation during tasting. This final chapter starts with an overview of the main findings, followed by a discussion and interpretation of the results. Additionally, we address methodological considerations, implications and suggestions for future research.

Main findings

An overview of the main findings of this thesis can be found in Table 7.1. Brain responses related to the consumption experience were modulated by content related factors, i.e. stimulus energy content and sugar type, character related factors, i.e. reward sensitivity and health interest and cognitive effects, i.e. food labels and selective attention.

Caloric content

In the first study described in this thesis, a comparison was made between taste activation evoked by caloric (maltodextrin and maltodextrin + sucralose) and non-caloric (sucralose) stimuli. In addition, there was examined in how far these taste responses were modulated by hunger state. No main effect of energy content was found. However, energy content and hunger state interacted in among other regions the bilateral middle cingulate cortex (also referred to as median cingulate cortex in this thesis), bilateral ventrolateral prefrontal cortex, right anterior insula and right thalamus (**Chapter 2**).

Sugar type: glucose versus fructose

Data from the first study was also used to investigate differences in taste activation between oral exposure to glucose and fructose during hunger and satiety. Tasting glucose compared to fructose induced greater brain responses in the right ACC during hunger and left precentral gyrus during hunger and satiety. Oral exposure to fructose relative to glucose elicited greater responses only during satiety in among others the left superior temporal pole and the left superior frontal gyrus (**Chapter 3**).

Reward sensitivity

Finally, a third research question was examined by studying data obtained during the first study. We assessed the associated between brain responses to oral calories (maltodextrin + sucralose minus sucralose) and trait reward sensitivity in different hunger states. Neural responses to oral calories from a simple maltodextrin solution correlated negatively with trait reward sensitivity in the right ventral

striatum (caudate), right amygdala and bilateral ACC during hunger. During satiety, there was a positive correlation between brain activation evoked by tasting calories and reward sensitivity in the left caudate. In addition, we examined the above association in data from another, previously executed, study. In this study, taste activation was obtained for two more complex products, a sucrose and non-caloric sweetened soft drink. Using this dataset, however, the results were not replicated **Chapter 4**).

Food labels and health interest

In the second study outlined in this thesis, we assessed to what extent brain responses induced by anticipation and receipt of a lemonade with different levels of perceived caloric content are influences by health interest. No differences were found for brain activation evoked by visual exposure to a low-caloric and high-caloric anticipatory cue. Tasting a perceived low- compared to high-caloric lemonade induced greater activation in the left putamen. Furthermore, health interest scores correlated significantly with taste activation induced by a perceived low- versus high-caloric lemonade in the right dorsal striatum (putamen). Additionally, explicit liking and wanting scores (explicit ratings) and implicit wanting scores (motivational reaction time task) that were obtained during the study were not significantly different between the lemonades (**Chapter 5**).

Selective attention

In the last study, we investigated the effect of selective attention to hedonics, intensity and caloric content on brain responses during tasting (fruit juice, tomato juice and water). Paying attention to either the taste intensity, pleasantness or caloric content of a beverage during consumption predominantly yielded similar brain responses. However, small differences were observed in the right putamen, right ACC and bilateral middle insula for paying attention to pleasantness relative to intensity. Moreover, paying attention to intensity compared to calories resulted in greater taste activation in the right middle OFC. Finally, paying attention to calories compared to intensity or pleasantness yielded no significant differences in taste activation. In addition, we assessed the relationship between taste activation during attentional focus on intensity, calories or pleasantness and subjective ratings. Intensity ratings correlated with taste activation during selective attention to intensity in the anterior insula and lateral OFC (**Chapter 6**). No correlations were found for the other selective attention conditions.

Discussion and interpretation of the results

Findings from this thesis combined with previous research (e.g. [1–3]) suggest that energy may be sensed in the oral cavity (**Chapter 2**). This puts the usage of non-nutritive sweeteners such as saccharin, aspartame and sucralose [4] as replacement of regular nutritive sweeteners such as sucrose in a new perspective. The usage of non-nutritive sweeteners was already under debate since the discovery that they likely disrupt the conditioned association between sweetness and energy content and thereby may increase food intake and body weight in rats [5]. However, to date, there is no consensus regarding the effect of non-nutritive sweeteners on weight gain. For instance, several short-term intervention studies found that the intake of non-nutritive sweeteners may help with weight reduction [6–9]. In line, a recent systematic review of evidence from human intervention studies also concluded that the usage of non-nutritive sweeteners in place of sugar, leads to reduced energy intake and body weight [10]. Nevertheless, others have pooled data of many animal and observational studies and reported that consumption of non-nutritive sweeteners over a period of many years was associated with increased risk of being overweight and obese [11]. Interestingly, several neuroimaging studies showed that the brains of frequent diet soda consumers tended to respond differently to sucrose [12], saccharin and sweet taste in general [13] compared to less frequent or non-diet soda consumers. For example, during oral exposure to saccharin, brain activation in the caudate, a brain reward area, correlated negatively with weekly diet soda consumption [13]. In sum, accumulating evidence suggests that non-nutritive sweeteners are unable to mislead our brain [14]. In particular because of the incapability of sweet taste without calories to elicit similar brain responses compared to sweet taste with calories. This possibly translates to a dissimilar consumption experience. There is however no consensus about how this affects eating behavior.

Besides caloric content, there are other factors, like personal characteristics, that may influence the consumption experience. In the obesogenic environment we currently live in, food cues are omnipresent and unescapable. Especially reward sensitive individuals may be affected by this environment and engage in overeating. To illustrate, reward sensitivity was found to correlate with BMI in normal-weight individuals [15] and has been associated with overweight and mild, but not morbid, obesity [16]. Indeed, individual differences in reward sensitivity tend to predict the brain response to palatable food images [17]. In this thesis, we additionally provide a proof of principle that brain reward activation induced by consumption of calories is also modulated by trait reward sensitivity (**Chapter 4**). To our knowledge, we were the first to examine the effect of reward sensitivity on brain responses evoked by food tasting. This finding serves as another step towards creating a more complete overview of factors that may influence the consumption experience. With regard to personality, this overview so far includes, but is not limited to the following traits: dietary restraint, impulsivity, external eating, diet importance and food addiction (for an overview see [18]). It

must however be noted that the number of studies on these topics is limited and findings are not very consistent [18].

Of major importance in the current obesogenic environment is to direct people towards making healthier choices. For one, this is done by nutrition-related claims (e.g. "calcium builds strong bones", "free from sugar", "light" and "organic"), information regarding ingredients and energy content and food logos (e.g. the Choices logo which is used in The Netherlands) on food labels in the supermarket [19–21]. In addition, taste-related claims (e.g. "now even tastier") are used by the food industry to market their products, irrespective of healthiness. Food claims and favorable information on food packages were found to improve consumer attitudes towards the product and their purchase intentions [22]. Information conveyed on food labels tends to be used the most by women, older consumers, more educated consumers, consumers in the higher social strata and consumers interested in health [23,24]. Information most read was on fat content and calories [24]. Second, educational campaigns regarding a healthy diet- and life-style are used to increase consumer health interest [25]. Previous research indicates that health interest is associated with healthier food choices [26–28]. In line with this, results presented in **Chapter 5** show that a greater brain reward response to the taste of a product that is perceived as healthy (low-caloric) compared to unhealthy (high-caloric) correlates positively with health interest. Moreover, since the perception of this product was manipulated by simple food claims (the word "low-caloric" versus "high-caloric"), it was additionally shown that such claims can be powerful tools to influence product attitudes.

In addition, the importance of attentional effects during food consumption has been addressed in this thesis. Limited attention to our food during eaten, e.g. due to watching television, listening to the radio, or eating in the company of others, can lead to a higher energy intake [29–33]. In accordance with this, brain responses to food viewing were absent or altered when participants were distracted in many brain regions involved in encoding taste intensity and pleasantness [34]. Rather than total distraction, food labels facilitate selective attention to one aspect (e.g. pleasantness, taste intensity or health) over others. Findings described in **Chapter 6** indicate that such attentional biases may alter taste-related brain activation to some extent and consequently may alter the consumption experience.

Methodological considerations

Experimental setup

In this thesis we sought to gain more insight into internal and external factors that influence the consumption experience. It must, however, be noted that the test setup used (see Figure 1.3) during taste-relate fMRI research is not similar to a real-life setting. For example, ordinarily foods are not

consumed when lying down, swallowing is voluntarily instead of cue-triggered, and the surroundings are not as noisy as in a MRI-scanner. Therefore, data gathered by means of an fMRI taste paradigm may not be completely representative for what happens in real life. However, effects of body position (seated versus supine) on flavor perception were found to be mild [35]. Moreover, several studies have shown that brain responses to food cues are linked to eating behavior outside the scanner and weight gain [36–40]. For example, Mehta et al. (2012) [36] showed that in normal-weight individuals, fullness ratings after a breakfast were correlated with activation in the dorsal striatum and that greater activation in the OFC, amygdala, insula and nucleus accumbens for fattening food cues correlated with greater consumption of calories from fat at an ad libitum buffet. Furthermore, Murdaugh et al. (2012) [37] found that in obese participants, brain responses to high-calorie food versus control images in regions involved in food motivation and attention before a weight-loss program predicted the success of the program and weight control over a follow-up period of 9 months. In addition, also Lawrence et al. (2012) [38] found an association between brain responses and eating behavior. Here, greater activation in the nucleus accumbence for food versus non-food images was associated with increased subsequent snack consumption. Finally, activation evoked by actual consumption in the scanner was also found to be associated with subsequent ad libitum intake and weight gain [39,40]. For instance, in a study of Spetter et al. (2012) [39] ACC activation induced by sips of tomato and fruit juice predicted subsequent ad libitum consumption of these beverages. Also Stice et al. (2010) [40] observed that individuals who gained weight over a period of 6 months showed a decrease in striatal activation compared to baseline for the receipt of a milkshake versus a tasteless solution, whereas in weight-stable individuals no such reduction was present. Thus, even though food-related fMRI paradigms are not similar to a real-life situation, they still provide valuable information about the functioning of the brain with regard to eating behavior and weight gain. New developments, like the development of an open upright MRI scanner that can be used for fMRI during the upright position [41] , may even increase the potential of food-related fMRI.

Test stimuli

The test stimuli used in the experiments described in the first part of this thesis are simple sugar solutions (**Chapter 2, 3 and 4)**. In this part of the thesis we focussed on the effect of energy and sugar content on the consumption experience. As these topics have not been broadly investigated before, we chose to use simple solutions to acquire a proof of principle with very controlled stimuli. A strength is the thorough matching of the stimuli on sweetness (when applicable), which made it possible to take into account any sweetness effects. It speaks for itself that follow-up research is needed to investigate whether the findings translate to real drinks and other food products. In the second part of this thesis **(Chapter 4-6)**, the practical implications for the topics (personality and cognitions) addressed became more relevant. Therefore, we intended to achieve more generalizability to a real life situation and used

beverages that were commercially available or similar to commercially available variants, i.e. lemonades (**Chapter 4 and 5**) and fruit and tomato juice (**Chapter 6**). A strength of several experimental setups used in this thesis was that the stimulus was kept constant over cognitive manipulations, thus eliminating any sensory differences. For example, one lemonade was presented as low- and as high-caloric. Whether the findings can be generalized to (semi) solids and real meals warrants further investigation.

Study population

In all studies of this thesis we studied young, normal-weight, right-handed, healthy females as participants. Only participants who met with these criteria were selected to create a homogeneous group that could be used to establish proof of principles in the relatively new field that we operate in. For fMRI a homogeneous group is necessary to minimize unwanted variation of the BOLD signal in the regions of interest. Participants were additionally screened and excluded on the basis of several more characteristics. These characteristics varied along with the relevance for the study and included restrained eating tendency, non-nutritive sweetener use and disliking of the products under study, but also, diseases, medicine use, smoking and alcohol consumption. This was done to take into account factors of no-interest that may influence taste responses (e.g. dietary restrained [42,43] and product liking [44]). In this thesis, we tried to map several more factors that may influence the consumption experience, so that in future taste-related neuroimaging research these can also be taken into account during the selection of the study population or can be used as covariates of (no-)interest during analyses. This may facilitate easier detection of proof of principles and/or help with the explanation of the found results. However, as a consequence of the homogeneous group used in this thesis, generalizability of the results to other groups must be done with caution since many individual factors such as gender [45,46], handedness [47,48], age [49,50] and BMI [51–53] have been shown to interact with taste-related brain responses. Nevertheless, this drawback is not specific for fMRI research but is common for a large part of research in the food domain.

Implications

In this thesis we show that the consumption experience varies with food content, personality traits, attitudes and cognitive effects. On the one hand, this provides valuable implications for the food industry. These new insights can be taken into account during the development of personalized nutrition, i.e. nutrition that is tuned to the needs of specific consumer groups. For example, we discovered that reward sensitive and health interested individuals respond differently to respectively actual and perceived oral calories. Food companies can use this information to their advantage and develop personalized products and product packages for these groups. The most evident would be to

make healthy products more appealing, thus to nudge these groups into making healthier choices. To date, an extensive debate is going on about the ethics regarding nudging [54–56]. In our opinion, nudging strategies that promote healthier food choices are a good development, provided that they preserve the freedom of choice [54]. We figure it is a win-win situation, where consumers make healthier food choices and food companies maintain sales. Finally, for our findings that selective attention to food characteristics, as well as caloric and sugar content of a product independent of sweet taste, may alter the consumption experience, implications may be related to the development of respectively food packages/advertisements and product recipes. However, it might be a little too soon to point to concrete implications in this area. Nevertheless, at the very least our findings provide a fundamental basis for more applied research in this field.

On the other hand, our results have implications for the data collection and processing in the food-related neuroimaging field. Firstly, the above mentioned factors, especially trait and attitude factors, can be taken into account during data analyses to explain variation and thereby to reduce noise. Secondly, the awareness that oral calories and sugars can pose an effect on their own, independent of sweet taste, may allow for better standardization of gustatory stimuli. Finally, our findings regarding selective attention emphasize the need for standardization of participant instructions during fMRI tasks across the field.

Suggestions for future research

First, it would be interesting to examine the generalizability of our results to other population groups and products. For example, we wonder if tasting a low- versus high-caloric lemonade also induces more brain activation in reward related areas in men. This might be very typical for women. Or, another example, do health-interested individuals also respond stronger to the healthy variant of more complex products, such as chocolate milk or ice-cream? These topics could be part of follow-up research. In addition, it goes without saying that reproducibility of our results needs to be established with the help of additional studies.

It must also be noted that, although we addressed some important factors for explaining variation in taste-related brain responses in this thesis, several other important factors still need to be examined further. So far, evidence for effects of hunger state, gender, age and BMI on food-related brain responses is mounting [57]. In contrast, factors related to personality traits have received little attention. Especially those that are important for eating behavior, such as impulsivity, external eating and dietary restraint are likely candidates for further research. How these traits and attitudes interact with cognitive factors such as food-claims and logo’s, product packages, advertisements, food content

information, shelve-position, store build-up, and government campaigns regarding food-education is also material worth studying. This because these factors can relatively easy be used to nudge consumers into making healthier choices. Hence, tailored supermarket-cognitions to the wants and needs of specific consumers may be the future.

Finally, a remark about fMRI research in general. During the past decades fMRI became a popular technique for investigating brain responses [58]. However, a problem of fMRI research that uses more complex fMRI-paradigms, is its low reproducibility [59–62]. Single study findings, especially in a young field such as food-related neuroimaging, are informative, but do not always show the complete picture. Therefore, meta-analyses that combine single study results could provide valuable information in the search for the truth [63]. Standardization of single study-setups, not only with respect to the above mentioned participant instructions, but also regarding task settings, food stimulus usage, participant characteristics, analysis procedures and data reporting may help to increase reproducibility and facilitates the execution of meta-analyses [57]. In addition, it is essential to go beyond univariate analysis and explore the communication between brain regions by means of relatively novel techniques such as functional connectivity and multivariate pattern analysis [64].

Main conclusions

In this thesis, we showed that food energy content, sugar type, trait reward sensitivity, health interest, food labels and selective attention all modulate taste-related brain activation. More specifically, we found that energy and sugar sensing in the oral cavity are hunger state dependent processes, in which food motivational, gustatory and reward regions play a central role. Secondly, brain reward activation in response to oral calories was modulated by reward sensitivity. Thirdly, we found that emphasizing a product's health benefits compared to its health risks by means of simple calorie labels, increased its reward value. Moreover, health-interest correlated positively with such taste-related reward responses. Finally, selective attention to pleasantness relative to intensity of a beverage affected taste activation in reward and gustatory regions.

In conclusion, these findings indicate that the formation of the final consumption experience is a very multifaceted process that dependents on numerous factors integrated by the brain, of which we are just beginning to grasp its complexity. Ongoing mapping of the impact of these factors is of fundamental importance for understanding why we eat the way we eat. Via the research in this thesis we hope to have provided just another piece to this complex puzzle.

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Summary

Food does not always ‘taste’ the same. During hunger, for example, food may be tastier compared to during satiety. Many other internal and external factors affect the way we experience our food and make it a dynamic process. Our brain is responsible for weighing and integrating these factors and forms the final consumption experience. Mapping the impact of all factors that influence the consumption experience is of fundamental importance for understanding why we eat the way we eat. Important drivers for food consumption are its rewarding capacity, healthiness and caloric content. Furthermore, in the current supermarket environment, advertisements and food claims are omnipresent, and may exert influence on our consumption experience by triggering all kinds of cognitive processes. Therefore, in this thesis we aimed to assess the effect of food content (caloric content and sugar type), character (personality trait reward sensitivity and attitude health-interest) and cognitive effects (labeling/claim effects and selective attention to food properties) on brain activation during tasting. Such taste-related brain responses were obtained with the use of functional Magnetic Resonance Imaging while administering small sips of liquid to young, normal weight female participants in a MRI scanner.

To begin with, we focussed on the effect of caloric content on taste responses (**Chapter 2**). An important function of eating is ingesting energy, and the ability to sense energy in the oral cavity would therefore be biologically relevant. However, in this thesis we showed that oral exposure to caloric (maltodextrin and maltodextrin + sucralose) and non-caloric (sucralose) stimuli does not elicit discriminable responses in the brain when averaged over hunger and satiety. Nevertheless, energy content did interact with hunger state in several brain regions involved in inhibition (approach-avoidance behaviors) and gustation: the middle cingulate cortex, ventrolateral prefrontal cortex, anterior insula and thalamus. Thus, brain activation in response to oral calories, irrespective of sweetness, seems to be dependent on hunger state.

In addition to the detection of oral calories in general, we examined whether different sugar types, glucose and fructose, can be sensed in the oral cavity (**Chapter 3**). Tasting glucose compared to fructose evoked greater food reward (anterior cingulate cortex, ACC) activation during hunger and greater food motivation (precentral gyrus) activation during hunger and satiety. Responses to oral fructose relative to glucose were greater only during satiety in an area associated with inhibitory control (superior frontal gyrus). It appears that oral glucose and fructose evoke differential brain responses, independent of sweetness.

Secondly, we investigated in how far reward sensitivity, a personality trait, affected brain responses to calories in the oral cavity (**Chapter 4**). This because a food’s reward value is highly dependent on its caloric content. Sensitivity to rewards was measured with the Behavioral Activation System Drive scale and was correlated with oral calorie activation from a simple maltodextrin solution and a sucrose

sweetened soft drink. Oral calorie activation was obtained by subtracting activation by a non-caloric solution (sucralose solution/non-caloric soft drink) from that by a caloric solution (maltodextrin + sucralose/sucrose sweetened soft drink). We found that neural responses to oral calories from a maltodextrin solution are modulated by reward sensitivity in reward-related areas such as the caudate, amygdala, and ACC. For soft drinks, we found no correlations with reward sensitivity in any reward related area. This discrepancy may be due to the direct detection of maltodextrin, but not sucrose in the oral cavity. However, the absence of this effect in a familiar soft drink warrants further research into its relevance for real life ingestive behavior.

In the last part of this thesis we explored how cognitions modulate the consumption experience. Perceived, rather than actual caloric content, inflicted by calorie food labels, induces cognitive processes that may influence the consumption experience on their own. We tested this in an experiment and found that receipt of a beverage perceived as low- compared to high-caloric induced more activation in the dorsal striatum, a region involved in coding food reward (**Chapter 5**). As low-calorie labels may appeal especially to the health-minded consumers, we correlated brain responses to the receipt of a beverage perceived as low- compared to high-caloric with health interest (measured with the General health interest subscale of the Health and Taste Attitude Scales). Indeed, health interest scores correlated positively with activation in the dorsal striatum.

Rather than focussing participants' attention on differences within one food aspect, in **Chapter 6** we focussed on selective attention to different food aspects, i.e. pleasantness versus taste intensity versus calories. In the supermarket, food labels and claims often do the same. In the first place, paying attention to hedonics, caloric content or taste intensity predominantly resulted in common brain activation in regions involved in the neural processing of food stimuli, e.g. the insula and thalamus. This likely resulted from 'bottom-up' sensory effects, which are more prominent than 'top-down' attentional effects. However, small differences were also observed; taste activation was higher during selective attention to intensity compared to calories in the right middle orbitofrontal cortex and during selective attention to pleasantness compared to intensity in the right putamen, right ACC and bilateral middle insula. Overall, these results indicate that statements regarding food properties can alter the consumption experience through attention-driven effects on the activation of gustatory and reward regions.

Finally, the general discussion (**Chapter 7**) describes main finding and conclusions of this thesis. In sum, we showed that food energy content, sugar type, trait reward sensitivity, health interest, food labels and selective attention all modulate taste-related brain activation. In conclusion, these findings indicate that the formation of the final consumption experience is a very multifaceted process that

dependents on numerous factors integrated by the brain, of which we are just beginning to grasp its complexity.

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Inge

About the author

Curriculum Vitae

Inge van Rijn was born on July 12th, 1987 in Leiden, The Netherlands. After completing secondary school at the 'Bonaventura College' in Leiden, she started the Bachelor's programme 'Nutrition and Health' at the Wageningen University. During her Bachelor she completed a Bachelor thesis (literature review) entitled 'Does the speed of eating affect appetite during a meal and bodyweight during a life time?' After obtaining her Bachelor's degree in 2009, she enrolled in the Master's program 'Nutrition in Health and Disease' at the Wageningen University. She completed a Master thesis in the field of 'Sensory Science and Eating Behavior' entitled 'The effect of protein status on food reward for foods differing in protein content (high and low) and taste (sweet and savory)'. Moreover, she did an internship of 6 months at Friesland Campina (department Sensory Science), where she performed research concerning the testing and validating of implicit liking and wanting techniques. Furthermore, she completed the minor 'Consumer Behavior, Preference and Perception' at Wageningen University. She graduated in 2011.

Inge started in 2012 as PhD candidate at the Division of Human Nutrition, Department Sensory Science and Eating Behavior, Wageningen University. Her research was part of the Food and Cognition Model Systems (FOCOM) project and focussed on the influence of external and internal factors on taste-related brain responses as described in this thesis.

During her PhD project, Inge attended several conferences, where she presented her work during oral talks and poster presentations. In addition, she followed several courses and was involved in teaching and supervising Bachelor and Master students with their theses. Inge was active in the Newtrition Committee, a committee involved in creating a newspaper for the division every 3 months.

List of publications

Publications in peer reviewed journals

Van Rijn, I., Wegman, J., Aarts, E., de Graaf, C., Smeets, P. A. M. Health interest modulates brain reward responses to a perceived low-caloric beverage in females. *Health Psychology* (2016), advance online publication.

van der Laan L. N., Charbonnier L., Griffioen-Roose S., Kroese F. M., **van Rijn I.**, Smeets P. A. M. Supersize my brain: a cross-sectional voxel-based morphometry study on the association between self-reported dietary restraint and regional grey matter volumes. *Biol. Psychol.* 117, 108–116 (2016).

van Rijn, I., Griffioen-Roose, S., de Graaf, C., Smeets, P. A. M. Neural processing of calories in brain reward areas can be modulated by reward sensitivity. *Front. Behav. Neurosci*. 9, 1–10 (2016).

van Rijn, I., de Graaf, C. & Smeets, P. A. M. Tasting calories differentially affects brain activation during hunger and satiety. *Behav. Brain Res*. 279, 139–147 (2015).

Griffioen-Roose S., Smeets P A. M, Weijzen P. L. G., **van Rijn I.**, van den Bosch I., de Graaf C. Effect of replacing sugar with non-caloric sweeteners in beverages on the reward value after repeated exposure. *PLoS One* 8, e81924 (2013).

Submitted papers

Van Rijn, I., de Graaf, C. & Smeets, P. A. M. It's in the eye of the beholder: Selective attention to food properties influences taste activation in gustatory and reward regions, submitted.

Papers in preparation for submission

Van Rijn, I., de Graaf, C. & Smeets, P. A. M. Glucose versus fructose: Differences in taste activation during hunger and satiety, in preparation for submission.

Abstracts and presentations

Van Rijn, I., de Graaf, C. & Smeets, P. A. M. Tasting calories differentially affects brain activation during hunger and satiety. Dutch Neuroscience Meeting (DNM), 11-12 June, 2015, Lunteren, The Netherlands. Oral presentation.

Van Rijn, I., Griffioen-Roose, S., de Graaf, C. & Smeets, P. A. M. Neural processing of calories is modulated by sensitivity to reward in the caudate and anterior cingulate cortex. 37th Annual Meeting of the Association for Chemoreception Sciences (ACHEMS), 22-25 April, 2015, Bonita Springs, United States of America. Poster presentation.

Van Rijn, I., Griffioen-Roose, S., de Graaf, C. & Smeets, P. A. M. Neural processing of calories is modulated by sensitivity to reward in the caudate and anterior cingulate cortex. 39th Annual Meeting

of the British Feeding & Drinking Group (BFDG), 9-10 April, 2015, Wageningen, The Netherlands. Poster Presentation.

Van Rijn, I., de Graaf, C. & Smeets, P. A. M. Tasting energy differentially affects brain activation during hunger and satiety. Annual Meeting of the Organization for Human Brain Mapping (OHBM), 8-12 June, 2014, Hamburg, Germany. Poster presentation.

Van Rijn, I., de Graaf, C. & Smeets, P. A. M. Tasting calories differentially affects brain activation during hunger and satiety. 38th Annual Meeting of the British Feeding & Drinking Group, 3-4 April, 2014, Portsmouth, United Kingdom. Oral Presentation.

Van Rijn, I., de Graaf, C. & Smeets, P. A. M. Energy sensing in the oral cavity: An fMRI study. PhD tour, 11-27 October, 2013, University of Deakin, Melbourne, Australia. Oral presentation.

Overview of training activities

Discipline specific activities

- Symposium 'A flavour of Neuroscience', University of Groningen, 2016, Groningen, The Netherlands
- Tübingen International Summer School: Matters of Taste, University of Tübingen, 2015, Tübingen, Germany
- Dutch Neuroscience Meeting, 2015, Lunteren, The Netherlands
- Course 'Advanced course in fMRI data analysis', Donders Centre for Cognitive Neuroimaging, 2015, Nijmegen, The Netherlands
- 37th Annual Meeting of the Association for Chemoreception Sciences , 2015, Bonita Springs, United States of America
- 39th Annual Meeting of the British Feeding & Drinking Group, 2015, Wageningen, The Netherlands
- Course 'Data Analysis in MATLAB', AMC Graduate School, 2014, Amsterdam, The Netherlands
- Annual Meeting of the Organization for Human Brain Mapping, 2014, Hamburg, Germany
- 38th Annual Meeting of the British Feeding & Drinking Group, 2014, Portsmouth, United Kingdom
- Course 'Linear Models', Graduate School PE&RC, 2013, Wageningen, The Netherlands
- Course 'Regulation of energy intake: the role of product properties', Graduate School VLAG, 2012, Wageningen, The Netherlands

- Course ‘SPM course’, Wellcome Trust Centre for Neuroimaging, University College London, 2012, London, United Kingdom
- MR-userday, Hospital Gelderse Vallei, 2014, Wageningen, The Netherlands
- 10th Dutch Endo-Neuro-Psycho Meeting, 2012, Lunteren, The Netherlands
- 11th International Conference on the Application of Magnetic Resonance in Food Science, 2012, Wageningen, The Netherlands

General courses

- Course ‘Good Clinical Practice’, Hospital Gelderse Vallei, 2015, Ede, The Netherlands
- Course ‘Career orientation’, WGS (Wageningen Graduate School), 2015, Wageningen, The Netherlands
- Workshop ‘Indesign’, Gildeprint, 2015, Wageningen, The Netherlands
- Course ‘Scientific writing’, WGS, 2014, Wageningen, The Netherlands
- Course ‘Effective behavior in your professional surroundings’, WGS, 2014, Wageningen, The Netherlands
- Course ‘Teaching and supervising thesis students’, Educational Staff Development Wageningen University, 2013, Wageningen, The Netherlands
- Course ‘How to give and receive feedback’, Wageningen University, 2012, Wageningen, The Netherlands
- PhD introduction week, Graduate School VLAG, 2012, Wageningen, The Netherlands
- Course ‘Scientific publishing’, WGS, 2012, Wageningen, The Netherlands

Optional courses and activities

- Staff seminars and research presentations, Division of Human Nutrition, 2012-2016, Wageningen, The Netherlands
- FOCOM meetings, 2012-2015
- PhD tour, 2013, Melbourne and Sydney, Australia
- Preparing PhD research proposal, 2012

Colophon

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