Between odours and overeating

Behavioural and neurobiological mechanisms of olfactory food-cue reactivity

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Chapter 1

General introduction

Background and aim

Food is available around every corner and cues of delicious calorie-dense foods tempt us to eat, even in the absence of hunger. Over the course of our evolutionary history food cue responses that stimulate energy intake have been beneficial in periods of scarcity, but are thought to stimulate overeating and obesity in an environment with an abundance of food cues [1-3]. Although there is a large group of individuals that struggle to balance food intake and metabolic needs in this obesogenic environment, most people are still capable of regulating their food intake and maintaining a healthy weight.

Internal metabolic signals together with signals from the environment provide essential input for the regulation of food intake [4]. It has been hypothesized that eating behaviour of obese individuals is more driven by external food cues than by their metabolic state [5,6]. Understanding the interaction between metabolic signals of hunger and external sensory signals from food is crucial for facilitating regulation of body weight and promoting health. Olfactory stimuli are potent cues for the availability of palatable and nutritious food sources. Although we are often unaware of the impact of food odours, they play a crucial part in our eating experience. The aim of the research described in this thesis was to elucidate the role of odours in (over)eating.

Food intake regulation: Metabolic signals

Metabolic signals related to appetite are important determinants of how much and how often food is consumed [7,8].

Increased appetite, or hunger, reflects the physical need for energy and nutrients [4,9]. Metabolic signals such as increased ghrelin concentrations stimulate food intake [10-12]. Feelings of hunger and satiety are not only a reflection of metabolic signals, but contain a large anticipatory cognitive component (*e.g.,* expectations, beliefs, attention). Sensory and cognitive factors during the anticipatory and ingestive phase of eating can induce conditioned responses called cephalic phase responses [8,9]. These physiological responses (*e.g.,* salivation, increased gastro-intestinal enzyme secretion and hormone release) have a rapid, short-lasting nature, and have a relatively small magnitude as compared to physiological responses during digestion and absorption [13-16]. Cephalic phase responses are thought to aid in the ingestion, digestion and absorption of consumed nutrients [13-15], but have also been argued to stimulate meal initiation [15,16]. Satiation includes all events that

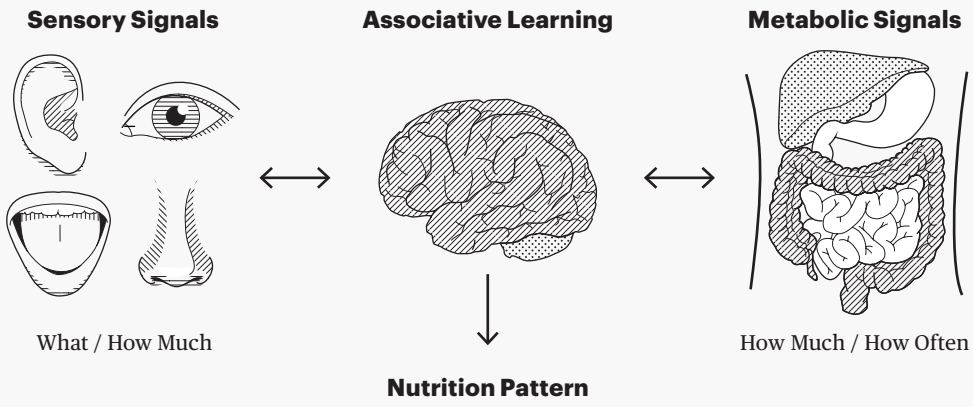

Figure 1.1. Factors affecting eating behaviour. Via associative learning, sensory signals related to food are linked to metabolic signals experienced after food intake. These, largely unconscious, learned associations shape our nutrition pattern (based on De Graaf & Kok [7], with permission).

operate during the course of a meal. During this phase, signals from the gastrointestinal tract (gastric distention, hormones, peptides) bring an on-going eating episode to an end [17-20]. Satiety describes the processes operating after termination of meal intake and is characterized by a suppressed drive to eat and continued inhibition of eating until a next eating episode is induced [19,20].

Food intake regulation: Sensory signals

Sensory signals in the anticipatory and ingestive phases of eating are thought to influence predominantly what we eat, but flavour (taste, smell) and texture may also be involved in how much we eat. How a food smells, looks, feels, tastes and sounds determines our likes and dislikes.

Already before birth [21] and throughout our lifetime there are numerous situations in which we experience sensory signals followed by physiological consequences after consumption. Via associative learning, food cues become predictors of physiological consequences (see **Figure 1.1.**) [22-24]. After repeated consumption

we can use sensory information to determine whether the physiological consequences of eating this product are positive or negative. Highly negative physiological experiences such as nausea or vomiting can lead to stimulus aversion, even after just one paired exposure [4,25]. This gives us the evolutionary benefit of quickly learning to avoid the dangers of eating unripe or spoilt food. On the other hand, we can learn to experience food cues as pleasant when the food they belong to provides a good source of energy and nutrients, such as protein, fat and carbohydrate [26].

SENSORY-SPECIFIC SATIETY

The general decrease of hedonic value of food-related sensory stimuli after food intake is referred to as *alliesthesia* [27-29]. This effect is less strong for cues of palatable food [29,30]. Rolls *et al*. [31] were the first to demonstrate *sensory-specific satiety*, a phenomenon that describes a more pronounced decrease in the rated pleasantness for the taste of a previously consumed food than for a food that was not eaten. This sensory-specific reduction was also observed for subsequent food intake. Greater similarity between the evaluated sensory signals and the consumed food may lead to more pronounced sensory-specific satiety. This entails that pleasantness decreases most for the specific sensory cues of the food that was consumed (*e.g.* a high-energy sweet chocolate bar), but also impacts evaluation of sensory cues of similar products to a lesser extent (*e.g.* a low-energy sweet strawberry). Sensory-specific satiety is thought to prevent dietary monotony and stimulate consumption of a variety of nutrients [8]. Because this type of satiety is sensory-specific, it leaves room for further consumption and perhaps also overconsumption when a large variety of food is available [32-34]. It has been demonstrated for taste, smell, texture and appearance [35-39]. Mere exposure to a food odour for the duration of a meal also decreased pleasantness for odour-related food cues [40], which suggests a role for olfaction during satiety. Next to this appetite inhibiting effect, odours are proposed to stimulate appetite in anticipation of ingestion [41,42].

The human olfactory system

The olfactory sense is involved in ingestive behaviour, avoidance of environmental hazards, and social communication [42,43], all vital for survival. In contrast to the visual system [44-46], the olfactory system is still poorly understood and has been underrepresented in scientific research. In 2004 Axel and Buck received the Nobel

Prize in physiology or Medicine for their work on identification of genes that encode families of olfactory-receptor proteins that pair to specific classes of odour molecules [47]. This gave olfactory science an impulse. In a recent study, humans were estimated to detect and distinguish at least 1 trillion olfactory stimuli [48]. The ability for odour naming is very limited in humans [49], but was found to be better for food compared to non-food odours [50]. Our poor language capacity to describe odours and rapid description in terms of pleasantness [51] indicate that naming is not necessary, as long as odours trigger the right association. These findings suggest that the sense of smell is more involved in detecting differences in the surroundings and determining quality rather than in defining the surroundings in detail. In the food domain, odours are seen as potent cues indicating food availability, pleasantness and safety [42,52–55]. Moreover, a loss of smell was proposed to lead to poor appetite, inappropriate food choices and reduced food intake [56].

Perception of olfactory food cues starts when volatile chemical compounds originating from food bind to olfactory receptors embedded in the olfactory epithelium, located at the roof of the nasal cavity (see **Figure 1.2.**) [57]. Olfactory receptors can be found at the ends of dendrites of olfactory sensory neurons that extend into the nasal cavity through a sieve-like bone structure called the cribriform plate [43]. On the other side of the cribriform plate, axons of these olfactory sensory neurons converge into a structures within the olfactory bulb called glomeruli [58,59]. Each specific odour molecule elicits a specific pattern of spatial activity in the glomerular layer of the olfactory bulb. This pattern is referred to as an 'odour image' and is regarded as a dynamic code for determination of odour identity and -concentration [59,60]. From the glomeruli, axons of olfactory sensory neuron make synaptic contact with mitral cells that relay the olfactory signals via the olfactory tract to the piriform cortex, entorhinal cortex and the amygdala [57,61]. The piriform cortex is thought to be involved in processing of the perceptual qualities of the odorant, needed to determine what is smelled. The absence of relay through the thalamus at this stage is unique to the olfactory sense. The orbitofrontal cortex receives input directly from the piriform cortex and indirectly from the amygdala and entorhinal cortex via the thalamus. In this network, the amygdala could shape the olfactory signal based on emotional salience. The orbitofrontal cortex is proposed to be involved in higher-order cognitive processing, integrating experiences and influences from other senses and determining the quality or value of the olfactory stimulus.

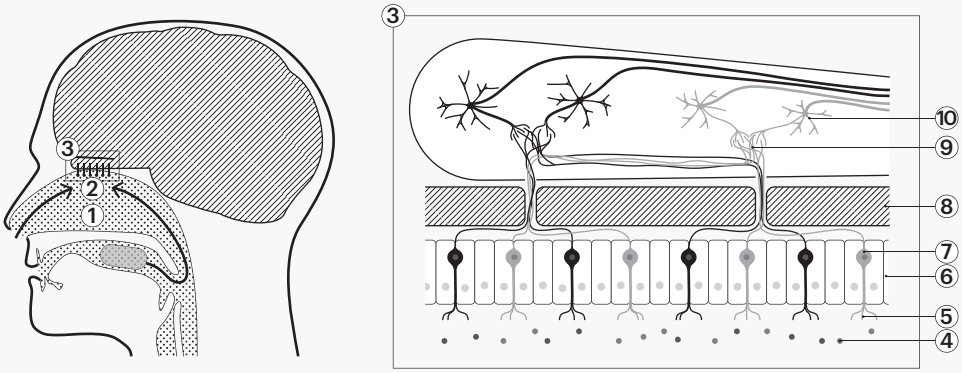

Figure 1.2. The human olfactory system. The arrow on the left represents the orthonasal route and the arrow on the right represents the retronasal route of olfaction. 1. Nasal cavity, 2. Olfactory epithelium, 3. Olfactory bulb, 4. Odour molecule, 5. Cilia, 6. Cells of the olfactory epithelium, 7. Olfactory sensory neuron, 8. Bone from the cribriform plate, 9. Glomerulus, 10. Mitral cell.

Volatile molecules can reach odour receptors at the olfactory epithelium via two routes: orthonasal and retronasal (see **Figure 1.2.**). Odours from the environment are sensed via the orthonasal route as they enter via the nostrils, go through the nasal passage and reach the top of the nasal cavity. Orthonasal stimulation occurs during anticipation of food intake. Retronasal olfaction has a prominent role when food has entered the mouth. During oral breakdown of the ingested food, volatile molecules are transported from the back of the mouth, via the nasopharynx to the olfactory epithelium [59]. Retronasal olfactory stimulation and gustatory stimulation of receptors on the tongue (taste) make up flavour.

Although the exact numbers are not substantiated by science, odours contribute substantially (~80–90%) to flavour perception. Nevertheless, most people overlook the influence of the sense of smell and solely refer to the taste component of flavour. Different sensory experiences and neural responses were found upon exposure via the retronasal and orthonasal route to the same odour. This was proposed to relate to whether the odorant represented an edible object [62,63]. The contribution of retronasal odours to increased (sensory-specific) satiety during food consumption

has been demonstrated, but these effects were small and did not impact food intake [64,65]. After orthonasal food odour exposure, previous research found an increased desire to eat [66,67]. Also, increased prospective pizza consumption and subsequent pizza intake in response to combined exposure to the sight and smell of pizza [68,69] were reported in previous research. These findings suggest that orthonasal odours play a role during food anticipation and possibly also during satiation, but what role they play exactly is still unknown.

Overeating and obesity

Many individuals are able to maintain a food intake that matches their biological needs in an environment where food cues are omnipresent. However, a large group of individuals eats in the absence of hunger and struggles to regulate their food intake. Over the long-term, food consumption that exceeds biological needs will lead to increased body weight [70,71]. Personality characteristics such as restraint, impulsivity and reward sensitivity have been proposed to mediate the association between food cue responsivity and increased risk for obesity [72-74].

Sensory cues of food can acquire emotional salience and rewarding properties through associative learning [75,76]. The motivation to consume a food product is largely determined by its reward value, and can be triggered by food cue exposure [77]. Increased reward responsiveness to environmental cues of palatable food in obese individuals may drive overeating [78-80]. Odour exposure led to marginally higher food intake in overweight compared to normal-weight children [81], but was not found to increase food intake more for overweight than normal-weight individuals in an adult population [68]. Several studies demonstrated increased neural reward responses to high energy-dense food cues in overweight and obese individuals [82-87]. Reward processing in the brain starts in regions of the midbrain, called the ventral tegmental area and the substantia nigra. These regions have dopaminergic projections to the ventral (nucleus accumbens) and dorsal striatum (putamen, caudate). Signals are then relayed to the amygdala, orbitofrontal cortex, anterior cingulate cortex and insula [88-90], regions that have also been implicated in processing of olfactory cues. Usually, reward value predicted by sensory signals and information about nutritional status conveyed by metabolic signals are integrated to balance food intake behaviour with energy expenditure [35,88,91,92]. However, in obese individuals, external signals of reward such as

olfactory cues of food are thought to overrule internal signals of hunger and satiety [5,93,94]. Moreover, heightened reward sensitivity after cue exposure is suggested to lead to over-activation of reward circuits and suppression of neural inhibitory control over food intake [95].

Decreased inhibitory control is thought to contribute to overeating in response to food cue exposure, which is linked to an increased risk of obesity [32,96,97]. Neural activation of brain regions involved in inhibitory control can provide insight in the underlying mechanisms of dysregulated food intake in obese individuals. In previous research, increased exertion of inhibitory control was related to greater activation of the medial-, ventrolateral- and dorsolateral prefrontal cortex and posterior parietal cortex [85,98-100]. Higher BMI was associated with increased behavioural impulsivity and hypo-activation of frontal inhibitory regions during response inhibition towards appetizing food pictures [101]. Moreover, there are indications that behavioural impulsivity and activation of the dorsolateral prefrontal cortex can predict weight loss success [97].

Roux-en-Y Gastric Bypass (RYGB) is an effective surgical intervention for long-term weight loss [102] that leads to a shift in preference and consumption from high to low energy-dense foods [103-105]. The underlying (neuro)biological mechanisms of this shift require further research. Decreased neural reward responses to pictures and words of high-energy foods after RYGB have already been demonstrated by Ochner *et al*. [106,107]. However, for this patient population it is still unknown whether neural responses to olfactory food cues are subjected to a similar change, changes in response inhibition have not yet been investigated and the role of metabolic factors needs clarification.

Rationale

Odours play an essential role in detection of food and determination of food quality. Previous research suggests a role for orthonasal odours during food anticipation, but how they influence eating behaviour is still unknown [66-69]. Changes in food preferences after RYGB were paralleled by changes in neural reward responses to pictures signalling high-energy food items [103,108,109], but their association with neural responses to olfactory food cues has never been studied. By investigating effects of orthonasal odour exposure on appetite, food preferences, food intake and neural responses we can elucidate their role in the regulation of eating behaviour.

_Choice of methodology

ODOUR EXPOSURE

In olfactory research there are different methods for odour exposure, ranging from semi-realistic to controlled exposure. Ambient exposure approximates real-life odour exposure most accurately. Ambient odours are suitable for measuring implicit effects on relatively slow and implicit processes influencing for example in-store consumer behaviour such as mood and food choice [110-112]. These types of research are less controlled in terms of exposure time and concentration. When studying the effects of a more extensive range of odours it is more effective to have participants sniff odorants from glass bottles. However, since participants are aware that they are smelling odours, this method is not suitable for researching implicit effects. A downside of ambient exposure and exposure from glass bottles is that the exact concentration that reaches the participants nose and the exact time of exposure cannot be controlled. In the study of fast behavioural (reaction time) and event-related physiological responses (skin conductance, heart rate, neural responses) a stable concentration and tight temporal control over odour exposure can be arranged with an olfactometer [113]. Some olfactometers provide an airflow heated to body temperature and humidified to > 80% relative humidity to prevent irritation to the nasal mucosa. Thanks to a quick switching systems, odorants can be delivered with a steep onset time and in a block-like fashion. This method of odour exposure is thus ideal for time-sensitive measures such as electro-encephalography (EEG) and functional magnetic resonance imaging (fMRI).

NEUROIMAGING TECHNIQUES

Neuroimaging techniques such as EEG and fMRI provide objective and non-invasive methods useful in gaining insight into neurobiological mechanisms underlying food cue responsivity. EEG is a relatively direct technique to measure neural activation. The recorded signal reflects differences in electrical potential across the scalp and represents the sum of multiple firing neurons. EEG has a high temporal resolution (milliseconds) and a poorer spatial resolution, which makes this method especially suitable for determining when differences in neural processing occur. Presentation of sensory stimuli can evoke changes in the EEG signal. When the EEG signals related to multiple trials of stimulus presentation are averaged, random brain activity is averaged out and a relevant stimulus related waveform, or event-related potential, remains. Within this waveform different components can be identified, with earlier components generally reflecting perceptual

processing and later components reflecting cognitive processing. EEG can be used to measure cortical responses, but it is not suitable for measuring neural activation in structures that are located deeper inside the brain.

In contrast to EEG, fMRI is a more indirect measure of neural activation with a poor temporal resolution (seconds) and a good spatial resolution (millimetres). Hence, it is more suitable for assessing where variations in neural activation occur. It is assumed that regional blood flow increases when oxygen, that was bound to haemoglobin particles on red blood cells, is used for brain activation [114,115]. Oxygenated and deoxygenated haemoglobin have different magnetic properties that can be detected using MRI. Changes in the relative concentrations of oxygenated and deoxygenated blood are captured in the blood oxygen level dependent (BOLD) [116]. The BOLD response follows the relatively slow blood flow to active neuronal tissue, which explains the limited temporal resolution of fMRI. MRI does allow for high resolution functional imaging of the cortex and structures deeper within the brain [117]. Together, as complementary neuroimaging techniques, EEG and fMRI can provide a full picture of when and where deviations in neural activation occur.

Aim and thesis outline

Orthonasal odours are considered as appetizing stimuli, but their role in food intake regulation and the underlying behavioural and neurophysiological mechanisms remain unclear. The overall aim of this thesis was to elucidate the role of odours in (over)eating. For this purpose we investigated the processes occurring upon odour exposure and before actual overeating: *Between odours and overeating*.

The following research questions were addressed in this thesis:

—1 How does food odour exposure affect appetite, preference and intake of the food that is cued?
—2 How does food intake affect food-cue reactivity?
—3 How does weight status influence behavioural and neurobiological responses to food cues?

We first examined whether food odours increase appetite. In addition, we assessed whether hunger state modulated these appetizing effects (**Chapter 2**). Following up on this, we explored how eating a food product to satiety changes subjective and implicit

neural evaluation of food odours and pictures (**Chapter 3**). We continued our research in semi-realistic context by studying effects of ambient odour exposure on food preference and intake. Next to this, we examined modulating effects of hunger state and weight status (**Chapter 4**). Effects of weight status on food cue responsivity were further explored in a group of obese patients before and after Roux-en-Y gastric bypass surgery. In this research we aimed to uncover underlying mechanisms of altered food preferences after surgery, by looking into changes in neural reward responses to food cues and changes in appetite related hormone concentrations (**Chapter 5**). In this population we also investigated pre- to post-surgery changes in neural measures of response inhibition to food cues (**Chapter 6**). In the final chapter of this thesis, the main findings are interpreted and discussed, implications of this research are described and recommendations for future research are given (**Chapter 7**). With the results of these studies we can create a better understanding of mechanisms of food selection and consumption that can be used to promote healthy eating behaviour.

Chapter 2

Food odours direct specific appetite

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Abstract

Background Olfactory food cues were found to increase appetite for products similar in taste. We aimed to replicate this phenomenon for taste (sweet/savoury), determine whether it extends to energy density (high/low) as well, and uncover whether this effect is modulated by hunger state.

Methods Twenty-nine healthy-weight females smelled four odours differing in the energy density and taste they signalled, one non-food odour, and one odourless solution (control), in random order, for three minutes each. Appetite for 15 food products was rated in the following two minutes.

Results Mixed model analyses revealed that exposure to an odour signalling a specific taste (respectively sweet, savoury) led to a greater appetite for congruent food products (sweet/savoury) compared to incongruent food products (savoury $p < .001$; sweet $p < .001$) or neutral food products ($p = .02$; $p = .003$). A similar pattern was present for the energy-density category (respectively high energy-dense, low energy-dense) signalled by the odours (low-energy products $p < .001$; high-energy products $p = .008$). Hunger state did not have a significant impact on sensory-specific appetite.

Conclusions These results suggest that exposure to food odours increases appetite for congruent products, in terms of both taste and energy density, irrespective of hunger state. We speculate that food odours steer towards intake of products with a congruent macronutrient composition.

Introduction

Olfactory cues of palatable food appear to work as appetizers while anticipating food intake. The smell of freshly baked bread entices you to buy and eventually eat a loaf.

From the day we are born we experience food odours during anticipation and consumption of food. Consumption is followed by nutritional consequences related to satiety, such as the digestion of available macronutrients. Over the course of our life we learn to use sensory food cues to (accurately) anticipate the energy density and taste (sweet/savoury) of the foods we are about to eat [22,23]. Through cephalic phase responses, our body is able to prepare for what will be ingested [8,13,118]. Food odours thus are an important guide in our food-rich environment, but their exact role needs to be clarified.

Olfactory food cues presented in the anticipatory phase of eating are found to increase the appetite for congruent products and decrease the appetite for incongruent products [119,120]. This phenomenon is referred to as sensory-specific appetite. Besides product-specific effects, Ramaekers *et al*. [119,120] found that savoury odours increased the appetite for (other) savoury foods and decreased the appetite for sweet foods, and *vice versa*. In earlier research, exposure to food odours (pizza, cookies) increased appetite, liking and craving for the food that was smelled in restrained eaters [66,67]. Moreover, Gaillet *et al.* [121,122] found that non-attentively perceived odours in the environment affected food choice. Participants placed in a waiting room with pear odour, chose fruity desserts more often compared to participants that had been waiting in an unscented room. Brief exposure to the smell and sight of pizza increased prospective intake for pizza and other savoury foods, but not for sweet foods [68,69]. After pizza cueing, the amount of pizza participants thought they could eat accurately predicted how much pizza they would actually eat [69]. Fedoroff *et al*. [66,67] and Larsen *et al*. [123] also found that participants ate more of an odour-cued food compared to non-cued food. Increased appetite likely parallels the effects of odour exposure on food choice, prospective intake and actual intake for matching products (*i.e.,* sweet/savoury, similar energy density, fruity).

The results of Ramaekers *et al.* [119,120] indicate the presence of sensory-specific appetite for taste category (sweet *vs*. savoury). Taste is important in the prediction of macronutrient content of a food. Savoury tastes are thought to indicate a high-protein content, whereas sweet tastes are suggested to point to a high-carbohy-

drate content (see *e.g.* [124-126]). Energy density of a food, such as fat content, is ecologically relevant as well. It has been suggested that humans are able to detect fat content of a food using their sense of smell [127]. Anticipation of the energy density of a food is important in the process of energy-intake regulation.

Internal cues of hunger and satiety impact how much we eat [9,128]. It is likely that these internal cues also play a modulating role in food-cue reactivity during the anticipation phase of eating and thereby influence our appetite (responses) and drive to ingest energy.

In this study we aim to replicate the influence of olfactory cues on sensory-specific appetite for a certain taste category and extend those findings to energy-density categories of foods. Additionally, we are interested whether hunger state plays a modulatory role in this effect. We expect that for both taste and energy density, exposure to food odours will lead to an increased appetite for products that are congruent to the odour and a decreased appetite for products that are incongruent to the odour. Additionally, we hypothesize that sensory-specific effects on appetite are more relevant and thus more pronounced in a hungry compared to a satiated state.

Methods

OVERALL DESIGN

This study followed a 2 × 6 within-subjects design, including the factors hunger state (hungry/satiated) and odour category (high-energy sweet: HESw, high-energy savoury: HESav, low-energy sweet: LESw, low-energy savoury: LESav, non-food control: NF, and no-odour: Baseline). We aimed to determine olfactory sensory-specific appetite for the taste and energy-density categories and assess whether hunger state modulates these effects.

PARTICIPANTS

Twenty-nine healthy-weight females (Body Mass Index (BMI): 21.3 ± 1.4 kg/m^2; age: 27 ± 11 years) participated in this study (see demographics in **Table 2.1.**). To ensure that all participants were normal weight (BMI: 18.5–25 kg/m^2), body weight (kg) and length (m) were determined. Further, participants had to be normosmic (scoring ≥ 12 on the Sniffin' Sticks 16-item identification test; [129]), in general good health (subjective), not using medication other than paracetamol and oral contraceptives, and had to be weight stable for at least two months. Restraint score (1-5) was determined by using the restraint subscale of the Dutch Eating Behaviour

Questionnaire (DEBQ; [130]). Individuals scoring higher than 2.8 on dietary restraint were excluded from participation. Respondents that did not like the products used in the study (< 40 mm on a 100 mm Visual Analogue Scale (VAS)) were excluded. We also did not include participants that had a smoking habit, had convictions that restricted consumption of certain products (vegetarian, vegan, not eating beef, *etc*.) or had mental or physical status that could hinder the study procedures (*e.g.*, food allergy, endocrine abnormality). Participants received a voucher of €25 for their contribution. All participants provided written informed consent before they participated in the study. This study was conducted in accordance with the Declaration of Helsinki of 1975, revised in 2013. The protocol was approved by the Medical Ethical Committee of Wageningen University (NL46034.081.13).

Table 2.1. Population (*N* = 29) description by demographic and personality characteristics.

Characteristic	**Mean ± *SD***
Age (years)	27.2 ± 11.5
BMI (kg/m^2)	21.3 ± 1.4
Olfactory performance (Sniffin' Sticks Identification 16)	13.6 ± 1.3
DEBQ: Restrained	2.2 ± 0.4

EXPERIMENTAL PROCEDURE

Participants attended two separate test sessions, once in a hungry state and once in a satiated state. For the hungry condition participants were asked to refrain from eating and drinking anything but water and weak tea in the three hours before the test session. For the satiated condition participants were asked to consume a comfortably satiating meal within the hour, but minimally 30 minutes before the test session. They were instructed to drink 0.5 L water 30 minutes before both test sessions to prevent dehydration. The order of the hunger state conditions was counterbalanced over participants. At the beginning of each test session participants rated their hunger (hunger, fullness, prospective consumption, desire to eat, and thirst) on a 100 mm VAS (EyeQuestion, Version 3.11.1, Logic8 BV, Elst, The Netherlands).

Every test session, participants were provided with six brown 50 mL bottles, each containing a different solution (5 mL). Participants were instructed to smell each bottle for three minutes. A short break of two minutes was used between odour exposures to avoid carry-over effects of the previous odour exposure [131]. In this time participants rated odour intensity and general appetite (100 mm VAS). Subsequently, participants indicated their appetite for 15 products belonging to five different categories (HESw, HESav, LESw, LESav, neutral control). The order in which the odours were presented was randomized between participants. Each participant received the same order of odours in both test sessions.

OLFACTORY STIMULI

We performed a pilot study in a separate sample of participants (n = 15), to select odours signalling either HESw food, HESav food, LESw food, LESav food, NF as control and a no-odour solution as baseline reference. Odour selection was based on similarities in perceived intensity and liking ratings, differences in the associated energy-density and taste categories, and consistent matching of the odour to a product/object. The selected odours included chocolate (HESw; International Flavors and Fragrances (IFF) 10810180; 5% in Propylene Glycol (PG)), beef (HESav; IFF 10878095; 0.04% in demi water), melon (LESw; IFF 15025874; 20% in PG), cucumber (LESav; IFF 73519595; 100%), fresh green (NF; AllSens-Voit Aroma Factory No. 819; 1% in PG), no-odour (baseline reference, 100% propylene glycol). Liking and familiarity ratings given by the 29 participants during a screening procedure can be found in the supplemental materials (Supplementary Table 2.1.). Odour intensity rated during the test sessions can also be found in the supplementary materials (Supplementary Table 2.2.).

PRODUCTS APPETITE QUESTIONNAIRE

Based on results of a second pilot study (n = 17), 15 food products were selected for the appetite questionnaire. The selection was based on ratings of liking, estimated caloric content, and on the indicated category (HESw, HESav, LESw, LESav, neutral) of the product. All of the selected products can be considered as snack foods. Three products were included for each category (HESw, HESav, LESw, LESav) and three neutral food products (in terms of taste) were added as control. HESw products included pieces of chocolate, cake and stroopwafel (a Dutch caramel syrup waffle); for HESav we selected beef croquette, cheese cubes and crisps; the

LESw products were a slice of melon, an apple and strawberries; for the LESav products we selected a piece of cucumber, tomato salad and raw carrot; bread, croissants and pancake were included as neutral controls. Liking and familiarity ratings for the products given by the 29 participants during a screening procedure are presented in Supplementary Table 2.3.

DATA ANALYSES

Hunger state—A paired samples *T*-test was performed on the hunger ratings (*i.e.*, hunger, fullness, prospective consumption, desire to eat, and thirst) to confirm that participants were in different hunger states in the two test sessions they attended.

Appetite ratings—All main analyses were performed following a linear mixed effects models procedure in IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). First-order ante-dependence was chosen as covariance structure. A *p*-value of < .05 was considered significant. Session order and participant number were added as repeated variables. Hunger state (hungry, satiated) was included as covariate. Differences in rated odour intensity between the odours were tested with a mixed model including odour category (HESw, HESav, LESw, LESav, NF, Baseline) as fixed effects factor.

General appetite ratings (100 mm VAS) were analysed by adding raw ratings of general appetite as dependent factor and odour category (HESw, HESav, LESw, LESav, NF, Baseline) as fixed effects factor.

We were interested in specific appetite effects induced by odorants signalling different categories. We used appetite ratings after exposure to the no-odour solution as a baseline reference, and subtracted these appetite ratings from ratings provided after smelling an odour, yielding Δ appetite scores. To determine sensory-specific effects of taste category, data were split by taste category (Sav, Sw, NF odours). Products were then pooled per taste category. Δ Appetite was used as outcome measure. Product taste category was added as fixed effects factor. Similarly, sensory-specific effects of the energy-density category were analysed by distinguishing the data by energy category (HE, LE odours). Products were then pooled per energy category. We added Δ appetite as outcome measure and product energy category as fixed effects factor. *Post hoc* paired comparisons were used to uncover effects of the odours on the separate product categories. To exclude the possibility that

the effects were merely driven by the specific odour-product match (*e.g.*, chocolate odour exposure = appetite for chocolate pieces), the analyses described above were rerun without the specific matches.

Results

HUNGER STATE (MANIPULATION CHECK)

Hunger ratings confirmed that feelings of hunger were significantly different between sessions, and according to the appropriate hunger states (hungry *vs*. satiated; see **Table 2.2**). In the hungry conditions the participants felt hungrier, less full, and indicated a higher prospective consumption and desire to eat (all $p < .001$). Ratings of thirst indicated that participants were equally thirsty in both test sessions.

Table 2.2. Hunger ratings (100 mm VAS) for both hunger states.

Parameter	Hungry (Mean ± *SE*)	Satiated (Mean ± *SE*)
Hunger ***	58 ± 4	11 ± 2
Fullness ***	22 ± 4	65 ± 3
Prospective consumption ***	61 ± 3	29 ± 4
Desire to eat ***	65 ± 4	18 ± 2
Thirst	38 ± 6	27 ± 4

*** *p*-value < .001, paired samples *T*-test.

GENERAL APPETITE

General appetite (100 mm VAS) differed significantly after exposure to different odours ($p = .015$) and also between hunger states ($p < .001$). General appetite after smelling chocolate (46 ± 3), beef (42 ± 4), melon (44 ± 3) and cucumber (47 ± 3) was significantly higher than after smelling fresh green (36 ± 3) or baseline reference (36 ± 3; all $p < .01$). General appetite in the hungry state (62 ± 3) was significantly higher compared to the satiated state (21 ± 3; $p < .001$).

SENSORY-SPECIFIC APPETITE (SSA): TASTE CATEGORY

Our results (see **Figure 2.1.**) show that by smelling Sw odours, appetite changes significantly for products of different taste cate-

gories ($F(2, 432) = 27.46$, $p < .001$). After exposure to Sw odours, Δ appetite was significantly higher for Sw products (4.0 ± 1.1) than for Sav (−0.4 ± 1.1; $p < .001$) or neutral products (2.2 ± 1.2; $p = .022$), and Δ appetite for Sav products was also significantly lower than appetite for neutral products ($p = .001$).

Similarly, Δ appetite after smelling Sav odours was significantly different between the product-taste categories ($F(2, 404) = 12.08$, $p < .001$). It was higher for Sav products (3.7 ± 1.2) than for Sw (−0.4 ± 1.3; $p < .001$) or neutral products (1.4 ± 1.3; $p = .003$), but did not differ between Sw and neutral products ($p = .017$).

Δ Appetite after smelling an NF odour was not significantly different for the different products ($F(2, 197) = 0.28$, $p = .750$). For Sw products (−1.0 ± 0.9), Sav products (−1.0 ± 0.9) or neutral products (−0.2 ± 0.9; all $p > .05$).

In addition to an increase in general appetite when hungry, participants' raw appetite ratings for products were higher in the hungry state (see **Supplementary Figure 2.1.**). However, there

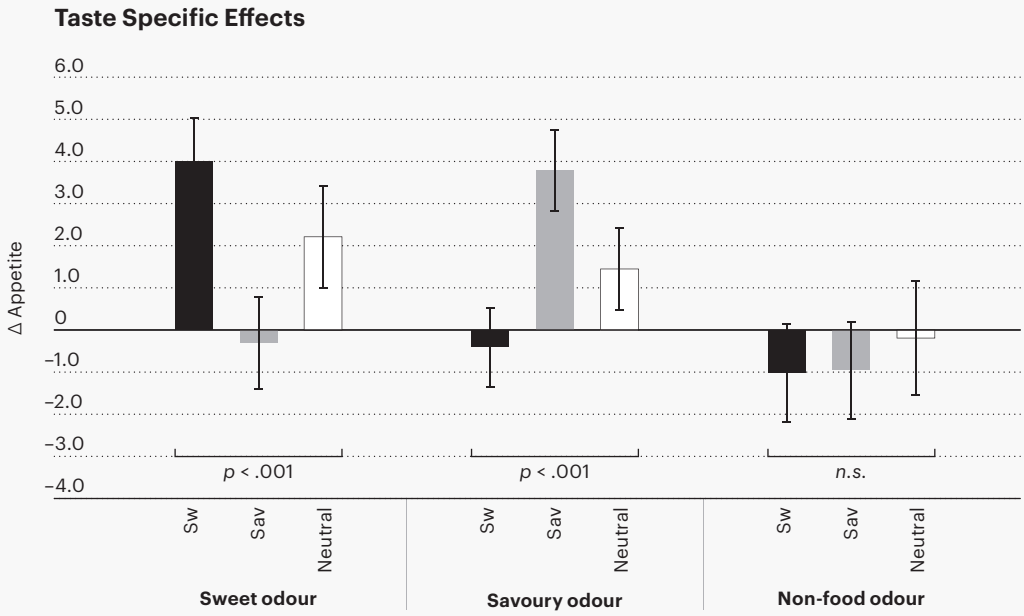

Figure 2.1. Δ Appetite (on 100 mm VAS; appetite after smelling an odour minus appetite after smelling a baseline reference) for sweet (Sw), savoury (Sav) and neutral products after smelling sweet, savoury and non-food odours.

were no significant differences in specific appetite (the difference between appetite after exposure to the no-odour control and appetite rated after exposure to an odour) between the hungry and satiated condition (difference in Δ appetite between hunger states after Sw odour: 0.7 mm; after Sav odour: 0.0 mm; after NF odour: 2.6 mm; all $p > .05$).

In order to exclude the possibility that the effects described above were driven by the specific odour-product match (*e.g.*, exposure to chocolate odour = appetite for chocolate pieces), these specific matches were excluded from the dataset and the analyses were rerun.

We found a significant main effect of Sw odour exposure on Δ appetite for different product taste categories ($F(2, 403) = 19.29$, $p < .001$). Δ Appetite after Sw odours remained significantly higher for Sw products (3.1 ± 1.1) than for Sav products (−0.4 ± 1.1; $p < .001$), but did not differ from Δ appetite for neutral products (2.0 ± 1.1; $p = .191$). Further, Δ appetite for Sav products also differed from Δ appetite for neutral products ($p = .003$).

Δ Appetite after smelling Sav odours was also significantly different between product categories that were based on taste ($F(2, 383) = 5.68$, $p = .004$). Δ appetite for Sav products (2.8 ± 1.3) remained significantly higher than for Sw products (−0.4 ± 1.3; $p < .001$), but was not significantly different from Δ appetite for neutral products (1.2 ± 1.3; $p = .052$). Δ Appetite for Sw products was significantly different from Δ appetite for neutral products ($p = .033$).

SSA: ENERGY CATEGORY

After smelling HE odours, Δ appetite for HE products (3.0 ± 1.1) was significantly higher than for LE (−0.3 ± 1.2; $p < .001$; see **Figure 2.2.**).

Paired comparisons also revealed that Δ appetite rated after smelling LE odours was significantly higher for LE products (2.9 ± 1.2) than for HE products (0.8 ± 1.1; $p = .008$). Δ Appetite rated after smelling an NF odour was not significantly different than for HE products (−0.5 ± 0.9) and LE products (−2.1 ± 1.1).

In Supplementary Figure 2.2. it is visible that raw appetite ratings for products are higher in the hungry state. Specific appetite (the difference between appetite after exposure to the no-odour control and appetite rated after exposure to an odour) was not significantly influenced by hunger state (difference in Δ appetite between hunger states after HE odour: 0.7 mm; after LE odour: 0.0 mm; both $p > .05$).

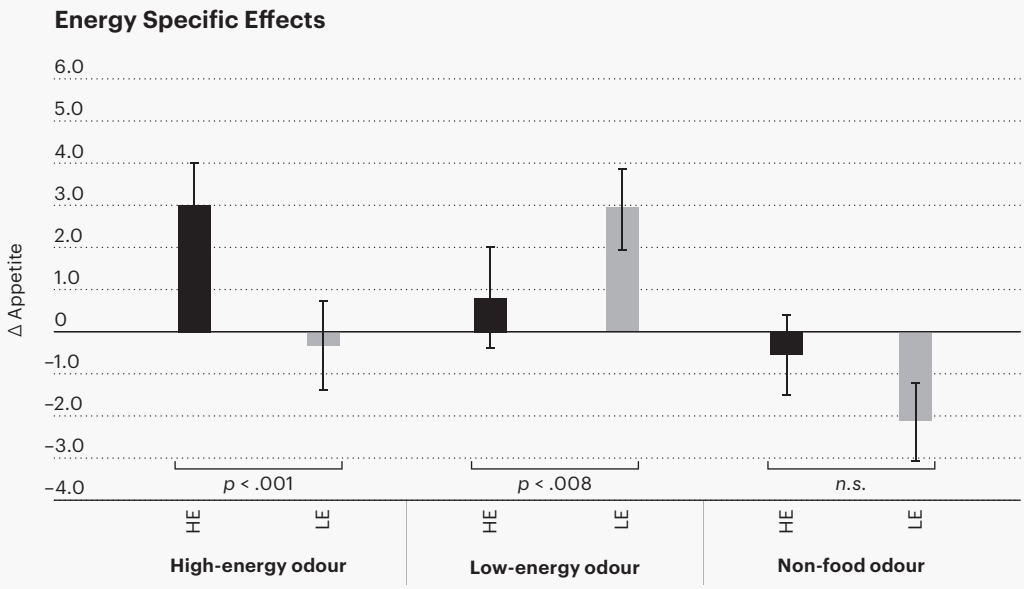

Figure 2.2. Δ Appetite (appetite after smelling an odour minus appetite after smelling a baseline reference) for high-energy (HE), low-energy (LE) products after exposure to high-energy (HE), low-energy (LE) and non-food (NF) odours.

As mentioned in the previous section, the analyses were rerun, excluding specific matches from the dataset, to account for the possibility that the effects described above were merely driven by the specific odour-product match.

Paired comparisons showed that after smelling HE odours, Δ appetite for HE products (2.3 ± 1.1) was significantly different from Δ appetite for LE products (−0.7 ± 1.1; $p < .001$). Δ Appetite after smelling LE products was not different for LE products (1.8 ± 1.2) and HE products (0.6 ± 1.1).

Discussion

With this research, we wanted to determine whether odours signalling specific categories (sweet/savoury taste; high-/low-energy density; non-food) can induce sensory-specific changes in appetite. Furthermore, we were interested in whether the strength of this effect was dependent on hunger state. The results reveal that

food odours increase appetite for products that are similar, both in taste and energy density. Hunger state did not significantly affect odour-induced sensory-specific appetite.

Our results indicate that odours that signal sweet products and odours that signal savoury products affect appetite in an opposite, but similar way. After smelling a sweet food odour, appetite for sweet products increases more compared to appetite for neutral (bread, croissant, and pancake) and savoury products. Similarly, after smelling a savoury food odour, appetite for savoury products increases significantly more than for neutral or sweet products. In line with this, the smell of flowers (fresh green), a non-food related odour, slightly decreased appetite ratings for sweet, savoury and neutral foods equally. These results are concurrent with those of Ramaekers *et al*. [119,120]. We suggest that the level of congruency between the odour and the product determines the strength of the appetizing effect of the odours. Interestingly, after removing the specific odour-product matches (*e.g.*, chocolate odour = appetite for chocolate pieces) from our dataset, appetite for taste-congruent products remained higher compared to non-congruent products. Olfactory sensory-specific appetite is a phenomenon that not only applies to a specific product, but also to categories of products related to taste quality (sweet *vs*. savoury). By repeated exposure to the combination of an odour and the nutritional consequences after eating the food we smell, it is proposed that we learn to use (olfactory) food cues as predictors for macronutrient content [22,23]. These associations are most often described in terms of taste quality. Sweet taste is related to a high-carbohydrate content, whilst savoury taste is associated with a high-protein content [124,125,132].

To our knowledge, this is the first study investigating olfactory sensory-specific appetite effects for energy density (high-energy *vs*. low-energy). When odours and products were categorized according to their associated energy density, we saw that appetite changed in a similar pattern as for taste category. Smelling odours associated with high energy-dense food increased the appetite for high-energy products significantly more than for \ products. The reverse occurred after smelling odours of low energy-dense foods. There was no difference in appetite for high energy-dense and low energy-dense products after smelling a non-food related odour. After removing the specific odour-product matches from the dataset, the pattern remained the same for high energy-dense odours. However, low-energy

odours did not induce category-specific appetite effects. High energy-dense odours appear to be more potent appetizers than low energy-dense odours. Sensory-specific appetite seems to exist for specific odour-product matches and for the high energy-density category more in general.

When comparing these effects to the effects of the taste category, it seems that olfactory signals of taste lead to larger increases in appetite than for olfactory signals of energy density. After removal of the specific odour-product matches, an interesting pattern in the results emerges. Category-broad effects for sweet, savoury and high energy-dense odours remain, whereas a significant category-broad effect for low-energy food odours is absent. Based on these results, we speculate that odours signal macronutrient content. Low-energy food cues signal products that do not have substantial content. This can explain the lack of category-broad effect for low-energy cues. As mentioned for the taste category, it is thought that sweet signals carbohydrates (sweet) and savoury signals protein [124,125,132]. On the contrary, the energy density of a food is not based on a single macronutrient *per se*, but can be composed of different macronutrient sources, mainly fats and carbohydrates. In essence, teasing apart energy and macronutrient contribution is complex since they are interrelated. A systematic and more complete research approach is necessary to uncover the appetizing effects of olfactory stimuli representing proteins, carbohydrates and fats. Deprivation or supplementation of a certain macronutrient by an experimental diet could unveil whether appetizing effects of odours are modulated by macronutrient status. This will help to determine whether the function of macronutrient signalling can indeed be ascribed to olfactory cues in the anticipatory phase of eating.

Intake regulation is largely dependent on feelings of hunger and satiation [133,134]. Individuals that maintain their energy balance should be able to adjust their food intake according to their hunger status and resist eating beyond satiety when tempted by environmental food cues [67]. Hunger ratings provided by the participants in our study confirmed that feelings of hunger (hunger, fullness, prospective consumption, desire to eat) were significantly different in the two hunger states (hungry *vs*. satiated; see **Table 2.2.**). Surprisingly, hunger state did not modulate odour-induced specific appetite: Δ Appetite for specific products after odour exposure was not different when participants were satiated or not. The theory that tempting food cues in the

environment stimulate eating in the absence of hunger has been posed before [7]. This idea is supported by research by Nijs *et al*. [135], where participants showed a similar bias in orientation and maintained attention to visual food cues in both a hungry and satiated condition. Appetite responses of our participants also show that the impact of food odour exposure is independent of hunger state.

Selective detection of nutritious foods has been a beneficial trait for survival throughout evolution. We propose that in the anticipation phase of eating, (olfactory) food cues induce appetite specific to the macronutrient content that is signalled by the odour. Besides changes in appetite behaviour [66,67], previous research revealed very rapid physiological changes (*e.g.*, salivation, endocrine responses [118,136]) following food odour exposure. It is not clear whether food cues signalling a specific macronutrient content cause specific physiological responses to facilitate macronutrient uptake. Over the course of evolution, it would seem advantageous to have built-in systems (physiological, behavioural) that work together to obtain food sources in the environment.

Although olfactory food cues in our surroundings (ambient exposure) clearly have a specific effect on appetite, the effect on food preferences or choice is less consistent. Food odours do not seem to affect the preference for one type of food over another, as measured by a forced choice computer task [128], but do influence what we decide to eat when choosing dishes from a menu [121,122]. These inconsistencies in choice are likely related to variations in context (controlled *vs*. real-life). Ferriday and Brunstrom [68,69] showed that smelling and viewing pizza increases the amount of pizza we think we can eat (prospective consumption), a measure closely related to appetite. This effect also transferred to dishes that were similar to pizza ("scrambled egg, chips and baked beans", "pasta and tomato sauce"), but not to dishes that were dissimilar (cake).What is more, after smelling a food (in combination with the sight), participants ate more of the cued food [66,67,69,123]. However, findings on intake are not consistently repeated [128] and seem to have specific prerequisites with regard to the context, cue exposure and also personality characteristics (restraint, impulsivity). Altogether, it seems that olfactory food cues have a more clear-cut role in the phase leading up to meal initiation [137], tempting us to start eating what is in front of us. Their influence may wane in later phases, when other cues such

as flavour (taste, retronasal odour), satiety signals and intake-inhibiting behaviours come into play, that work toward cessation of eating. In the context of food scarcity and low food security, it is highly beneficial to have the ability to detect nutritious food sources. Subsequent increases in appetite and changes in physiology may promote and facilitate adequate food intake and uptake, thereby increasing chances of survival. In most Western societies, however, energy-dense foods are readily available. In this case, appetizing olfactory food cues are part of an environment that promotes overconsumption, ultimately contributing to a higher incidence of nutrition-related diseases (*e.g.*, obesity, diabetes).

We cannot exclude that our sample size was too small to detect modulatory effects of hunger state on specific appetite. However, we do not find this plausible since significant effects of hunger state on general appetite were found. Confirmation of the absence of this effect may be provided by future research in a larger study population. Additionally, in the current study we only included females, limiting the possibility to generalize our findings to a broader population that includes males.

Conclusions

In conclusion, we show that food odours increase appetite for products with a similar taste and/or energy density. Odours thus steer towards intake of congruent food products and perhaps help to prepare the body for digestion of this product. This process seems to rely more on taste quality (related to macronutrient content) than on the amount of calories a food provides (energy density). We propose that food odours in the anticipatory phase of eating transfer information about the macronutrient content of the food. Moreover, food odours appear to elicit their appetizing effects in both hungry and satiated states, indicating that exposure to food odours can promote overeating and could contribute to obesity. Conversely, these effects may be used to stimulate appetite and meal initiation in people that are undernourished.

Acknowledgements

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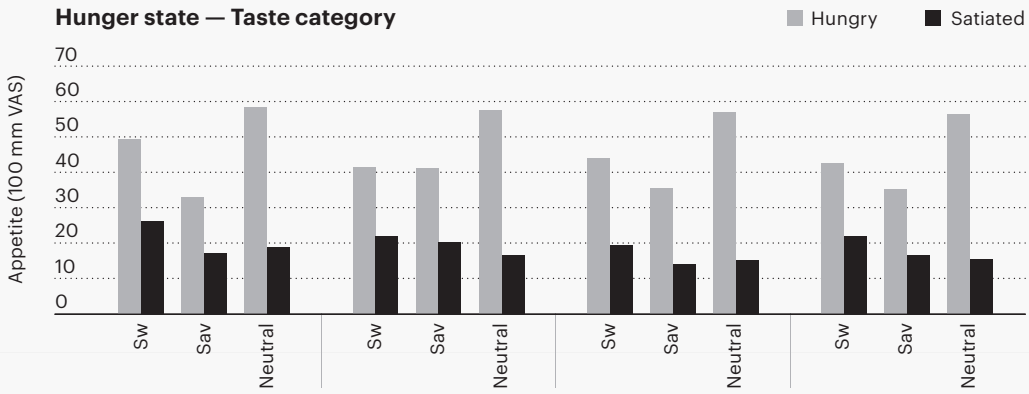
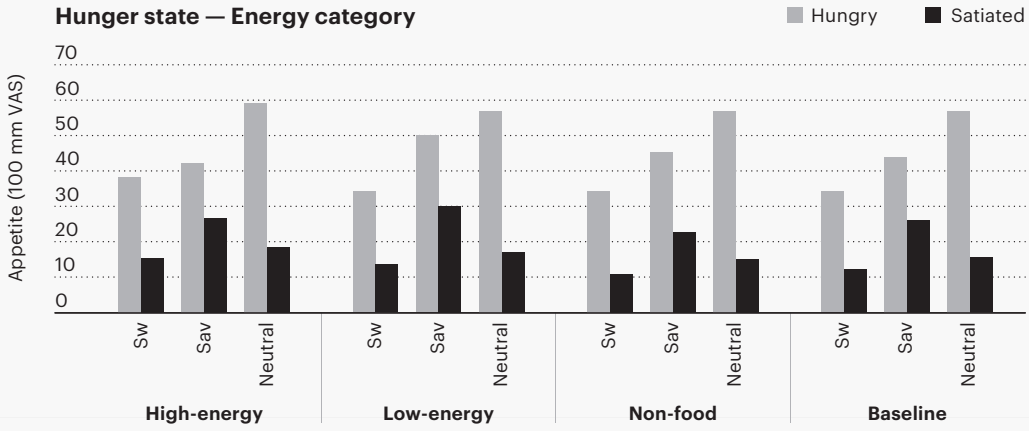
Supplementary materials



Supplementary Figure 2.1. Raw appetite ratings for products from different taste categories: Sweet (Sw), savoury (Sav), neutral. Participants provided ratings in hungry and satiated states after exposure to odours from different categories: Sweet, savoury, non-food and baseline (no-odour).



Supplementary Figure 2.2. Raw appetite ratings for products from different energy categories: High-energy (HE), low-energy (LE), neutral. Participants provided ratings in hungry and satiated states after exposure to odours from different categories: high-energy, low-energy, non-food and baseline (no-odour).

Supplementary Table 2.1. Liking and familiarity ratings (100 mm VAS) of the odours that were used in the test sessions. Ratings for all odours > 40 mm.

Odour	Category	Liking (Mean ± *SE*)	Familiarity (Mean ± *SE*)
Chocolate	High-energy - Sweet	75 ± 21	82 ± 22
Beef	High-energy - Savoury	53 ± 27	68 ± 29
Melon	Low-energy - Sweet	65 ± 26	76 ± 23
Cucumber	Low-energy - Savoury	64 ± 20	81 ± 22
Fresh Green	Non-food control	63 ± 22	64 ± 24
No Odour	Baseline reference	49 ± 16	35 ± 22

Supplementary Table 2.2. Average odour intensity ratings (100 mm VAS) after 3 min of smelling.

	(Mean ± *SE*)
Chocolate	75 ± 3
Beef	74 ± 3
Melon	73 ± 2
Cucumber	58 ± 3
Fresh Green	76 ± 4
No Odour	7 ± 2

Supplementary Table 2.3. Liking and familiarity ratings (100 mm VAS) of the products that were used in the appetite questionnaire.

Product	Category	Liking (Mean ± *SE*)	Familiarity (Mean ± *SE*)
Pieces of chocolate	High-energy – Sweet	93 ± 9	97 ± 4
Cake		84 ± 12	94 ± 8
Stroopwafel		84 ± 16	92 ± 12
Beef croquette	High-energy – Savoury	81 ± 15	86 ± 14
Cheese cubes		81 ± 15	91 ± 14
Crisps		88 ± 11	96 ± 7
Slice of melon	Low-energy – Sweet	90 ± 11	92 ± 9
Apple		84 ± 13	96 ± 8
Strawberries		94 ± 11	96 ± 6
Piece of cucumber	Low-energy – Savoury	81 ± 13	97 ± 4
Tomato salad		80 ± 14	81 ± 20
Raw carrot		76 ± 19	92 ± 11
Bread	Neutral control	80 ± 15	97 ± 5
Croissant		79 ± 14	92 ± 10
Pancake		87 ± 10	95 ± 6

Chapter 3

Modulation of event-related potentials to food cues by sensory-specific satiety

Harriët F.A. Zoon, Kathrin Ohla, Cees de Graaf, Sanne Boesveldt

In preparation for submission

Abstract

Background

Tempting environmental food cues and metabolic signals are important factors in appetite regulation. Food intake reduces pleasantness of food cues that are congruent to the food eaten (sensory-specific satiety). This study was aimed to disentangle effects of sensory-specific satiety on perceptual and evaluative processing of meal-congruent and incongruent food cues.

Methods

Twenty healthy female subjects (age: 20 ± 2 years; BMI: 22 ± 2 kg/m^2) participated in two separate test sessions during which they consumed an *ad libitum* amount of a sweet or savoury meal. Before and after consumption event-related potentials were recorded in response to visual and olfactory cues signalling high-energy sweet, high-energy savoury, low-energy sweet and low-energy savoury food and non-food. Also, appetite, liking and wanting for food products of these categories were rated.

Results

In general, we observed that food intake lead to more negative amplitudes in the event-related potentials for food, but also non-food cues. Changes were more pronounced in response to high-energy sweet food pictures after a sweet meal, and also occurred in early processes of perception (~80–150 ms) and later processes of cognitive evaluation (~300–700 ms).

Conclusion

Food intake appears to lead to general changes in neural processing that are related to motivated attention, and sensory-specific changes that reflect decreased positive valence of the stimuli and/or modulation of top-down cognitive control over processing of cues congruent to the food eaten to satiety.

Introduction

With an abundance of tempting environmental cues (*e.g.,* visual, olfactory), effective regulation of food intake is vital. Sensory properties of food play an important role in the selection of food for adequate and diverse nutrient intake, but also in the cessation of eating [134,137]. With this study we aim to get a better understanding of the neural processes involved in the interplay between metabolic and sensory factors involved in appetite control and food intake regulation.

When a particular product is consumed to satiety, the hedonic value of the sensory cues (tastes, odours) related to that product declines while pleasantness for products that have not been consumed decreases less [31,35]. This phenomenon is referred to as sensory-specific satiety. It decreases the likelihood of continued intake of food with the same or similar sensory characteristics. Hence, sensory-specific satiety is thought to promote a more varied diet [31,126,138].

Sensory-specific satiety has been extensively described using subjective measures of pleasantness and food intake, but the underlying neural mechanisms need more clarification. Previous research revealed sensory-specific satiety changes in facial expressions and skin conductance that indicate increased boredom upon consumption of a product that was previously eaten to satiety [139]. Neuroimaging studies revealed decreases in orbitofrontal cortex activation [35,140] upon sensory-specific satiety. These changes were related to decreased reward, or increased aversion, when people did not want to eat more of the product. Moreover, changes in perception of odour intensity after food intake have been related to sensory-specific satiety [40,141]. Together these results suggest that sensory-specific satiety has modulatory effects on processes of sensory perception and hedonic evaluation. Increased understanding of the modulatory role of food intake on temporal dynamics of neural processing of food cues is required.

The dynamics of neural processing from early stages of perception to later stages of cognitive evaluation can be captured using electro-encephalography (EEG). Several EEG studies have reported decreased amplitudes late positive potentials (P3/LPP; ~300–700 ms) elicited by visual food cues after food intake [135,142-145]. It is proposed that internal state modulates neural processes of attention and reward, and thereby stimulates behaviours aimed at restoring energy levels [92,144], perhaps also in a sensory-specific fashion.

With this study we aimed to assess whether sensory-specific satiety leads to changes in event-related potentials for different categories of food cues. We hypothesize that food intake will lead to a general decrease in amplitude for late event-related potentials that reflect decreased reward, which will be most pronounced for food cues that are congruent to the food eaten to satiety.

Methods

OVERALL DESIGN

This study followed a within-subject design, including the factors test moment (pre-/post-meal) and stimulus category (high-energy sweet: HESw, high-energy savoury: HESav, low-energy sweet: LESw, low-energy savoury: LESav, non-food control: NF, and no-odour: Baseline). We looked at two different meal types (sweet/savoury) and used cues of two stimulus modalities (visual/olfactory).

PARTICIPANTS

Twenty normal-weight females (age: 20 ± 2 years; Body Mass Index (BMI): 22 ± 2 kg/m^2) participated in this study. Included participants were normosmic (scoring ≥ 12 on the Sniffin' Sticks 16-item identification test; [129]), in general good subjective health, not using medication other than paracetamol and oral contraceptives, were weight stable for at least two months and were not considered restrained eaters (score ≤ 2.8 on the Dutch Eating Behaviour Questionnaire [130,146]. We also did not include participants that had a smoking habit, had convictions that restricted consumption of certain products (*e.g.,* vegetarian, vegan) or a mental or physical status that could hinder the study procedures (*e.g.*, food allergy, epilepsy). Respondents that did not like the products used in the study (< 40 mm on a 100 mm Visual Analogue Scale (VAS)) or did not like the odours used in the study (< 30 mm on a 100 mm VAS) were excluded. Participants received monetary compensation for completing the study. All participants provided written informed consent before they participated in the study. This study was conducted in accordance with the Declaration of Helsinki of 1975, revised in 2013. The protocol was approved by the Medical Ethical Committee of Wageningen University (NL52713.081.15).

EXPERIMENTAL PROCEDURE

In two separate test sessions event-related potentials were acquired before and after meal intake. Each participant received

a sweet meal in one test session and a savoury meal in the other. The order of the meals was counterbalanced over all participants. Participants were instructed not to eat and drink anything but water and weak tea in the three hours before the test session. Upon arrival, they were seated in a comfortable chair and the EEG equipment was fitted. Further, participants rated their hunger, fullness, prospective consumption, desire to eat, and thirst on a 100 mm Visual Analogue Scale (VAS) and filled in an appetite questionnaire (100 mm VAS; see [147]. The EEG paradigm lasted approximately one hour, divided over six separate blocks with short breaks in between to stay focused. During the blocks, event-related potentials to visual and olfactory stimuli were recorded. After completion of the first EEG paradigm the equipment was disconnected and an *ad libitum* rice meal was provided. Participants were instructed to eat as much as they wanted and to drink all of the water. When they were satiated (± 15 minutes after meal intake started), the equipment was connected again and the EEG paradigm was repeated for another hour.

AD LIBITUM MEAL

Between the two EEG measurements an *ad libitum* amount of rice product (± 800 kCal; ± 575 g) and 200 ml of tap water were provided. In each test session, a sweet rice dish (chocolate flavour) or a savoury rice dish (flavoured with beef gravy) was served. Both dishes were equal in energy density and similar in macronutrient content (see nutritional values in **Table 3.1.**).

Table 3.1. Nutritional values per 100 g of the sweet and savoury rice meals

Nutrients	Sweet	Savoury
Energy (kcal)	141	139
Protein total (g)	2.4	2.4
Fat total (g)	3.2	3.2
Carbohydrates total (g)	25.1	25.0
Mono- en disaccharides total (g)	14.7	1.8
Dietary fibres total (g)	1.0	0.2
Sodium (mg)	20	564

APPETITE QUESTIONNAIRE

Participants provided ratings (100 mm VAS) of their general appetite and their appetite for 15 different food products in five categories (HESw, HESav, LESw, LESav, Neutral [147]). The order in which the products were presented was randomized. HESw products included pieces of chocolate, cake and stroopwafel (a Dutch caramel syrup waffle); HESav products included beef croquette, cheese cubes and crisps; LESw products included a slice of melon, an apple and strawberries; LESav products included a piece of cucumber, tomato salad and raw carrot; bread, croissants and pancake were included as taste neutral products.

OLFACTORY STIMULI

We used odours signalling either HESw, HESav, LESw, and LESav food, and included a non-food (NF) odour and a no-odour solution as controls (see [147]). The selected odours included chocolate (HESw; International Flavors and Fragrances (IFF) 10810180; 5% in Propylene Glycol (PG)), beef (HESav; IFF 10878095; 0.08% in demi water), melon (LESw; IFF 15025874; 10% in PG), cucumber (LESav; IFF 73519595; 100%), fresh green (NF; AllSens-Voit Aroma Factory No. 819; 1% in PG), no-odour control (100% PG). Odours were presented birhinally by means of a computer-controlled olfactometer [113].

VISUAL STIMULI

The selection of pictures was based on the category (HESw, HESav, LESw, LESav, NF) and match to the selected odours. For each odour we selected three different pictures to reduce boredom. We selected chocolate muffin, brownie and chocolate bonbons for chocolate odour; steak, tranches of steak and meat balls for beef odour; cantaloupe, Galia melon and melon salad for melon odour; cucumber slices with peel, cucumber salad and cucumber chunks for cucumber odour; green soap, tulips and white flowers for fresh green odour. Standardized food images used in the EEG paradigm were provided by the Image Sciences Institute, UMC Utrecht, and created as part of the Full4Health project (www.full4health.eu), funded by the European Union Seventh Framework Program (FP7/2007-2013) under grant agreement nr. 266408, and the I.Family project (http://www.ifamilystudy.eu), grant agreement nr. 266044 [148].

EEG PARADIGM

The EEG paradigm consisted of six individual blocks with an

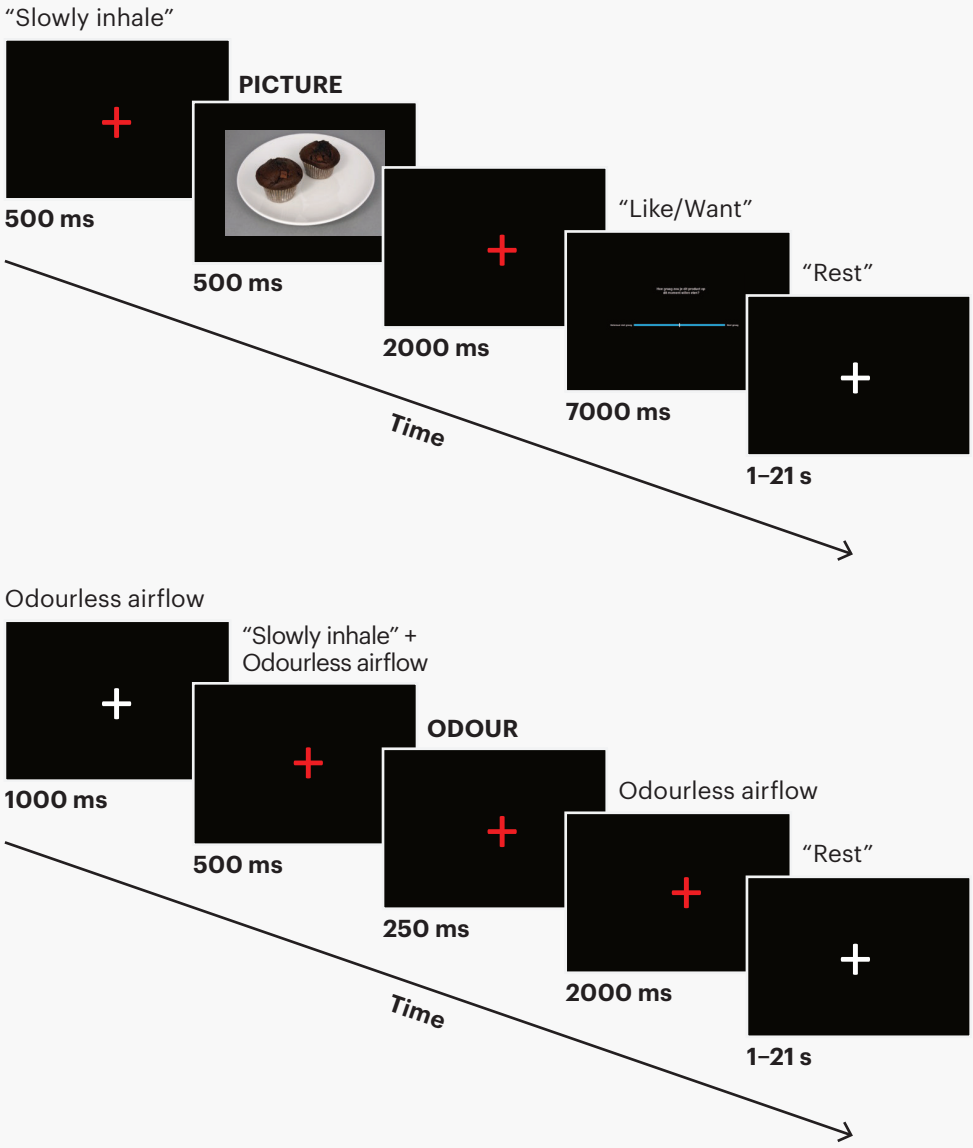


Figure 3.1. Schematic overview of odour (below) and picture trials (above) in the EEG paradigm. Throughout the paradigm participants were occasionally asked to provide ratings of wanting, liking and odour intensity on a visual analogue scale for the cue that preceded the question (see picture trial above).

approximate duration of 8 min each. E-Prime 2.0 Professional (Psychology Software Tools Inc.) was used to run the paradigms. In each block, 18 visual and 18 olfactory cues of HESw, HESav, LESw, LESav food, NF products and controls (odourless air/ empty plate picture) were presented one by one, in pseudo randomized order, resulting in a total of 108 odour- and 108 picture presentations for each EEG paradigm. During odour trials (see **Figure 3.1.**), a red fixation cross was presented from 500 ms before onset to 2000 ms after offset of odour presentation. Participants were instructed to slowly inhale via the nose when the red fixation cross appeared on screen. The odorized air flow (3 L/min) was switched on for 250 ms. In order to eliminate effects of tactile stimulation on event-related potentials (ERPs), an odourless airflow with the same flow rate (3 L/min) was presented in the 1500 ms before onset and 2000 ms after offset of odour presentation. Picture trials (see **Figure 3.1.**) started with a red fixation cross (500 ms), after which the picture was presented (500 ms), followed by the red fixation cross (2000 ms). To maintain focus on the task, participants were occasionally asked to provide liking and wanting ratings for each stimulus, and intensity ratings for the odours (100 mm VAS; 7000ms), throughout the entire EEG paradigm. Between trials we included a rest period (1-21 s) during which a white fixation cross was visible, and a constant airflow of 0.5 L/min was active. The inter-stimulus interval between odour presentations was jittered between (17-31 s) to prevent olfactory adaptation.

EEG RECORDINGS AND PRE-PROCESSING

The BioSemi Active-Two amplifier system (BioSemi, Amsterdam, the Netherlands) was used to continuously record electrical brain activity. Participants were fitted with an elastic cap that was mounted with 64 Ag/AgCl active electrodes placed according to the extended 10-20 system. Eight external electrodes were placed to monitor horizontal and vertical eye-movements and blinks, heart rate, and mastoid reference signal. The signals were referenced to CMS, while DRL served as ground. Data were recorded with a sample rate of 512 Hz. Full bandwidth data were stored on hard disk for off-line analysis. EEG data were processed using the EEGLAB toolbox (version 13.3.2 [149]) in MATLAB (version 7.12.0, Mathworks, Natick, MA). After importing the data and merging of pre- and post-meal EEG paradigms for each test session, power line noise between 50-100 Hz was removed. A high pass band filter of 0.2 Hz (transition band width 0.1 Hz) and a low pass band filter of 30 Hz

(transition band width 2 Hz) were applied to the data. Data were then segmented into epochs of 2000 ms (-500 to 1500 ms relative to stimulus onset). Epochs with unique, non-stereotypic artefacts were manually rejected. Following this, independent component analysis (ICA) was performed. Artefactual components (*i.e.,* eye blinks, movement, and alpha band activation) were identified and rejected after visual inspection. Data were then re-referenced to an average reference.

DATA ANALYSES

Food intake & hunger ratings—Paired samples *T*-tests in IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA) were performed on food intake (in grams and kcal) and on the hunger ratings (*i.e.,* hunger, fullness, prospective consumption, desire to eat, and thirst) provided pre- and post-meal to explore effect of food intake on general feelings of hunger.

Appetite ratings—Pre-meal appetite ratings were subtracted from post-meal ratings, yielding Δ appetite scores. A linear mixed effects models procedure in SPSS was applied to test sensory-specific effects of meal intake on Δ appetite. Participant number was added as a random factor. Meal type (sweet/savoury) and product category (HESw, HESav, LESw, LESav, Neutral) were included as fixed effects factors. First-order ante-dependence was chosen as covariance structure. A p-value of $< .05$ was considered significant. *Post hoc* paired comparisons were used to uncover effects of the meal type on the separate product categories.

Liking, wanting and intensity ratings—Pre-meal ratings for liking, wanting and odour intensity provided during the EEG paradigm were subtracted from post-meal ratings, yielding scores for Δ liking, Δ wanting, and Δ odour intensity. Mixed effects models in SPSS were applied to determine sensory-specific satiety effects. Separate analyses were performed for picture and for odour stimuli. We included participant number as random factor and meal type (sweet/savoury) and stimulus category (HESw, HESav, LESw, LESav, NF) as fixed effects factors. First-order ante-dependence was chosen as covariance structure. A p-value of $< .05$ was considered significant. *Post hoc* paired comparisons were used to uncover effects of the meal type on the separate stimulus categories. Results for odour intensity can be found in the supplemental materials (Supplementary Table 3.1.).

EEG data—A STUDY file was created in EEGLAB. For each meal type and each stimulus modality four separate study designs were added (savoury meal - picture; savoury meal - odour; sweet meal - picture; sweet meal - odour). Test moment (pre-meal/post-meal) and stimulus category (HESw, HESav, LESw, LESav food, and NF) were included. All ERPs within the design were precomputed and a baseline correction (-500-0 ms) was performed. Paired *T*-tests were performed to test pre- and post-meal differences in ERP responses for each of the food cues. Differences were explored by visualising pre- and post-meal group-averaged ERP waveforms from electrodes Fz, Cz, and Pz. Only values exceeding the statistical threshold of $p \leq .05$ at 30 contiguous sampling points were considered significant. Significant differences were only reported for picture responses between 100-1000 ms and odour responses between 250-1000 ms, latencies at which event-related potentials are reliably reported (see [150,151]. Olfactory ERPs have a low signal-to-noise ratio and olfactory stimulation does not elicit clearly identifiable negative and positive peaks in everyone [152]. For the analyses of olfactory ERPs we therefore only included participants ($n = 11$) that showed a recognizable ERP waveform averaged over all olfactory stimulations.

Results

FOOD INTAKE & HUNGER RATINGS

Intake of the sweet and savoury meal was similar (in grams: sweet 223 ± 114, savoury 244 ± 113, $p = .330$; and in kCal sweet: 315 ± 161, savoury 339 ± 157, $p = .413$). After the savoury and after the sweet meal, ratings of hunger, prospective consumption, and desire to eat decreased significantly and ratings of fullness increased significantly (all $p < .001$, see **Table 3.2.**). Ratings of thirst were significantly higher after sweet meal intake ($p = .019$) and did not change after the savoury meal ($p = .252$).

CHANGES IN SPECIFIC APPETITE RATINGS

The type of meal and the product category for which appetite was rated had a significant interaction effect on the change in appetite from pre- to post-meal $F(4,488) = 5.15$, $p < .001$). Pairwise comparisons revealed that the decrease in appetite for HESw products was significantly greater after the sweet compared to the savoury meal ($p = .001$) and the decrease in appetite for LESav was significantly greater after the savoury compared to the sweet meal ($p = .028$, see **Figure 3.2.**). No significant differences between

Table 3.2. Pre- and post-meal ratings (Mean ± *SD*) of hunger feelings (100 mm VAS)

Meal type	Parameter	Pre	Post	Significance (*p*-value)
Sweet	Hunger	65 ± 16	16 ± 15	< .001*
	Fullness	27 ± 23	67 ± 20	< .001*
	Prospective consumption	64 ± 12	27 ± 18	< .001*
	Desire to eat	69 ± 14	26 ± 20	< .001*
	Thirst	34 ± 21	22 ± 19	.019*
Savoury	Hunger	59 ± 20	16 ± 14	< .001*
	Fullness	24 ± 18	65 ± 22	< .001*
	Prospective consumption	62 ± 15	21 ± 16	< .001*
	Desire to eat	66 ± 21	19 ± 16	< .001*
	Thirst	27 ± 21	21 ± 22	.252

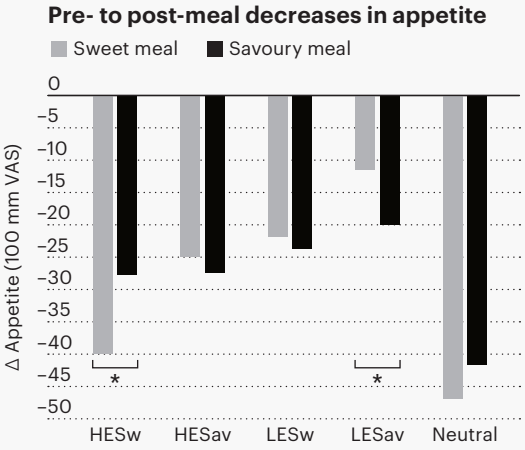


Figure 3.2. Pre- to post-meal decreases in appetite (Δ appetite) for several categories of food products, indicated for consumption of sweet meal and savoury meal. Δ appetite for HESw products was significantly greater after intake of a sweet compared to a savoury meal (p = .001) and Δ appetite for LESav was significantly greater after intake of a savoury compared to a sweet meal (p = .028).

meals were found for changes in appetite for HESav, LESw and Neutral products.

CHANGES IN LIKING FOR FOOD CUES

Odours—We found a significant interaction effect between the type of meal and stimulus category on liking of odours ($F(4,337) = 3.31$, $p = .011$; see **Figure 3.3.**). Pairwise comparisons showed a significantly greater decrease in liking for HESw odour after the sweet compared to the savoury meal ($p = .014$) and a significantly greater decrease in liking for HESav odour after the savoury compared to the sweet meal ($p = .013$). No significant differences were observed for the other stimulus categories.

Pictures—There was a significant interaction effect between the type of meal consumed and the stimulus category on the change in liking for pictures ($F(4,495) = 3.02$, $p = .018$; see **Figure 3.3.**). No significant effects of meal type were found for any of the stimulus categories in the *post hoc* pairwise comparisons.

CHANGES IN WANTING FOR FOOD CUES

Odours—The category of the stimuli and the type of meal had a significant interaction effect ($F(4,317) = 3.48$, $p = .008$; see **Figure 3.3.**). In the pairwise comparisons we observed significantly greater decreases in wanting for HESw odour after the sweet meal compared to the savoury meal ($p = .002$) and for HESav odour after the savoury compared to the sweet meal ($p = .035$). For the other stimulus categories no significant effects of meal type were found.

Pictures—The interaction between stimulus category and the type of meal had a significant effect on the change in wanting ($F(4,492) = 3.17$, $p = .014$; see **Figure 3.3.**). Pairwise comparisons revealed significantly greater decreases in wanting for HESw pictures after the sweet compared to the savoury meal ($p = .019$) and for HESav pictures after the savoury compared to the sweet meal ($p = .011$). Meal type did not significantly affect wanting for the other stimulus categories.

CHANGES IN ERP RESPONSES

Overall, we observed more negative amplitudes in event-related potentials (ERP) post- relative to pre-meal, both after a sweet and after a savoury meal (for a schematic overview of the results see **Supplementary Table 3.2**. and **Supplementary Table 3.3.**).

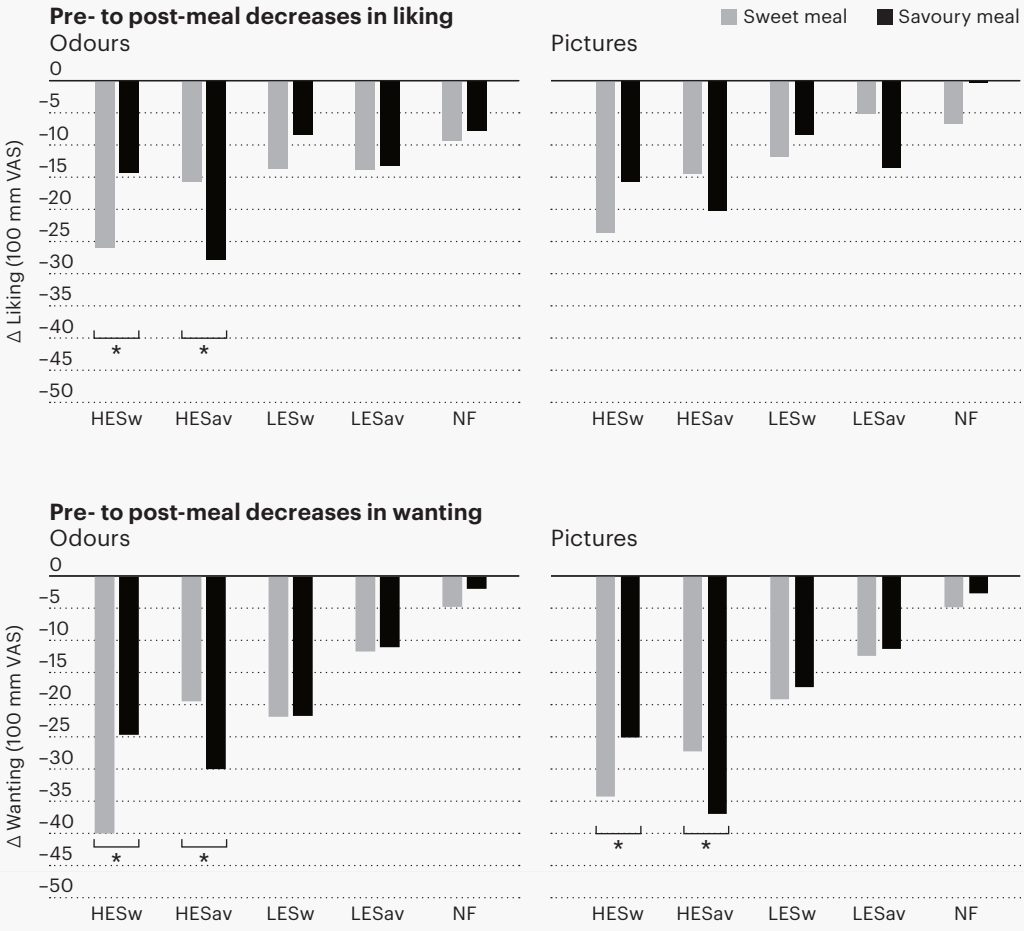


Figure 3.3. Pre- to post-meal differences in liking (Δ liking) and wanting (Δ wanting) for several categories of (food) cues, indicated for sweet meal and savoury meal consumption. Above left: Δ liking for HESw odours was significantly greater after intake of a sweet compared to a savoury meal (p = .014) and Δ liking for HESav odours was significantly greater after intake of a savoury compared to a sweet meal (p = .013). Above right: no significant differences were found in Δ liking for food and non-food pictures between sweet and savoury meal conditions. Below left: Δ wanting for HESw odours was significantly greater after intake of a sweet compared to a savoury meal (p = .002) and Δ wanting for HESav odours was significantly greater after intake of a savoury compared to a sweet meal (p = .035). Below right: Δ wanting for HESw pictures was significantly greater after intake of a sweet compared to a savoury meal (p = .019) and Δ wanting for HESav pictures was significantly greater after intake of a savoury compared to a sweet meal (p = .011).

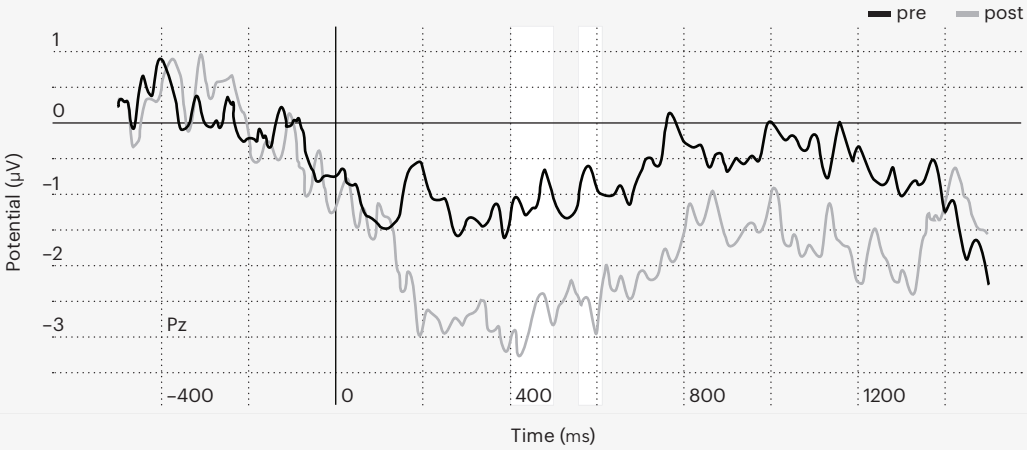


Figure 3.4. ERP-waveforms for HESav odour pre- (black line) and post- (grey line) savoury meal. The white bars indicate significantly increased post-meal amplitude of negative potential between 402–500 ms and 559–611 ms at Pz (*n* = 11).

Odours—After a sweet meal, olfactory ERPs to HESav odour showed significantly decreased amplitude of late positive potentials (623-668 ms) at Cz. We also found increased amplitude of negative potentials at Cz (420-455 ms and 490-525 ms) to LESw odour after sweet meal intake. Responses to HESw (congruent), LESav and NF odours were not significantly different pre- and post- sweet meal.

After a savoury meal, ERPs to HESav (congruent) odour showed significantly increased amplitude of negative potential at Pz (402-500 ms and 559-611 ms, see **Figure 3.4.**). Next to this, we observed increased amplitudes of late positive potentials at Cz (695-736 ms) to NF odour after compared to before savoury meal consumption. No significant pre- to post- savoury meal differences were found in ERP to HESw, LESw and LESav odours.

Pictures—After a sweet meal, negative amplitudes in ERP responses to HESw pictures at Fz were more pronounced in early (84-150 ms) and later stages (170-494 ms, 545-576 ms and 568-623 ms, see **Figure 3.5. A.**) of information processing. HESw pictures responses at Cz we also showed more negative amplitudes (273-307 ms, 320-375 ms and 434-477 ms, see **Figure 3.5. B.**) after

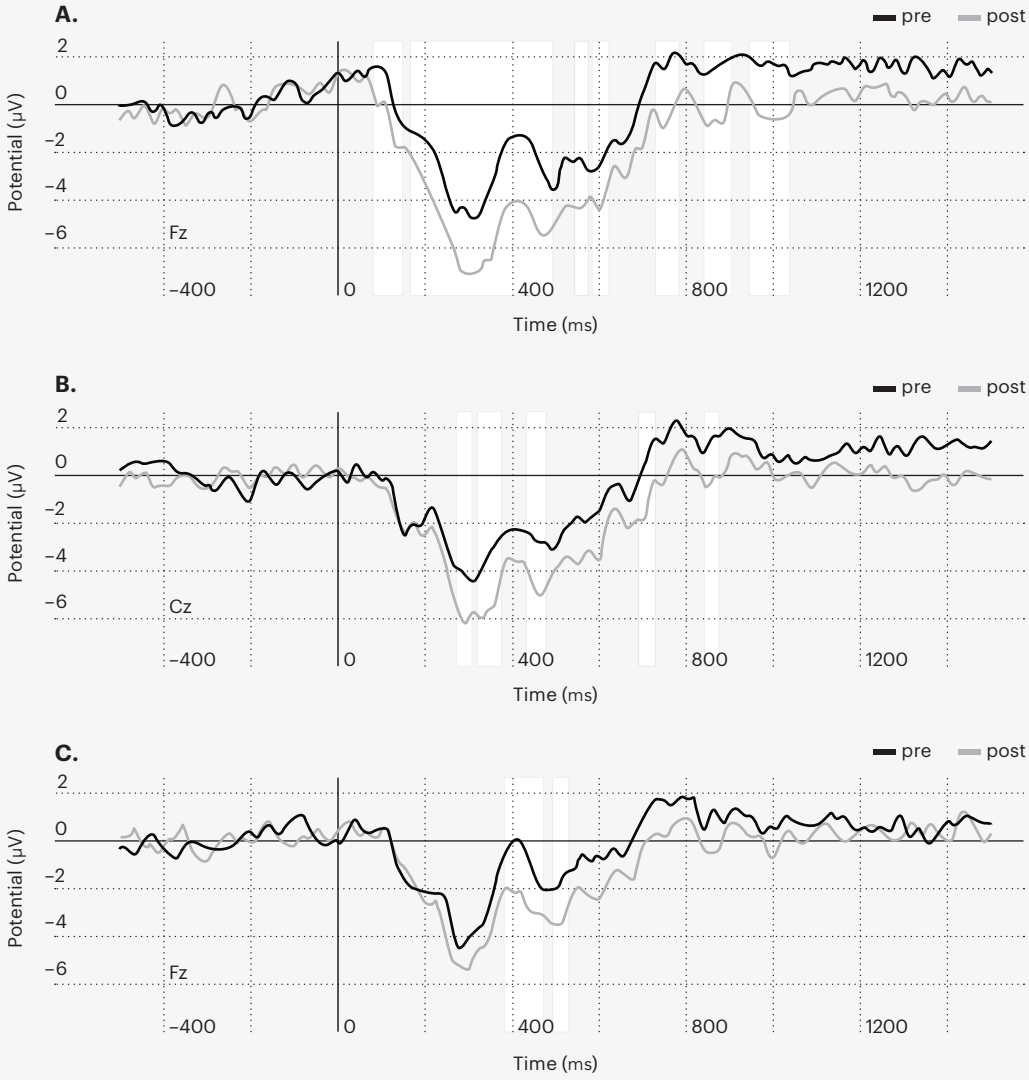


Figure 3.5. A. ERP-waveforms for HESw pictures pre- (black line) and post- (grey line) sweet meal. The white bars indicate significantly increased post-meal amplitude of negative potentials between 84–150 ms, 170–494 ms, 545–576 ms and 568–623 ms at Fz. **B.** ERP-waveforms for HESw pictures pre- and post- sweet meal. The white bars indicate significantly increased post-meal amplitude of negative potentials between 273–307 ms, 320–375 ms and 434–477 ms at Cz. **C.** ERP-waveforms for LESav pictures pre- and post- savoury meal. The white bars indicate significantly increased post-meal amplitude of negative potentials between 383–471 ms and 494–529 ms at Fz.

sweet meal consumption. We also found significantly higher amplitudes pre- compared to post- sweet meal in late positive potentials of ERPs for HESw pictures at Fz (730–783 ms, 842–902 ms and 945–1040 ms) and Cz (689–729 ms and 842–873 ms). After intake of a sweet meal, ERP responses to HESav pictures showed significantly greater amplitudes for negative potentials at Cz (149–182 ms) and Fz (643–691 ms). Further, in response to LESw pictures we found more negative amplitudes after sweet meal intake at Cz (385–420 ms and 494–557 ms) and Pz (88–121 ms and 432–623 ms). LESav pictures elicited less positive amplitudes at Fz (379–436 ms) and ERP responses to NF pictures showed decreased amplitudes of early and late positive potentials at Cz (373–418 ms, 576–643 ms, and 799–838 ms) and Fz (846–889 ms and 902–936 ms).

After a savoury meal, amplitudes of late positive potentials for HESw pictures were reduced at Cz (725–787 ms and 809–850 ms). Also, more positive amplitudes for HESw pictures were found at Pz (92–125 ms and 291–404 ms) post- compared to pre-meal. We did not find significant effects of savoury meal intake on ERP responses to HESav pictures. LESw pictures elicited greater amplitudes of negative potentials and lower amplitudes of positive potentials at Fz (170–215 ms and 344–375 ms) after compare to before a savoury meal. ERPs for LESav pictures showed increased negative amplitude post- compared to pre-meal ERPs at Fz (383–471 ms and 494–529 ms, see **Figure 3.5. C.**). Responses to NF pictures showed more negative amplitudes and decreased amplitude of late positive potential at Fz (598–645 ms, 826–887 ms and 900–957 ms) and increased amplitudes of late positive potentials at Pz (787–818 ms).

Discussion

To our knowledge, this study was the first to investigate the impact of sensory-specific satiety on neural processes of sensory perception and cognitive evaluation, reflected in event-related potentials to olfactory and visual food cues (visual and olfactory). We observed that overall, food intake led to more negative amplitudes in the event-related potentials (ERPs) to all cues. Interestingly, differences in event-related potentials before and after intake of a sweet meal indicated sensory-specific satiety changes in early components reflecting sensory perception (< 150 ms for visual stimuli) and in late components reflecting cognitive evaluation and motivated attention (~300–700 ms for

visual stimuli). The subjective ratings of liking, wanting and appetite confirm that participants reached sensory-specific satiety after both meals.

Subjective ratings of liking and wanting for the cues confirm that participants reached sensory-specific satiety after meal intake. Liking and wanting ratings for cues congruent to the consumed food decreased more than ratings for food cues that were incongruent to the consumed food. Intake of a sweet meal led to a more pronounced decrease of appetite for high-energy sweet products, while intake of a savoury meal reduced appetite more for low-energy savoury products.

In general, during later stages of ERP responses to all odours and pictures we observed more negative amplitudes after meal intake. Previous research findings indicate decreased amplitudes of positive potentials (170–310 ms, ~300–700 ms) to food cues associated with satiety [135,144,145,153]. It has been proposed that certain aspects of information processing are modulated based on their relevance to the motivational state [154–157]. For example, selective attention towards food cues is probably higher in a state of hunger because food cues are relevant for upregulating energy intake. However, this motivated attention theory does not fit with the increased negativity in late positive potentials we found (~600–650 ms, ~730–1000 ms) for frontal and central ERP responses to non-food stimuli, after meal intake. The non-food stimuli we selected were considered pleasant. Perhaps, satiety reduces attention towards cues that are salient (palatable, arousing), irrespective of the category they belong to. Alternatively, boredom or decreased attention to the paradigm could explain changes in neural processing after food intake, since post-meal EEG recordings were always performed after the pre-meal recordings in the current study design. However, studies that tested their participants on two separate days, thereby limiting effects of boredom, found similar changes [135,144,145,153].

Next to these general effects of food intake, we found pronounced differences in ERP responses to high-energy sweet cues after sweet meal consumption that suggest sensory-specific changes in neural information processing. As expected, amplitudes of late positive potentials (~300–700 ms) to high-energy sweet pictures were lower after compared to before sweet meal intake, which could reflect decreased hedonic evaluation of congruent food cues, in line with sensory-specific satiety. Consumption to satiety has been suggested to increase aversion for the consumed

food [140], which is reflected in a shift in activation of the orbitofrontal cortex from the medial part associated with processing of pleasant stimuli, to the lateral part associated with processing of aversive stimuli. The reduced late positive potentials are possibly related to increased boredom or decreased arousal with regard to the specific food product that was consumed. Interestingly, effects of boredom have been proposed to explain part of sensory-specific satiety [158] and reduced skin conductance and changes in facial expressions also suggest increased boredom to foods consumed to satiety [139].

Interestingly, we also demonstrated that sweet meal intake leads to more pronounced early negative amplitudes (< 150 ms) in frontal ERP responses to high-energy sweet pictures. This suggests sensory-specific satiety alterations in processes of sensory perception. Unpleasant stimuli were found to modulate early ERP amplitudes to a greater extent than pleasant or neutral stimuli, which suggests that stimuli with a negative valence are prioritized early in the information stream [159]. Sensory-specific satiety, characterised by decreased pleasantness for meal-congruent stimuli, could determine a predisposition in which top-down control of attention modulates early stages of stimulus processing, specifically for context congruent stimuli.

After savoury meal intake we observed more negative amplitudes (~400–600 ms) in posterior ERP responses to meal-congruent high-energy savoury odour, but not pictures. However, we did find that savoury meal intake led to more negative amplitudes (~400–500 ms) in frontal ERP response to low-energy savoury pictures. This is in accordance with the pattern of results we find for pre- to post-meal changes in appetite for foods and liking for pictures. Contrary to our expectations, appetite for low-energy savoury products, but not high-energy savoury products, decreased more after intake of a savoury meal compared to a sweet meal. Although non-significant, savoury meal intake led to a similar decrease in liking for low-energy savoury pictures. Overall, it seems that the savoury (beef) meal was less potent in achieving sensory-specific satiety compared to the sweet (chocolate) meal. This was also observed previously when beef stimuli were used to study sensory-specific satiety [31,160]. These diminished effects of sensory-specific satiety for beef could be related to a relatively low fat content, as greater sensory-specific satiety was found for products with a higher fat content [160], but could also be specific to the product beef.

This study was one of the first to demonstrate sensory-specific modulation of early neural processing of visual food cues. We did not observe changes in early neural processing of food odours, which is possibly related to a lower signal-to-noise ratio in the olfactory ERP response [152] in combination with a small sample size (N = 11). By using subjective ratings (liking, wanting and appetite) and implicit electrophysiological measures, we were able to relate changes in behavioural attitude towards food to changes in neural processing. The length of the test sessions (± 3 h in total) can be seen as a limitation of this study, as boredom may have influenced the results. However, reducing the number of trials would likely lead to a decrease in statistical power. Moreover, separating pre- and post-meal recordings will likely reduce boredom, but recording on the same day provides a better pre-meal baseline condition because day-to-day variations in the data are limited. In the current study we used a passive viewing/smelling paradigm. Perhaps, this is associated with a lower responsivity to food cues than in the context of an active task, which could explain why we also found significant differences in event-related responses to non-food items after food consumption [155]. Future studies should consider adopting a more interactive paradigm (*e.g.*, go/no-go, choice task) to investigate the influence of sensory-specific satiety on neural processing of food cues during behaviour related to appetite control. In addition, in order to better understand the modulation of ERPs by sensory-specific satiety found in the present study, it is relevant to look into sensory-specific changes in activation of the neural structures that generate these ERPs.

Conclusion

In conclusion, we found general effects of satiety on late event-related potentials to food and non-food cues, which may be related to decreased motivated attention to food cues during satiety. Modulation of non-food cue processing suggests that satiety may affect attention to a wider range of environmental cues. Interestingly, we also observed more pronounced modulation of early event-related potentials after sweet meal intake in response to high-energy sweet (congruent) food pictures, suggesting a less positive attitude and/or modulated top-down cognitive control towards meal-congruent stimuli by sensory-specific satiety. No such changes were found in relation to sensory-specific satiety with a savoury meal, indicating lower sensory-specific effects for savoury compared to sweet products.

Acknowledgements

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Supplementary materials

SUPPLEMENT 3.1: CHANGES IN ODOUR INTENSITY

There was no interaction effect between stimulus category and the type of meal on changes in rated odour intensity ($F(4,320) = 0.03$, $p = .999$). Also, pairwise comparisons did not show significant effects of meal type on any of the stimulus categories. Further, no significant interaction effect was found for test moment (pre-/post-meal) and stimulus category ($F(4,626) = 1.67$, $p = .155$). However, pairwise comparisons revealed significant pre- to post-meal changes in perceived intensity of HESav odour after both the sweet ($p = .028$) and the savoury meal ($p = .022$), and also of LESw odour after the savoury meal ($p = .047$).

Supplementary Table 3.1. Pre- and post-meal ratings of odour intensity (100 mm VAS) provided during the EEG paradigm

		Pre	Post	
Meal type	**Stimulus category**	**Mean ± *SE***	**Mean ± *SE***	**Significance *p*-value**
Sweet	HESw	70 ± 3	68 ± 3	.593
	HESav	68 ± 4	59 ± 4	.028*
	LESw	70 ± 4	63 ± 4	.089
	LESav	63 ± 4	60 ± 3	.310
	NF	63 ± 4	62 ± 4	.831
Savoury	HESw	74 ± 4	70 ± 4	.347
	HESav	67 ± 4	57 ± 4	.022*
	LESw	73 ± 4	65 ± 4	.047*
	LESav	61 ± 3	57 ± 3	.253
	NF	67 ± 4	66 ± 4	.823

Supplementary Table 3.2. Intervals and approximate potentials for significant differences in ERP responses to odours between pre- and post-meal measurements (*n* = 11).

		Cz			Pz		
			potential (μV)			**potential (μV)**	
Meal type	**Stimulus category**	interval (ms)	pre	post	interval (ms)	**pre**	**post**
Sweet	HESav	623 – 668	1.1	–0.4			
	LESw	420 – 455	0.4	-0.9			
		490 – 525	1.3	-0.7			
Savoury	HESav (congruent)				402 – 500	–1.2	–2.8
					559 – 611	–0.7	-2.7
	LESw	369 – 406	–0.5	1.2			
	NF	695 – 736	1.0	2.9			

Supplementary Table 3.3. Intervals and representative amplitudes for significant differences in ERP responses to pictures between pre- and post-meal measurements.

		Fz			Cz			Pz		
Meal type	**Stimulus category**	**interval (ms)**	**potential (μV) pre**	**post**	**interval (ms)**	**potential (μV) pre**	**post**	**interval (ms)**	**potential (μV) pre**	**post**
Sweet	HESw (congruent)	84 – 150	0.7	−0.7	273 – 307	−4.2	−6.2			
		170 – 494	−3.9	−6.5	320 – 375	−3.2	−5.5			
		545 – 576	−2.5	−4.3	434 – 477	−2.8	−4.9			
		586 – 623	−2.5	−4.1	689 – 729	0.9	−1.1			
		730 – 783	1.7	−0.6	842 – 873	1.8	−0.1			
		842 – 902	1.6	−0.7						
		945 – 1037	1.7	−0.5						
	HESav	643 – 691	−1.9	−3.2	149 – 182	−3.6	−2.2			
	LESw				385 – 420	−0.9	−2.5	88 – 121	−0.7	−2.2
					494 – 557	−1.1	−3.1	432 – 623	2.6	0.5
	LESav	379 – 436	−0.1	−2.7						
	NF	846 – 889	0.8	−1.0	373 – 418	−3.2	−4.2			
		902 – 936	1.5	−0.6	576 – 643	−1.3	−2.8			
					799 – 838	1.3	0.2			
Savoury	HESw				725 – 787	2.3	0.5	92 – 125	−2.3	−1.1
					809 – 850	1.8	−0.7	291 – 404	−0.4	1.8
	LESw	170 – 215	−1.8	−3.1						
		344 – 375	−2.1	−4.1						
	LESav	383 – 471	−0.2	−2.4						
		494 – 529	−1.5	−3.2						
	NF	598 – 645	−2.3	−3.7						
		826 – 887	1.0	−0.7						
		900 – 957	0.9	−0.5						

Chapter 4

Food preference and intake in response to ambient odours in overweight and normal-weight females

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Abstract

Background

In our food abundant environment food cues play an important role in the regulation of energy intake. Odours can be considered as external cues that signal energy content in the anticipatory phase of eating. This study aims to determine whether exposure to olfactory cues associated with energy dense foods leads to increased food intake and greater preference for energy-dense foods. In addition, we assessed whether BMI and hunger state modulated this effect.

Methods

Twenty-five overweight (mean BMI: 31.3 kg/m^2, *SE*: 0.6) and 25 normal-weight (mean BMI: 21.9 kg/m^2, *SE*: 0.4) females, matched on age and restraint score, participated. In 6 separate sessions they were exposed to odours of three different categories (signalling non-food, high-energy food and low-energy food) in two motivational states (hungry and satiated). After 10 minutes of exposure food preference was assessed with a computerized two-item forced choice task and after 20 minutes a Bogus Taste Test was used to determine energy intake (kcal and grams).

Results

In a hungry state, participants ate more ($p < .001$) and preferred high-energy products significantly more often ($p < .001$) when compared to the satiated state. A trend finding for the interaction between hunger and BMI suggested that the food preference of overweight participants was less affected by their internal state ($p = .068$). Neither energy intake (kcal: $p = .553$; grams: $p = .683$) nor food preference ($p = .280$) was influenced by ambient exposure to odours signalling different categories.

Conclusion

Future studies need to explore whether food odours can indeed induce overeating. More insight is needed regarding the possible influence of context (*e.g.,* short exposure duration, large variety of food) and personality traits (*e.g.,* restraint, impulsive) on odour-induced overeating.

Introduction

The sensory properties of food, such as the sight and smell, are important factors for regulating what and how much we eat [7,134]. Such food cues are omnipresent in our food abundant environment. When walking through a random shopping street you most likely encounter the sight of delicious burgers and the smell of sweet pastry. Food cues may provide information about the nutritional consequences related to the food they signal as we have learned to associate them with the post-ingestive effects after frequent combined exposure [22]. Mere exposure to these food cues may activate cephalic phase responses (*e.g.,* salivation, gastric activity) that prepare the gastro-intestinal tract for better absorption of nutrients [8,13,118]. This may stimulate food craving and intake [161,162].

Together with the sensory properties of food, internal signals related to metabolic state play an important role in determining food preference and intake [74,163,16474,163,164]. Metabolic state can modulate hedonic responses to the sensory properties of food and can thereby stimulate energy intake in a state of high energy demand (hunger). Findings of Jiang *et al.* [29] demonstrated that hedonic ratings of visual and olfactory food cues decrease after food intake. The strength of this effect is proposed to be dependent on the average hedonic values of food categories [165]. Highly palatable and energy dense foods (*e.g.,* ice cream, chocolate, cake and pie) received higher hedonic ratings in a hungry state, whereas ratings for low energy products such as fruits did not differ between metabolic states [29]. Food preference has been considered as the outcome of the hedonic value of the foods, the metabolic state and the context [166]. Altogether, it appears that the interplay between internal hunger cues and external food cues may influence the attitude towards food (hedonic ratings, preference). However, the interaction effect of the two factors has not been widely investigated and it is at present unclear how these factors affect actual food intake.

Eating behaviour of overweight people is thought to be less directed by internal cues, as these can be easily overruled by external cues [5,6]. A heightened cephalic phase response to palatable food cues in our food abundant environment may contribute to overeating and overweight. Ferriday & Brunstrom [68] already demonstrated that the motivation to consume food after exposure to the sight and smell of pizza was higher in overweight compared to lean individuals. BMI was found to positively affect portion size selection of visual and olfactory cued food [167]. Moreover, differences in actual food intake after cue exposure have been shown as well. Food in-

take during a Bogus Taste Test was marginally higher in overweight children after intensely smelling a food that was put in front of them than after a non-food control condition, whereas it was lower in normal-weight children [81]. On the other hand, pizza intake (g), after 1 min of pizza odour exposure, did not differ significantly between normal-weight and overweight participants in the study of Ferriday & Brunstrom [68].

If food cues indeed signal the body to gear up for optimized ingestion of food, it would not be surprising if this is directed toward the intake of specific food categories or products. Appetite for a product may not only be stimulated or suppressed by external cues that are related to the product category, but also by cues that are specific to the product. Rolls & Rolls [40] demonstrated that signs of satiety for a specific product occur after smelling their respective odour. They found that pleasantness ratings for banana and chicken odours decreased after 5 minutes of smelling a plastic cup containing banana or chicken, respectively. Recently, a similar study found opposing evidence for increased appetite specific to products that were cued by ambient odours [120]. Even though pleasantness of the odour and appetite for a product are not the same thing, it is plausible that odour exposure would modulate such ratings in the same direction. Perhaps the opposing effects can be explained by the fact that smelling odour-filled cups is more conscious, while ambient odour exposure represents a more implicit and realistic way of experiencing odours.

Previous research into the effects of food cues has often used a combination of visual and olfactory cues (*e.g.*[68,81]), or used 'artificial' methods of smelling (*e.g.* [40]). Independent from visual food cues, food odours alone might also lead to physiological responses that prepare for food intake. Although anecdotal evidence does suggest an important contribution of food odours to the regulation of energy intake, there is currently insufficient scientific evidence to substantiate these reports. Studies that have looked into behavioural responses to food cues mainly use subjective ratings (*e.g.*[40,68,120,167]). These ratings may provide some indication about food preferences, but they may not represent actual food choice and intake. Research into the effects of olfactory food cues on actual eating behaviour is scarce. Larsen *et al.* [123] examined this, but did not find an effect of ambient cookie odour exposure on cookie intake. Additionally, in order to learn more about odour-induced overeating in our food abundant environment, it is important to use experimental set-ups that mimic odour exposure as it occurs in a natural context.

To get a better grip on the complex issue of overeating, the mechanisms behind actual food intake regulation need to be clarified. In this study we explore a combination of several key factors: hunger state, BMI and energy density of the food cue. By examining effects of food odours alone with a more naturalistic method of odour exposure (ambient exposure) the current study provides new information on ecologically relevant food-cue responses. Our primary interest was to determine the effect of different categories of olfactory cues (signalling high-energy food, low-energy food and non-food) on eating behaviour (food preference and intake). In addition, we were interested whether this effect would be modulated by BMI and hunger state. Exposure to an odour of an energy-dense food product was expected to lead to an increased food intake and a stronger tendency to choose high-energy products. We hypothesized that this effect would be more pronounced in the overweight participants and that hunger state would influence food-cue responses less in overweight than in normal-weight individuals.

Methods

OVERALL DESIGN

This study followed a 2 × 2 × 3 mixed model cross-over design, including BMI group (overweight; normal-weight) as a between-subject factor and hunger state (hungry; satiated) and odour category (signalling high-energy food, low-energy food and non-food) as within-subject factors (see **Table 4.1.**).

Table 4.1. Schematic overview of the study design. In six separate sessions, overweight and normal-weight participants were exposed to odours of three different categories (signalling high-energy food, low-energy food and non-food) in two motivational states (hungry and satiated).

Between-subjects	**Within-subjects**			
BMI group	**Hunger state**	**Odour Type**	**Sweet**	**Savoury**
Normal weight (18.5-25 kg/m^2)	Hungry (8h fasting)	High-energy food	Chocolate	Peanut
Overweight (> 27 kg/m^2)	Satiated (breakfast)	Low-energy food	Strawberry	Cucumber
		Non-food	Fresh Green (flowery)	Wood

PARTICIPANTS

25 overweight (mean BMI: 31.3 kg/m^2, *SE*: 0.7) and 25 normal-weight (mean BMI: 21.9 kg/m^2, *SE*: 0.4) females participated in this study. They were matched on age (mean: 33, *SE*: 1.6) and restraint score (mean: 3.0, *SE*: 0.1). Restraint score (1-5) was determined using an online version of the restraint subscale of the Dutch Eating Behaviour Questionnaire (DEBQ; [130]). Higher scores indicate higher dietary restraint. Inclusion criteria were: weight stable for at least 6 months, no psychological or physical abnormalities or use of medication that could influence their sense of smell, eating behaviour or body weight. Individuals who met the inclusion criteria were invited for a screening session at Wageningen University.

During the screening session, BMI (kg/m^2) was measured. Individuals that were either normal-weight (BMI: 18.5-25 kg/m^2) or overweight (BMI > 27 kg/m^2) were included. Further, individuals were tested with the identification part of the Sniffin' Sticks task to ensure that they were normosmic (≥ 75% correct; [129]).

In order to keep participants naïve to the actual goal of the study (to study the effect of odour exposure on eating behaviour), they were informed that the study was aimed at assessing differences in the pleasantness ratings for several types of sandwich spreads between overweight and normal-weight participant. All participants provided written informed consent before entering the study. This study complied with the rules and regulations of the Medical Ethical Committee of Wageningen University and was executed in accordance with the ethical principles of the Declaration of Helsinki (2008).

OLFACTORY STIMULI

In six separate sessions participants were exposed to ambient odours of three different categories; signalling either high-energy food (HE), low-energy food (LE) and non-food (NF). Test sessions were conducted with two different odour sets since we were interested in odour category effects instead of effects of specific odours (see **Table 4.1.**). One set included sweet odours: Dark Chocolate (HE; IFF 10810212; 5% in Propylene Glycol (PG)), Strawberry (LE; IFF 10809989; 6% in PG) and Fresh Green (NF; AllSens No. 819; 2% in PG); and the other set included savoury odours: Peanut (HE; IFF 15038990; 3% in PG), Cucumber (LE; IFF 15032189; 100%) and Wood (NF; AllSens No. 821; 10% in PG). Participants were randomly assigned to one of the two odour sets. Because we were not interested in the specific effects of sweet

and savoury odours, results of the two sets were pooled together. Test sessions with the two odour sets were conducted in separate periods with four days in between to prevent possible odour contamination in the rooms. In addition, each room was designated to one odour to prevent odour contamination. Odours were released into the rooms using vaporizers (AllSens Geurbeleving, Oosterhout, the Netherlands). Air-conditioning was used to establish a continuous airflow. To ascertain similar odour intensities in each room, vaporizers were set to release a puff of odorous air for one second with intervals of one minute, for Dark Chocolate and Peanut (9.3 L/min) and for Strawberry and Cucumber (10.1 L/min). Wood and Fresh Green were released into the rooms with a continuous flow (10.7 L/min). Odours were clearly detectable but mild. Results from the debriefing after completion of the six test sessions indicated that some participants were aware of the odours in the test rooms.

In a pilot study, a separate sample of participants rated twelve different odours on estimated calories and liking (see **Supplementary Table 4.1.**). High energy-dense and low energy-dense food odours within one odour set had to be rated significantly different on expected amount of calories, according to their respective categories. From the remaining odour options we chose the ones that scored best on liking. To determine odour intensity, several participants were asked to rate three different intensity levels of ambient odours. All ratings were given on a 100 mm visual analogue scale (VAS). The data of this pilot were used to adjust ambient odours such that they were all presented at clearly perceivable but mild intensities.

EXPERIMENTAL PROCEDURE

Each participant visited the test location on six separate occasions, at approximately the same time in the morning (8:00-12:00 AM). Three participants could be tested simultaneously in three individual but similar, undecorated rooms of the same size, each containing a different odour. Over all six test sessions, every participant was exposed to odours of three different categories (signalling high-energy food, low-energy food and non-food), both in hungry and in satiated state. The order of conditions was semi-randomized. At baseline (non-odour room) the participant rated her appetite (hunger, fullness, prospective consumption, desire to eat, and thirst) on a 100 mm VAS scale. After 10 minutes, the participant was escorted to one of the three rooms that was

filled with one of the ambient odours. Here she was instructed to complete several tasks. After 10 minutes of exposure, food preference was measured and after 20 minutes of exposure food intake was measured. In spite of a significant decrease in odour intensity ratings over time, Ramaekers *et al.* [120] did not find an effect of exposure duration on appetite. Still, they found a significant increase in appetite after 18 min of odour exposure. This supported our choice to measure food intake after 20 minutes of odour exposure. For the timeline of the study procedure see Figure 4.1.

The experimental procedure of this study also included physiological measurements (heart rate, skin conductance and salivary response) and measurements of mood. Participants carried the wireless heart rate and skin conductance equipment on them throughout the test session. Results of these extra measurements will be reported elsewhere.

Figure 4.1. Schematic timeline of study procedure: After rating hunger in a non-odour room (10 min), the participant was guided to a room filled with an ambient odour. After 10 min of odour exposure, the food preference task commenced and after 20 min of odour exposure participants started a task in which ad libitum food intake was measured. The dark grey blocks indicate periods in which other measures were taken that will be described elsewhere (*e.g.*, mood and physiological responses)

HUNGER STATE

In three of the test sessions participants were asked to refrain from consuming anything but weak tea and water for eight hours before the test session (hungry state). In the remaining three sessions participants were instructed to consume the amount and

type of food and drinks they would normally consume during breakfast, before arrival at the test location (satiated state). Participants were instructed to consume the same type of breakfast before each of the three satiated test sessions. To ensure that participants were in the required hunger state, they rated their appetite at the beginning of each session, as described above.

ENERGY AND FOOD INTAKE

Food intake (g) was measured after 20 minutes of odour exposure with the use of a Bogus Taste Test. Participants were unaware that all the products provided in the taste test were weighed before and after the test session to determine food intake. Energy intake (kcal) was calculated afterwards based on product label information. The test products included chocolate paste (Albert Heijn Chocoladepasta puur), peanut butter (Albert Heijn Pindakaas Naturel), strawberry jam (light; Albert Heijn Halvajam aardbeien) and a grated cucumber salad (Albert Heijn komkommer), which were to be eaten on cream crackers (LU cream crackers). The products were all provided in amounts that allowed for *ad libitum* intake. We used familiar products that are known to be eaten for breakfast (and lunch) in the Netherlands. Water was supplied for rinsing in between the tasting of different products. Participants were instructed to consume all the water they were provided with so water intake would be equal in all participants. Audio-instructions informed the participants that they had to taste at least two bites of each product and give ratings on five questions in the provided questionnaire. They were given 15 minutes to complete the taste test, and participants were told that they were free to eat whatever they liked after finishing the taste test.

The selection of products used in the Bogus Taste Test was based on results of a pilot study that was conducted in a separate sample of participants. A plate with 15 products was served and participants had to smell five different odours, one by one. For each odour, participants had to make a top three ranking of products that best matched the odour (*e.g.,* after smelling Dark Chocolate odour: 1. Chocolate paste, 2. Chocolate sprinkles, 3. Chocolate flakes). We selected the best matching product for each odour that also fitted within their respective energy-density category.

FOOD PREFERENCE

After 10 minutes of odour exposure, food preference was assessed with a computerized paired picture food-preference task

(E-prime, v2.0; Psychology software tools, Sharpsburg, PA, USA) that was based on the Leeds Food Preference Questionnaire [168]. 16 different pictures of food items (83 × 124 mm; see **Supplementary Figure 4.1.**) were presented on a 15.4 inch laptop in all possible combinations of two, adding up to a total of 120 paired picture items. Participants could indicate which of the two presented food products they would like to eat most at that particular moment by button press ('c' or 'm' on the keyboard), which triggered the presentation of the next picture pair. The position of picture presentation was balanced between left and right. The food pictures were taken under the same conditions (background, lighting, camera angle, *etc*.). Food items were either high or low in energy-density, sweet or savoury, sandwich topping or not, and congruent or incongruent to the odours used in the study (see **Supplementary Figure 4.1.**). There were two congruent products for each of the four food odours. The food preference task took approximately 10 min to complete (see **Figure 4.1.**).

DATA ANALYSES

All analyses were performed following a linear mixed effects models procedure in IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk NY).As fixed effects BMI group, hunger state and odour category were included. Session number and participant ID were added as repeated variables. Compound symmetry was chosen as covariance structure. A p-value of $< .05$ was considered significant.

The analysis was performed on participants' total intake (kcal and g) during the Bogus Taste Test. Next to that, separate analyses were performed for intake of each of the four products (chocolate paste, strawberry jam, peanut butter and cucumber salad) to gain more insight into the sensory-specific effects of the different odours. Specific intake of cucumber for one of the participants was removed from the database (Z-score > 5), as this intake approached the total amount of this product offered, removing the *ad libitum* eating character of the test.

Food preference score was calculated as the percentage of times that a high-energy product was preferred over a low energy product. During the food preference task a subset of 64 items with picture combinations of ['high-energy food product' - 'low energy food product'] were presented (*e.g.,* crisps - carrots). In order to assess sensory-specific effects of the odours, the percentage of preference was calculated for the four product types

(chocolate products, strawberry products, peanut products and cucumber products; see **Supplementary Figure 4.1.**). For this, the frequency of preference for a specific product type was divided by the total number of presented items (*i.e.* 120) and multiplied by 100. Each product type was presented in 28 items, which is related to a maximum sensory-specific preference score of 23.3%.

Results

HUNGER STATE (MANIPULATION CHECK)

Hunger ratings taken at baseline confirmed that feelings of hunger were significantly different in the two hunger states (hungry *vs.* satiated). In the hunger conditions participants felt more hungry, less full, showed an increased prospective consumption, a higher desire to eat and were more thirsty compared to the satiated conditions (all $p < .001$; see **Table 4.2.**).

ENERGY AND FOOD INTAKE

Total energy intake during the Bogus Taste Test was 105 kcal higher in a hungry (mean ± *SE*: 321 ± 19 kcal) than a satiated state (216 ± 19 kcal; $F(1,240) = 91.00$, $p < .001$). Food intake was 32 g higher in a hungry state (295 ± 6 g) compared to a satiated state (263 ± 6 g; $F(1,240) = 87.55$, $p < .001$). Intake did not differ significantly between the overweight and normal-weight participants (kcal: $F(1,48) = 0.12$, $p = .72$; g: $F(1,48) = 1.22$, $p = .28$), nor was there a significant interaction between hunger state and BMI (see **Figure 4.2.**; kcal: $F(1,240) = 1.84$, $p = .18$; g: $F(1,240) = 1.02$, $p = .31$).

Table 4.2. Hunger ratings for both motivational states as measured on a 100 mm VAS, for all participants combined

	Hungry state (Mean ± *SE*)	**Satiated state (Mean ± *SE*)**
Hunger***	62 ± 2	15 ± 2
Fullness***	20 ± 2	62 ± 2
Prospective consumption***	59 ± 2	25 ± 2
Desire to eat***	65 ± 2	20 ± 2
Thirsty***	44 ± 3	32 ± 3

*** *p*-value < .001

Additionally, total intake was not influenced by the odour condition (see **Figure 4.3.**; kcal: $F(2,240) = .63$, $p = .53$; g: $F(2,240) = .38$, $p = .68$). No significant interaction was found between odour category and BMI (kcal: $F(2,240) = 0.56$, $p = .57$; g: $F(2,240) = 0.45$, $p = .64$) nor hunger (kcal: $F(2,240) = 0.61$, $p = .55$; g: $F(2,240) = 0.27$, $p = .76$).

Analyses of the intake (kcal and g) of the four products separately did not show significant differences between odour conditions (chocolate, strawberry, fresh green, peanut, cucumber or wood) for intake of chocolate paste ($F(5,146) = 1.02$, $p = .41$), strawberry jam ($F(5,161) = 0.58$, $p = .71$), peanut butter ($F(5,151) = 0.10$, $p = .99$) or cucumber salad ($F(5,145) = 0.24$, $p = .94$).

FOOD PREFERENCE

In a hungry state, participants preferred the high-energy products 6.4% more often than when they were in a satiated state (hungry: 43.6% ± 2.3; satiated: 37.2% ± 2.3; $F(1,240) = 32.18$, $p < .001$). This effect of hunger appeared to be less pronounced for overweight participants (4.3% difference) than for normal-weight participants (8.4% difference; see **Figure 4.4.**; $F(1,240) = 3.37$, $p = .07$).

Exposure to the different odour categories did not result in a significant difference in food preference (see **Figure 4.5.**; $F(2,240) = 1.28$, $p = .28$). Also, no significant interaction was found between odour category and BMI ($F(2,240) = 0.10$, $p = .91$) nor hunger ($F(2,240) = 0.77$, $p = .47$).

Separate analyses for each product did not reveal significant effects of odour exposure (chocolate, strawberry, fresh green, peanut, cucumber or wood) on food preferences for chocolate products ($F(5,140) = 1.29$, $p = .27$), strawberry products ($F(5,137) = 1.30$, $p = .27$), peanut products ($F(5,146) = 0.55$, $p = .74$) or cucumber products ($F(5,136) = 1.59$, $p = .17$).

Discussion

To our knowledge, this is the first study to examine food preference and food intake in response to ambient odour exposure in normal-weight and overweight participants. Participants were exposed to three different categories of odours (signalling high-energy food, low energy food and non-food) in both a hungry and a satiated state. Our results show that participants ate more when they were hungry than when they were satiated. In addition, they show a significantly greater preference for high-energy food in hungry state compared to satiated state. Moreover, our results point to a stronger effect of hunger on high energy-dense food preference

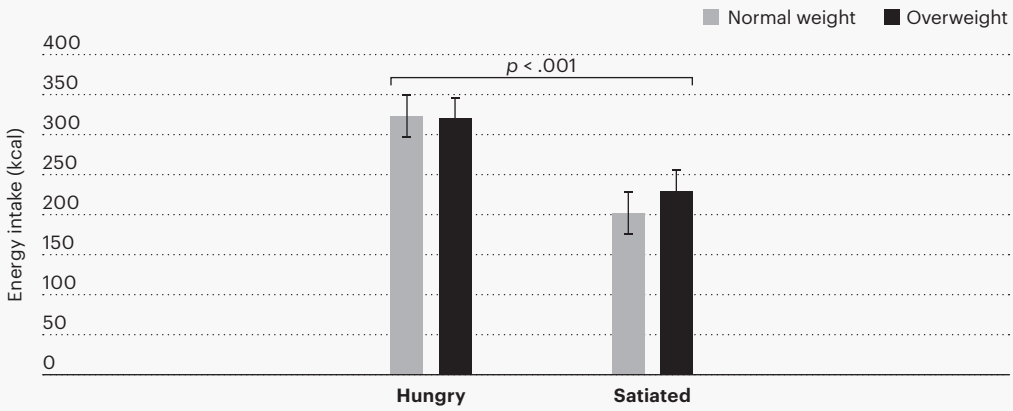

Figure 4.2. Energy intake – Hunger state: Total energy intake (kcal) after 20 min of odour exposure for the two hunger states, separated for overweight (black bars) and normal-weight (grey bars) participants. Results show higher intake in hungry state compared to satiated state. No significant interaction between hunger state and BMI was found.

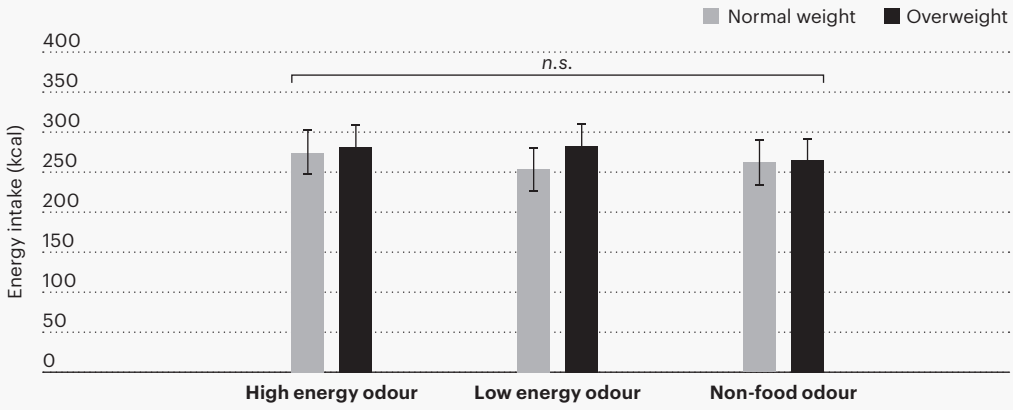

Figure 4.3. Energy intake – Odour type: Total energy intake (kcal) after 20 min of odour exposure for three odour categories, separated for overweight (black bars) and normal- weight (grey bars) participants. Total energy intake did not significantly differ between odour conditions. No significant interaction was found between odour category and BMI.

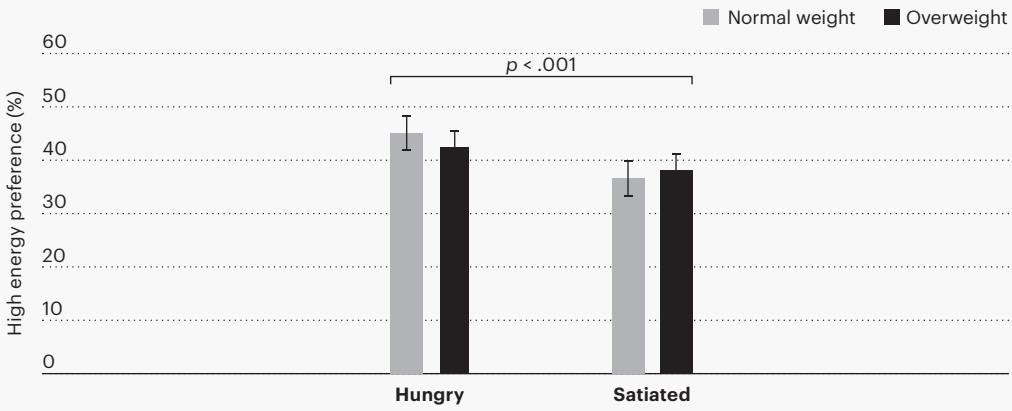

Figure 4.4. Food preference – Hunger state: Preference for high-energy products measured after 10 min of odour exposure using a two item forced-choice task, depicted for the two hunger states, separated for overweight (black bars) and normal-weight (grey bars) participants. Results show more high-energy preference in hunger state compared to satiated state. A trend level interaction was found between hunger state and BMI (p = .07).

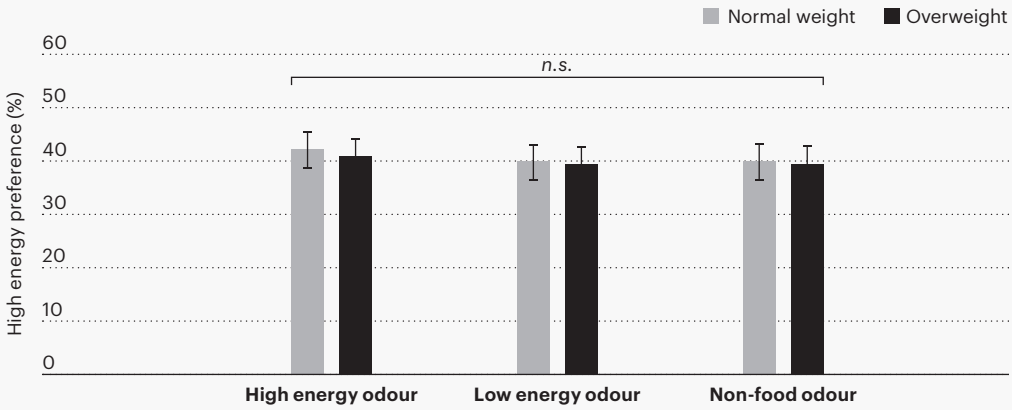

Figure 4.5. Food Preference – Odour type: Preference for high-energy products measured after 10 min of odour exposure using a two item forced-choice task. Results depicted for the three odour categories, separated for overweight (black bars) and normal-weight (grey bars) participants. Food preference did not significantly differ between odour conditions. No significant interaction was found between odour category and BMI.

in normal-weight than overweight participants. In spite of the clear presence of odours, no significant difference in food preference and intake was found between the different odour conditions.

Feelings of hunger and satiety are crucial in the regulation of energy intake [134,169]. Hunger signals ensure increased energy intake after deprivation whereas feelings of satiety put a brake on intake when the energy balance is restored. As expected, our results indicate that overnight fasting for eight hours was enough to induce feelings of hunger. Internal state of hunger was in turn associated with higher food intake in both overweight and normal-weight participants compared to a satiated state. These results are in line with a study by Nederkoorn *et al.* [74], where physiological hunger was related to increased food intake. Although a difference in food intake of 32 g seems small (11% of the total intake in grams), the effect of hunger state on intake was possibly reduced because participants were always instructed to eat at least one bite of each product. Notably, the effect of hunger state on energy intake is relatively higher (Δ 105 kcal; 33% of the total energy intake). In our study, hunger increases the amount of food intake and increases the preference for products that are most effective in quickly restoring energy balance (high-energy products).

Normal-weight participants showed a higher preference for high-energy products in a hungry state than in a satiated state. In overweight people this difference in high-energy preference between the two states was less pronounced. This trend level interaction between hunger state and BMI group seems to indicate that overweight individuals are less sensitive to their internal hunger cues compared to normal-weight individuals, which is in line with earlier suggestions by Herman & Polivy [5] and Schachter [6]. Moreover, they argue that overweight individuals' eating behaviour is more determined by external cues of palatable food. Energy dense foods are typically regarded as more palatable than low energy foods. However, this heightened responsivity to external cues could not be confirmed by our current findings that did not show an effect of odour exposure on food preference and intake. Possibly, a decreased sensitivity to hunger signals may increase the number of times people with overweight eat after temptation by food cues instead of increasing portion size per eating occasion, which in turn could still result in overeating. Normal-weight individuals may be more resistant to external food temptations because their internal state signals are more powerful in inhibiting the response to external cues. Overweight and normal-weight participants both indicate

feelings of hunger that are concordant with the state of hunger they we instructed to be in, however it seems that their eating behaviour is affected differently.

Contrary to our hypothesis, no significant differences in food intake and food preference were found between the ambient odour conditions (high-energy food, low energy food and non-food), even though the odours were presented at supra-threshold intensities. Previous studies have demonstrated increased food craving [67,69] and food intake [81,161] after food-cue exposure. However, those studies used a combined exposure to visual and olfactory cues of food that may have strengthened the food-cue response (see [170,171]) by increasing awareness of the food cues or the ability to identify the cued product. On the other hand, identification of an odour may alter odour perception and subsequent behavioural responses (see *e.g.* [172,173]) and it has been suggested that odours are able to influence behaviour outside of awareness [174]. A recent study by Ramaekers *et al.* [120] showed increased appetite after ambient exposure to food odours (supra-threshold) alone, whereas non-food odours were found to suppress appetite. Moreover, the food odours used in that study specifically affected appetite for the products cued by the food odour. The differences between our findings and those of Ramaekers *et al.* [120] may be related to the fact that self-report measures such as appetite ratings cannot be directly extrapolated to food intake, *i.e.* the type and amount of food people indicate that they want to eat is not necessarily equivalent to what they eventually consume (*e.g.* [175-177]). Future studies need to provide increased insight into the distinction between prospective measures of eating (*e.g.*, appetite ratings, preference) and actual food intake.

The relatively long period of odour exposure (10 minutes for food preference; 20 minutes for food intake) could explain why we did not obtain odour-induced effects on eating behaviour, as cue-related responses may fade as result of adaptation [178] or habituation [179]. In previous research, decreased food intake was found after long exposure to visual and olfactory food cues (5–10 min; [81,180]), whilst brief exposure times seemed to increase (prospective) intake (1–3 min; [68,69,161]. Nevertheless, Ramaekers *et al.* [120] found that ambient food odour exposure already resulted in significantly increased appetite ratings after one minute and did not change over time (5, 9, 13 and 18 min), suggesting no adaptation or habituation after prolonged odour exposure. However, appetite ratings and actual food intake might be affected differently by exposure duration.

Almost all participants in this study were restrained eaters, who have the intention to restrict their intake [13]. This could have reduced the likelihood to find significant differences in food intake between normal-weight and overweight participants and the odour conditions. Prolonged exposure to food odours might trigger cognitive control mechanisms that counteract initial behavioural responses, especially in restrained eaters [181]. After exposure to palatable food cues, successful restrained eaters even showed 'neural alarm bell responses' that suggested activation of self-regulation [182].

Even though the current study adopted a naturalistic method of odour exposure, examining the effects of ambient odour exposure on eating behaviour in a more conventional eating context such as a restaurant, might provide further insights about actual food choice and intake. Moreover, in a restaurant setting the assortment of food products is generally larger than in a taste test, increasing the probability of covering personal preferences. This may in turn lead to a higher variability in food intake and increased likelihood of discovering significant effects of odour. Furthermore, food odour-induced overeating may not be apparent in all overweight individuals. In previous studies, overeating was only observed in highly restrained eaters that also scored higher on impulsivity [73,74]. A specific combination of personality traits (*e.g.* restraint and impulsivity) might be the constraint under which overeating after cue exposure can be found. Future studies that will examine eating behaviour in a restrained population should also include measures of impulsivity.

This is one of the first studies that systematically examined the effect of olfactory food cues on actual food intake and preference. In conclusion, we found that hunger is an important determinant for food intake and food preference. Overweight individuals appeared less affected by their hunger state than normal-weight individuals. Despite the clear presence of ambient odours, our participants' eating behaviour was unaffected by odours that signal different energy densities and food products. By exploring a combination of key factors in eating behaviour (BMI group, hunger and energy density associated with the food cue) this study has provided a fundament for further research into cue-induced overeating. How olfactory cues play a role in overeating remains to be clarified. Future studies are needed to systematically examine under which circumstances (*e.g.*, odour exposure duration, personality traits, metabolic state and setting) odour-induced overeating can take place.

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Supplementary materials

Supplementary Table 4.1. Pilot study results: Ratings of 'expected amount of calories' (very little–very much) and 'liking' (not at all–extremely) for selected and non-selected odours as measured on a 100 mm VAS.

Odour Set	Category	Odour	Calories (Mean ± *SE*)	Liking (Mean ± *SE*)
Set 1: sweet	High-Energy Food	Dark chocolate	78 ± 2	55 ± 7
	Low-Energy Food	Strawberry	38 ± 9	48 ± 7
	Non-Food	Fresh Green	–	33 ± 5
Set 2: savoury	High-Energy Food	Peanut	42 ± 8	33 ± 6
	Low-Energy Food	Cucumber	10 ± 3	73 ± 4
	Non-Food	Wood	–	52 ± 6
not selected		Coconut	53 ± 6	60 ± 5
		Cheesecake	53 ± 7	31 ± 5
		Banana	47 ± 8	52 ± 6
		Tomato	24 ± 4	26 ± 7
		Lavender	–	52 ± 7
		Grass	–	37 ± 6

Calories: *** (Dark chocolate vs. Strawberry)

Liking: n.s. (Dark chocolate vs. Strawberry); ** (Strawberry vs. Fresh Green); * (Dark chocolate vs. Fresh Green)

Set 2: savoury — Calories: ** (Peanut vs. Cucumber)

Set 2: savoury — Liking: *** (Peanut vs. Cucumber); * (Cucumber vs. Wood); * (Peanut vs. Wood)

*p-value < .05, **p-value < .01; ***p-value < .001

Supplementary Figure 4.1. Food Preference Task – Stimuli: During the food preference task, 16 food pictures (83 × 124 mm) were presented on a 15.4 in. laptop in 120 possible pair combinations. Food items were either high or low in energy-density, sweet or savoury, sandwich topping or not, and congruent or incongruent to the odours used in the study.

Chapter 5

Altered neural responsivity to food cues in relation to food preferences, but not appetite-related hormone concentrations after RYGB-surgery

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Abstract

Background After Roux-en-Y gastric bypass (RYGB) surgery, patients report a shift in food preferences away from high-energy foods.

Objective We aimed to elucidate the potential mechanisms underlying this shift in food preferences by assessing changes in neural responses to food pictures and odours before and after RYGB. Additionally, we investigated whether changes in neural responses are associated with changes in plasma endocannabinoid and ghrelin concentrations.

Methods 19 RYGB patients (4 men; age 41 ± 10 years; BMI 41 ± 1 kg/m^2 before; BMI 36 ± 1 kg/m^2 after) participated in this study. Before and two months after RYGB surgery, they rated their food preferences using the Macronutrient and Taste Preference Ranking Task. BOLD fMRI responses towards pictures and odours of high-energy foods, low-energy foods and non-food items were measured before and two months after surgery. Blood samples were taken to determine plasma endocannabinoid and ghrelin concentrations pre- and post-surgery.

Results Patients demonstrated a shift in food preferences away from high-fat/sweet and towards low-energy/savoury food products, which correlated with decreased superior parietal lobule responsivity to high-energy food odour and a reduced difference in precuneus responsivity to high-energy versus low-energy food pictures. Deactivation in the anteroventral prefrontal cortex (superior frontal gyrus) was less towards low-energy food pictures and more similar to high-energy versus non-food odours. The precuneus was less deactivated in response to all cues. Plasma concentrations of anandamide were higher after surgery, while plasma concentrations of other endocannabinoids and ghrelin did not change. Changes in neural responses were not correlated with changes in appetite-related hormone concentrations.

Conclusions RYGB leads to changes in top-down control towards high-energy food compared to low-energy food and non-food cues orchestrated by the frontoparietal control network, rather than changes in activation of reward related brain regions, in a satiated state. Together with correlations with the shift in food preference from high- to low-energy foods this indicates a possible role in new food preference formation.

Introduction

Roux-en-Y gastric bypass (RYGB) surgery is currently the most effective long-term treatment for morbid obesity [102]. After RYGB, patients report decreased hunger and lower caloric intake. In addition, a shift in food preferences from high- to low-energy foods is observed [103]. This change in food preferences has been related to alterations in taste perception and food reward, with fMRI studies showing decreased activation in the mesocorticolimbic reward network in response to high-energy compared to low-energy food cues [108]. However, the exact mechanism behind this shift in food preferences and related neural responses is not completely understood. Potential mediators include changes in subjective hedonic evaluation of food (cues), changes in gut hormones signalling hunger and satiety, post-ingestive side effects of surgery, and changes in nutrient sensing in the gut [183]. A better understanding of the mechanisms behind changed food preferences may help to identify factors responsible for the success of this weight-loss intervention, and might guide novel non-surgical strategies that have less risk of complications.

As previously shown, the decreased desire to eat high-energy foods correlates with decreased activation in reward-related brain areas to pictures of high-energy compared to low-energy foods [103,108,109]. In a scanner setting it is difficult to realistically mimic an eating environment and thus far, neuroimaging studies used pictures as food cue. However, other sensory modalities might be equally or more important for food choice and anticipation of food intake. Specifically, food odours play a crucial role in initiating food intake [41,137,184] by steering appetite [147] and cravings for specific foods [67,123]. Given their largely unconscious role in priming eating decisions, it is of interest to investigate (alterations in) neural reward responses to palatable food odours as well, in relation to changes in food preferences after RYGB.

There is mounting evidence that gut hormones play a role in altered food preferences after RYGB [185]. In general, gut hormones play an important role in food choice and food intake, by signalling nutritional status and food reward value to the brain [186]. Most gut hormones are anorexigenic, with higher circulating plasma concentrations resulting in a suppression of food intake. Ghrelin, however, is an orexigenic gut hormone that can stimulate food intake [187]. Moreover, ghrelin is not only involved in regulating homeostatic eating; eating to fill a need for energy or nutrients, but also in hedonic eating; eating for pleasure [188,189]. The orexigenic effect of

ghrelin appears to be mediated through the endocannabinoid (eCB) system [190]. The eCB system is a neuromodulatory system that consists of endogenous ligands, so called endocannabinoids, their receptors and enzymes involved in their synthesis and breakdown. Ghrelin and eCB plasma concentrations increase in anticipation of hedonic eating [189,191], and neuroimaging studies suggest a role for ghrelin and the eCB system in reward processing [192-194]. Thus far, only a few studies examined the role of ghrelin, and none have explored the eCB system, in relation to altered brain reward activation after RYGB and food preferences [195,196].

To improve our understanding of the changes in food preferences typically seen after RYGB, we assessed neural changes in response to high- and low-energy appetizing food cues in two different sensory modalities: odours and pictures. Additionally, to better explain these neural mechanisms, plasma eCB and ghrelin concentrations, as well as food preferences were measured and correlated to changes in brain activation pre- to post-surgery.

Participants and Methods

PARTICIPANTS

Twenty-one morbidly obese individuals participated in this study. All participants were enlisted to undergo RYGB surgery at Rijnstate hospital, Arnhem, the Netherlands. Requirements for the surgery were: Body Mass Index (BMI) of > 40 kg/m^2 or > 35 kg/m^2 with co-morbidity that was expected to improve after surgically-induced weight loss, long-lasting obesity (> 5 years), proven failed attempts to lose weight in a conventional way, intention to adhere to a postoperative follow-up program. Reasons not to consider individuals for surgery were being pregnant or lactating, psychiatric disorders, alcohol or drug dependency, life threatening conditions or being dependent on the care of others. Individuals were screened for participation in the study at Rijnstate hospital. All participants were right-handed, non-smoking, did not have conditions that interfered with the MRI measurements (*e.g.*, claustrophobic, metal implants, pacemaker, neurological disorders), had a normal sense of smell (scoring ≥ 10 on the identification part of the Sniffin' Sticks [129]), were not vegetarian and did not have allergies or intolerances to the foods used and cued (visual/olfactory stimuli) in the study. Participants received a monetary reward for their contribution. All participants provided written informed consent before entering the study. The protocol was approved by the Medical Ethical Committee of Wageningen University (NL45837.081.13)

and was executed in accordance with the ethical principles of the Declaration of Helsinki of 1975, as revised in 2013.

OVERALL DESIGN AND EXPERIMENTAL PROCEDURE

This study had a 2 × 2 × 3 within-subject design, including the factors time (pre- and post-gastric bypass surgery), stimulus modality (visual/olfactory) and stimulus category (high-fat/high-sugar (HFHS) food, low-fat/low-sugar (LFLS) food, Non-Food (NF)).

Participants visited the test facilities at three occasions. First, they were familiarized with the MRI test environment and the experimental task and stimuli used, in a dummy MRI scanner at Wageningen University (training session). Following the training session, there were two identical test sessions during which the actual measurements were taken. The first test session took place 3.4 (*SD* 1.8) weeks before and the second test session took place 9.2 (*SD* 1.3) weeks after RYGB. Each participant was scanned at approximately the same time of day for both sessions, between 14:00-17:00 at hospital Gelderse Vallei (Ede, The Netherlands). Participants were instructed to refrain from eating and drinking anything but water and weak tea in the three hours before a test session. Upon arrival at the hospital, blood samples were taken for analysis of plasma endocannabinoid and ghrelin concentrations. In order to measure responses underlying hedonic eating (eating for pleasure, in the absence of hunger), participants were offered orange juice, and after a short break they consumed a standardized meal consisting of bread roll(s), cheese, ham and butter (see **Supplementary Table 5.1.** for more detailed information), to evoke a state of satiety (**Table 5.1.**). Following this, they waited for 15 minutes to allow digestion. Before entering the MRI room, participants were presented with the odours and pictures used in the reward task to familiarize with the stimuli and reinforce the appropriate association with the stimuli. They also rated their hunger, fullness, prospective consumption, desire to eat, and thirst on 100-unit Visual Analogue Scales (VAS). During the scan session, first, a reward task was performed while functional MR images were acquired. Second, structural MR images were collected. Thereafter, participants took part in two additional functional runs in which a food-related go/no-go task was performed (data reported elsewhere). At the end of the test session, olfactory performance was assessed using the Sniffin' Sticks (threshold, discrimination, identification [129]). The regular Identification 16 was used during screening, thus to prevent a potential learning effect, we used the Identification 16+ for the sec-

ond test session [197]. Paired sample *T*-tests revealed that the overall olfactory performance (TDI score) was not different between test sessions (before: 33.7 ± 4.7; after: 35.0 ± 4.2; $p = .328$).

Table 5.1. Weight and hunger ratings (100-unit VAS) pre- and post- RYGB

	Pre-surgery Mean ± *SD*	**Post-surgery Mean ± *SD***	**Significance**
Weight (kg)	120 ± 14	104 ± 15	$p < .001$
BMI (kg/m^2)	41 ± 3	36 ± 4	$p < .001$
Hunger	13 ± 21	11 ± 24	$p = .738$
Fullness	70 ± 25	68 ± 34	$p = .867$
Prospective consumption	27 ± 25	8 ± 18	$p = .020$
Desire to eat	22 ± 20	12 ± 23	$p = .197$
Thirst	66 ± 26	51 ± 30	$p = .022$

BMI: body mass index; RYGB: Roux-en-Y Gastric Bypass; VAS: visual analogue scale

FOOD PREFERENCES (MTPRT)

Food preferences were assessed two weeks before and two months after RYGB using the online version of the macronutrient and taste preference ranking task (MTPRT[198]). In this task, participants were presented with four pictures of different food products at a time and asked to rank the products according to what they most desire to eat at that moment. Food products included in this task were either high in carbohydrate, high in fat, high in protein or low in energy, and had a sweet or savoury taste. The MTPRT was presented in EyeQuestion software (Logic8 BV).

FOOD STIMULI (ODOURS AND PICTURES)

Odours and pictures were selected to signal either high-fat, high-sugar food (HFHS), low-fat, low-sugar food (LFLS) or non-food (NF) items (as control) by means of pilot studies in separate samples of participants. Odours were selected to be similar in perceived intensity and liking, different in the associated energy-density, and correctly associated to the

corresponding food product/object. The selected odours were Chocolate (HFHS; International Flavors and Fragrances (IFF)

10810180; 8.5% in Propylene Glycol (PG)), Caramel (HFHS; IFF 10895342; 20% in PG), Tomato (LFLS; IFF 15039016; 24% in PG), Cucumber (LFLS; IFF 73519595; 34% in PG), Fresh Green (NF; AllSens-Voit Aroma Factory No. 819; 2.2% in PG), Wood (NF; AllSens-Voit Aroma Factory No. 821; 2.2% in PG). Pictures were selected to be similar in liking, and consistently matched to a food product/object and to one of the selected odours. For each odour we selected three different pictures to reduce effects of boredom. We selected Chocolate muffin, Brownie, and Chocolate bonbons for Chocolate odour; Caramel ice-cream, Stroopwafel (Dutch caramel syrup waffle), and Boterkoek (Shortbread) for Caramel odour; Tomato slices with pepper, Tomato slices, and Tomato slices with basil for Tomato odour; Cucumber slices with peel, Cucumber salad, and Cucumber chunks for Cucumber odour; Green soap, Tulips, and White flowers for Fresh Green odour; and Chunk of wood, Pine branches, and Purple soap for Wood odour. Standardized food images used in the fMRI task were provided by the Image Sciences Institute, UMC Utrecht, and created as part of the Full4Health project (www.full-4health.eu), funded by the European Union Seventh Framework Program (FP7/2007-2013) under grant agreement nr. 266408, and the I.Family project (http://www.ifamilystudy.eu), grant agreement nr. 266044 [148].

(F)MRI PARADIGM AND MEASUREMENTS

The reward task lasted ± 40 min and consisted of olfactory and visual cues of HFHS food, LFLS food and NF were presented one by one, in pseudo random order (see **Figure 5.1.**). Six olfactory stimuli and 18 visual stimuli (3 related to each odour) were presented. Each odour was presented 15 times and each picture was presented 5 times, resulting in a total of 90 odour and 90 visual presentations. Trials started with the presentation of a red fixation cross (1s) during which participants were instructed to slowly inhale via the nose. Following this, either an odour or a picture (2 s) was presented. During presentation of an odour the red fixation cross remained on the screen. Over the entire run participants were asked to provide liking and wanting ratings twice for each stimulus, and intensity ratings for the odours (7 s). Between trials a rest period (3–11 s) was included, during which a white fixation cross was visible. The inter-stimulus interval between odour presentations was kept between 17–24 s to prevent adaptation and was jittered to prevent habituation. Olfactory stimuli were presented using an fMRI-compatible computer-controlled 8-channel olfac-

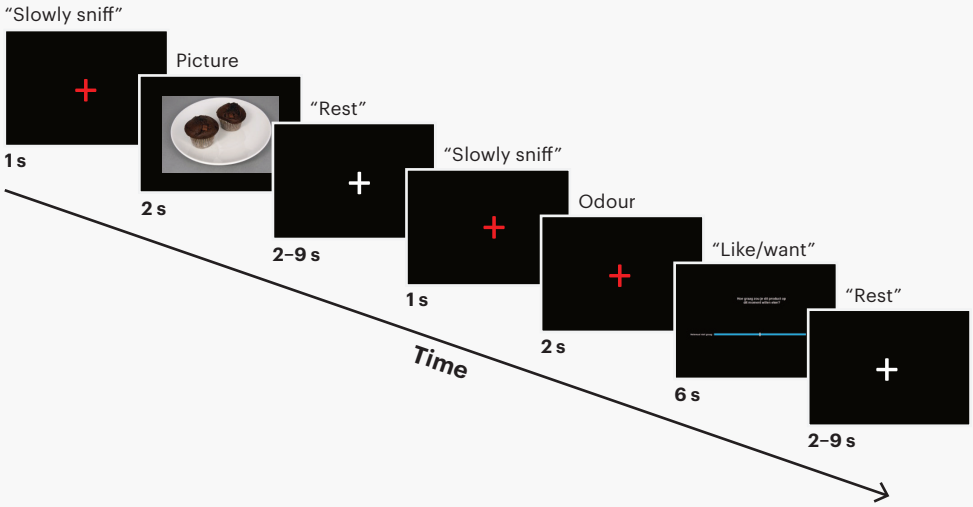

Figure 5.1. Design of the fMRI reward paradigm. Trial presentation started with a red fixation cross for 1 s during which participants were instructed to slowly inhale via the nose ('slowly sniff'), an odour or picture was then presented for 2 s. Occasionally participants provided liking, wanting or intensity ratings on a 100 mm VAS within 7 s. Between trials a rest period (3–11 s) was included, during which a white fixation cross was visible.

tometer (Burghart, Wedel, Germany) that delivered the odours via a small nasal cannula in a constant air flow (8 L/min) that was heated to 37 °C and humidified to 80% relative humidity to prevent irritation of the nasal mucosa. Visual stimuli were projected using a back-projection screen, which could be viewed by the participants via a mirror positioned on the head coil. The reward task ran in E-Prime 2.0 Professional (Psychology Software Tools Inc.). An MR compatible button box was used to answer questions in the task. Head movements were restricted by placing foam cushions next to the participants' head. In addition, surgical tape was placed across the forehead to provide feedback on head movements. Earplugs were provided for noise reduction.

A 3-Tesla Siemens Magnetom Verio MRI scanner in combination with a 32-channel head coil was used, to acquire 993 T_2^*-weighted gradient echo images with BOLD contrast (repetition time = 2240 ms, echo time = 25 ms, flip angle = 90°, field of view = 192 × 192

mm, 45 axial slices, ascending order, voxel size 3 × 3 × 3 mm) in one functional run. The imaging volume was tilted at an oblique angle of 30° to the anterior-posterior commissure line to reduce signal dropout in the orbitofrontal and ventral temporal lobes [199]. A high-resolution T_1-weighted anatomical MRI scan was acquired (MPRAGE: repetition time = 1900 ms, echo time = 2.26 ms, flip angle = 9°, field of view = 256 × 256 mm, 192 sagittal slices, voxel size = 0.5 × 0.5 × 1 mm).

DATA ANALYSES

Results are expressed as means ± *SD* unless otherwise specified. Behavioural data were analysed in SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). Results were considered statistically significant at $p < .05$. Paired-samples *T*-tests were used to test differences in weight, BMI and hunger ratings pre- and post-surgery. Mixed-models were used to analyse the differences in liking, wanting and intensity ratings that were provided in the test sessions before surgery and after surgery (see **Supplementary Table 5.2.**).

Food preferences—Sixteen participants completed the online MTPRT pre- and post-surgery. Preference scores for high-fat sweet and low-energy savoury products were calculated based on rankings in the MTPRT using the formula below. The higher the rank, the higher the score. The preference scores can range from 1 to 4 [198].

$$preference\ score = \frac{4 * (\#\ rank\ 1) + 3 * (\#\ rank\ 2) + 2 * (\#\ rank\ 3) + 1 * (rank\ 4)}{8}$$

Paired samples *T*-test were used to compare preference scores for high-fat sweet and low-energy savoury pre- and post-surgery.

Ghrelin and endocannabinoids—Blood was collected in tubes with EDTA as anticoagulant. To one tube, 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (Sigma-Aldrich) was added to reach a concentration of 1 mg/ml in the collected blood. The tubes were centrifuged at 1300 x g for 10 minutes at 4 °C. Plasma was then portioned into aliquots and stored at -80 °C. Prior to storage, hydrochloric acid was added to a final concentration of 0.05 N to

the plasma that contained 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride. This plasma was later used for ghrelin analyses. To another aliquot, phenylmethylsulfonyl fluoride (Sigma-Aldrich) and URB602 (Sigma-Aldrich) were added to a final concentration of 100 μM for both. This was used to measure plasma concentrations of the eCBs anandamide and 2-arachidonoyl glycerol (2-AG), and the related N-acylethanolamines docosahexaenoyl ethanolamide (DHEA), dihomo-γ-linoleonyl ethanolamide (DLE), oleoyl ethanolamide (OEA), palmitoyl ethanolamide (PEA), stearoyl ethanolamide (SEA), using an LC-MS/MS technique described elsewhere [200]. ELISA was used to measure total ghrelin concentrations in plasma (Millipore).

For each biochemical parameter, normality was checked. Only DHEA was normally distributed and a paired-samples *T*-test was used to compare plasma concentrations before and two months after surgery. For all other parameters Related-Samples Wilcoxon Signed Rank Tests were used. Correlations between pre- to post-surgery changes in gut hormones (eCBs, ghrelin) and changes in BMI, changes in body weight, and changes in liking and wanting of the stimuli were tested in SPSS using Spearman's rho correlation coefficient.

fMRI___Two out of 21 datasets were excluded from further analyses, because realignment parameters indicated substantial movement artefacts (> 5 mm). Whole brain functional images of 19 individuals (4 men and 15 women; age 41 ± 10 years) were preprocessed and analysed using the SPM12 software package (Wellcome Trust Centre for Neuroimaging, London, UK) run with MATLAB 7.12.0 (R2011a, The Mathworks Inc.). Functional images were slice timed, realigned and coregistered. A DARTEL framework was used to create a study-specific template and participant-specific deformation fields [201]. The images were then spatially normalized to the MNI standard brain using the study-specific DARTEL template and the participant-specific deformation fields. Smoothing was applied to the normalized images using an isotropic Gaussian kernel with a 6 mm full width at half maximum.

Subject level analyses: Each test session (pre-/post-surgery) was modelled separately. Motion related variance was corrected for by including motion-correction parameters in the model. Subject level analyses included calculation of six contrast images for odour, and six contrast images for picture presentations (HFHS vs rest; LFLS vs rest; NF vs rest; HFHS vs LFLS; HFHS vs NF; LFLS vs NF). Image

calculation was used to subtract the contrast images post-surgery from the contrast images pre-surgery, creating a contrast image in which the within-subject changes from pre- to post-RYGB were captured.

Group level analyses: For our main contrast of interest, pre- to post-surgery changes in the difference between BOLD responses to HFHS and LFLS cues (HFHS > $LFLS_{pre}$–HFHS > $LFLS_{post}$) were analysed for odours and pictures in two separate *T*-tests. Six *T*-tests were performed to analyse pre- to post-surgery differences in BOLD responses to the different stimulus categories ($HFHS_{pre}$-$HFHS_{post}$; $LFLS_{pre}$–$LFLS_{post}$; NF_{pre}–NF_{post}), for visual and olfactory cues separately. Also, we performed four *T*-tests to analyse differences in BOLD responses between HFHS and NF stimuli and between LFLS and NF stimuli (HFHS > NF_{pre}–HFHS > NF_{post}; LFLS > NF_{pre}–LFLS > NF_{post}), separate for odours and pictures. We used a whole brain approach, with a significance level of $p = .001$ (unc.) and a cluster extent threshold of $k = 8$ contiguous voxels. For all contrasts, the mean beta values of significant clusters were extracted with use of the MarsBar toolbox (http://marsbar.sourceforge.net/). Mean beta values of each significant cluster were subsequently correlated with pre- to post- surgery changes in endocannabinoid and ghrelin concentrations, changes in BMI, changes in body weight, changes in liking and wanting of the stimuli, and changes in preference for high-fat sweet and low-energy savoury products. Correlation analyses were performed in SPSS using Spearman's rho correlation coefficient.

Results

After RYGB surgery, the mean body weight of our study population decreased from 120 ± 3 to 104 ± 3 kg, a mean weight loss of 16 ± 4 kg. This weight change led to a decrease in BMI from 41 ± 1 to 36 ± 1 kg/m^2 (see **Table 5.1.**).

HUNGER, LIKING AND WANTING RATINGS

Our standardized meal was successful in achieving a state of satiety, as observed from the hunger, fullness and desire to eat ratings, and were similar before and after RYGB surgery. Ratings for prospective consumption and thirst were significantly higher pre- compared to post-surgery (see **Table 5.1.**).

HFHS pictures were significantly less liked (pre: 47 ± 6, post: 29 ± 5) and wanted (pre: 42 ± 6, post: 26 ± 5) after surgery (both $p < .001$). Similarly, HFHS odours were less liked (pre: 50 ± 8, post:

30 ± 7; $p < .001$) and less wanted (pre: 40 ± 7, post: 24 ± 6; $p = .018$) after surgery. Liking and wanting ratings remained the same in both test sessions for LFLS pictures and odours, and for NF pictures. NF odours were significantly less liked (pre: 35 ± 7; post: 23 ± 7; $p = .008$) and wanting ratings were similar pre- and post-surgery. Intensity ratings for HFHS, LFLS and NF odours were similar between the two test sessions (for all, see **Supplementary Table 5.2.**).

FOOD PREFERENCES

Preference for high-fat/sweet products decreased after surgery (pre: 2.6 ± 0.72, post: 2.0 ± 0.8; $T(1,15) = 3.39$, $p < .05$). Preference for low-energy/savoury products increased after surgery (pre: 2.3 ± 0.6, post: 2.7 ± 0.6; $T(1,15) = -3.50$, $p < .05$).

FUNCTIONAL IMAGING DATA

Pictures: Pre- to post-surgery differences—In the left precuneus, the difference in brain responses to HFHS and LFLS pictures was significantly smaller after compared to before surgery (see **Table 5.2.** and **Figure 5.2.**). Before surgery, viewing LFLS cues led to greater deactivation of the left precuneus than viewing HFHS cues. After RYGB, deactivation in response to food pictures was minimal in this region and did not differ between HFHS and LFLS. Participants showed significantly smaller responses to LFLS pictures in the left superior frontal gyrus (anteroventral PFC) after surgery (see **Table 5.2.**). The pre-surgical deactivation of this region to LFLS pictures, was no longer present in the post-surgery session.

Right superior parietal lobule responses to NF pictures were significantly different pre- and post-RYGB, showing deactivation pre- and activation post-surgery. Further, the difference in right precuneus response to HFHS compared to NF pictures was reversed from pre- to post-surgery. Deactivation in response to HFHS pictures was greater than deactivation to NF pictures before surgery, and smaller than deactivation to NF pictures after surgery.

Odours: Pre- to post-surgery differences—During HFHS odour exposure significantly greater deactivation was found in the right precuneus and left superior parietal lobule pre- compared to post-surgery (see **Table 5.2.**). We also observed significantly different precuneus responses to LFLS odours pre- versus post-sur-

Table 5.2. Significant differences in neural activation by food and non-food cue exposure (picture or odour), pre- to post-RYGB

			Cluster size	*Z*-score	Peak coordinates x	y	z
Picture							
$HFHS\text{-}LFLS_{pre} > HFHS\text{-}LFLS_{post}$	L	Precuneus	9	3.43	–3	–54	69
$LFLS_{pre} < LFLS_{post}$	L	Superior Frontal gyrus/ anteroventral PFC	13	3.73	–21	51	9
$NF_{pre} < NF_{post}$	R	Superior Parietal lobule	11	3.45	24	–66	63
$HFHS\text{-}NF_{pre} < HFHS\text{-}NF_{post}$	R	Precuneus	8	3.49	15	–54	21
Odour							
$HFHS_{pre} < HFHS_{post}$	R	Precuneus	9	3.92	15	–42	51
	L	Superior Parietal lobule	8	3.66	–21	–54	51
$LFLS_{pre} < LFLS_{post}$	R	Precuneus	11	3.57	9	–48	72
	R	Superior Parietal lobule		3.52	15	–54	72
	L	Precuneus	10	3.51	–3	–66	45
$NF_{pre} < NF_{post}$	L	Superior Parietal lobule	20	3.89	–21	–54	69
	L	Precuneus	12	3.69	–12	–69	63
$HFHS\text{-}NF_{pre} > HFHS\text{-}NF_{post}$	L	Superior Frontal gyrus / anteroventral PFC	8	3.92	–21	63	3

HFHS: high-fat/high-sugar; LFLS: low-fat/low-sugar; L: left; NF: non-food; R: right; RYGB: Roux-en-Y Gastric Bypass; Whole brain results; $p < .001$ (unc.)

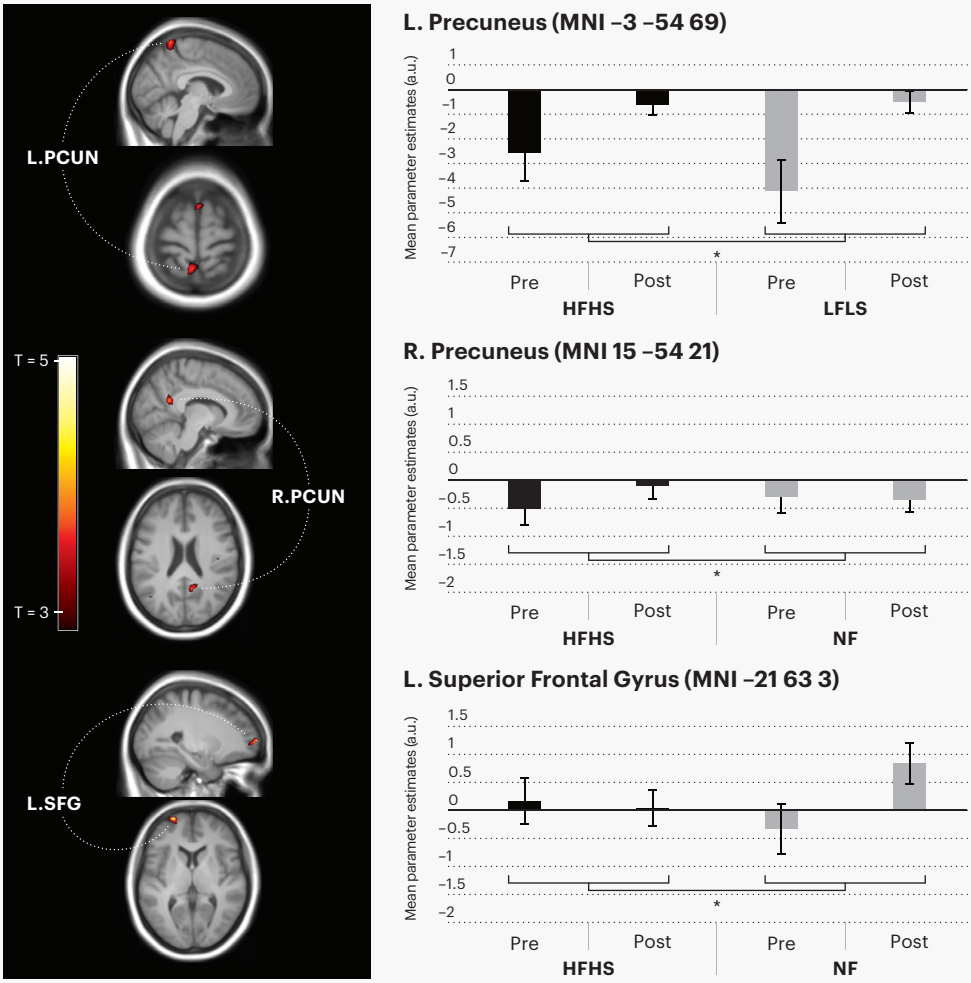

Figure 5.2. Significant differences in neural activation by food and non-food cue exposure (picture or odour), pre- to post-RYGB. Results for the brain images were thresholded at $p = .005$ for visualization. **Upper:** Post-surgery, the left precuneus (L.PCUN, MNI: -3 -54 69) showed significantly less difference between activation to low-fat/low-sugar (LFLS) and high-fat/high-sugar (HFHS) food pictures ($k = 9$, $z = 3.43$). Pre-surgery, more deactivation was observed in response to LFLS compared to high-fat/high-sugar (HFHS) pictures while post-surgical responses to these two stimuli appeared similar. **Middle:** Post-surgery, the right precuneus (R.PCUN, MNI: 15 -54 21) showed significantly more difference between activation to high-fat/high-sugar (HFHS) food and non-food (NF) pictures ($k = 8$, $z = 3.49$). Pre-surgical deactivation in response to HFHS food pictures was higher, while post-surgical deactivation to HFHS food pictures was lower compared to non-food pictures. **Lower:** Post-surgery, the left superior frontal gyrus (L.SFG, MNI: -21 63 3) showed significantly less difference between activation to high-fat/high-sugar (HFHS) food and non-food (NF) odours ($k = 8$, $z = 3.92$). Post- compared to pre-surgery, activation to HFHS odour appeared similar, while increased activation was observed in response to NF odour.

Table 5.3. Plasma concentrations of endocannabinoids and ghrelin pre- and post-RYGB surgery[1]

Compound	Pre-surgery Mean ± *SD*	Post-surgery Mean ± *SD*	Difference Mean ± *SD*
Anandamide*	0.4 ± 0.2	0.5 ± 0.2	0.1 ± 0.2
2-arachidonoyl glycerol	8 ± 3	14 ± 17	6 ± 17
Docosahexaenoyl ethanolamide	0.5 ± 0.1	0.4 ± 0.1	-0.1 ± 0.2
Dihomo-γ-linoleonyl ethanolamide	0.1 ± 0.05	0.09 ± 0.04	-0.01 ± 0.05
Oleoyl ethanolamide	2.6 ± 1	2.6 ± 1	0.0 ± 1
Palmitoyl ethanolamide	1.7 ± 0.7	1.6 ± 0.4	-0.17 ± 0.8
Stearoyl ethanolamide	2.5 ± 1	2.2 ± 1	-0.3 ± 1
Ghrelin	459 ± 190	442 ± 171	-17 ± 159

[1]Concentrations in ng/mL; n = 18, for endocannabinoids, n = 17 for ghrelin.
*Significant difference (p = .006) pre- and post- Roux-en-Y Gastric Bypass (RYGB).

gery (see **Table 5.2.**). The right precuneus was deactivated pre- and activated post-surgery. Before surgery there was an absence of response in the left precuneus, but after surgery this region was activated in response to LFLS odours. In response to NF odours deactivation of the left precuneus and left superior parietal lobule was observed pre- surgery, this was significantly different after surgery, when activation was seen in these regions. The difference in response of the left superior frontal gyrus (anteroventral PFC) to HFHS odours vs NF odours was significantly different pre- and post-surgery. This effect appears to be mainly driven by increased activation in response to NF odours after surgery.

Correlations between neural changes and behavioural measures—No significant correlations were found between changes in neural responses to odours or pictures, changes in BMI or body weight, and changes in liking, wanting and intensity ratings for odours (all $p > .05$). An increased reduction of deactivation of the left superior parietal lobule to HFHS odour after RYGB was correlated with a greater increase in preference for low-fat/savoury food ($r = -.550$, $p = .027$), and albeit not significant, associated with a greater decrease in preference for high-fat/sweet food ($r = .417$, $p = .108$). An increased reduction in activation of the left precuneus to HFHS pictures relative to LFLS pictures was

correlated with a greater decrease in preference for high-energy/sweet relative to low-energy/savoury food (r = -.530, p = .035).

ENDOCANNABINOIDS AND GHRELIN

Plasma concentrations of anandamide were significantly greater post- than pre-surgery (before: 0.38 ± 0.17 ng/ml, after: 0.48 ± 0.15 ng/ml. Z = -2.77, p = .006. For the other eCBs, 2-AG, DHEA, DLE, OLE, PEA, SEA, and ghrelin, concentrations did not change significantly (see **Table 5.3.** for values).

Changes in eCB and ghrelin concentrations did not correlate with changes in neural activation, nor with changes in liking and wanting of pictures and odours, nor with BMI or body weight changes (all p > .05).

Discussion

The aim of this study was to provide additional insights into the neural mechanisms underlying changes in food preferences after RYGB, using appetizing olfactory cues representing high- and low-energy foods, and by including measures of appetite-related hormones (eCBs and ghrelin). Indeed, patients demonstrated a shift in food preferences away from high-fat/sweet and towards low-energy/savoury food products. This shift correlated with less deactivation of the superior parietal lobule to high-energy food odours and a smaller difference in activation of the precuneus to high-energy versus low-energy food pictures, after RYGB surgery. Main findings further included less deactivation of the precuneus, found in response to all cues. Deactivation in the superior frontal gyrus was less towards low-energy food pictures and more similar to high-energy versus non-food odours. Further, anandamide concentrations were increased post-surgery, while other eCB and ghrelin concentrations did not change post-surgery. Neural changes did not correlate with changes in eCB and ghrelin concentrations.

In line with previous research [103], participants reported a shift in food preferences away from high-energy foods, and towards low-energy food products, on the MTPRT [198]. These alterations in food preferences may (in part) underlie the success of RYGB surgery as weight-loss intervention.

Post- compared to pre-surgery, the left superior frontal gyrus was less deactivated in response to low-, but not to high-energy pictures. Previous research into the effects of RYGB reported postsurgical reductions in prefrontal cortex activation that were

more pronounced for high- relative to low-energy cues [202]. Moreover, decreased differences in desire to eat high- versus low-energy foods were predicted by a reduced difference in a more posterior part of the superior frontal gyrus response to high-energy versus low-energy food cues [109]. Unfortunately, these studies only compared surgery-related changes in differences between high- and low-energy food responses, rather than considering the separate changes in responses to high as well as low-energy foods. It is possible that the decreased difference in activation between high- and low-energy food cues are in fact largely driven by a diminished response to low-energy food cues, similar to our observations. The anteroventral region of the superior frontal gyrus we found has been implicated in subjective reward value [203] and in processes of self-control [204]. Several studies have reported that activation patterns in this region can act as a predictor of food choices [203,205,206]. Changes in superior frontal activation could be related to the beneficial shift in food preferences, from high-energy towards more healthy low-energy foods, that is frequently reported after RYGB surgery [104,106,108,207] and also confirmed by the current study.

With this study we have extended previous research into RYGB by including olfactory food cues. Food odours are potent appetitive cues that predict the immediate presence of food and the rewarding effects of eating [184]. In contrast to visual cues, olfactory cues are processed largely unconsciously [59]. We observed less deactivation of the superior parietal cortex after compared to surgery, in response to food odours but not pictures. Increased superior parietal activation was previously found during anticipation of reward [208]. This region was also proposed to be part of a top-down control system for attentional processes that is modulated by implicit contextual cues, with greater deactivation being associated with increased attentional demands [208,209]. The diminished deactivation of the superior parietal cortex we found after surgery could indicate a decrease in reward valuation of high-energy food odours, and thereby a lowered attentional demand. In line with this, the diminished activation was positively, albeit not significantly, correlated with (lowered) preference for high-energy foods, and negatively correlated with heightened preference for low-energy foods.

After surgery, decreased activation of the bilateral precuneus was found in response to high-, but most pronounced to low energy-dense food cues (pictures and odours). In previous research,

decreased precuneus responsivity to high- versus low-energy food cues was related to decreased food liking [109]. A role in food reward anticipation has been proposed by other studies [209–217]. Alternatively, the difference in precuneus activation before and after surgery could be related to changes in attentional control [209,218]. Greater deactivation in this region indicates increased attentional demand. In relation to our data this would mean that food cues in general, but low-energy food cues in particular, recruit less attention after surgery. Further research should focus in more detail how (changes in) attentional processes relate to changes in food preference and choice rather than food-cue reactivity.

When we look at the neural regions (the superior frontal gyrus, superior parietal lobe and precuneus) in which we find changed responsivity to different food cues, it is interesting that they are all part of a frontoparietal control network involved in adaptive top-down control [206,219]. In a study by Schonberg *et al.* [206] participants who were trained to choose their less preferred food item showed decreased activation in this frontoparietal network during low-value food choices. The authors propose that over the course of extensive training the need for top-down frontoparietal control reduces as the food preference response moves from goal-directed to more habitual. Speculative, a similar process is set in motion after RYGB. In RYGB patients, aversive consequences associated with consumption of sugary and fatty foods (*e.g.*, nausea, light-headedness, flushing, and diarrhoea [220]) could lead to a relative preference for low-energy food products. Around two months after surgery, we see a neural food cue response that is in line with a more habitual rather than a goal-directed pattern.

Alterations in concentrations of appetite related hormones were proposed to mediate the changes in neural processing that are observed after RYGB [185,221,222] and could ultimately contribute to beneficial changes in food preference and intake [220]. In this study we did not find pre- to post-surgery differences in plasma ghrelin concentrations. In line with previous studies, we see a large variation in pre- to post surgery differences in plasma ghrelin concentrations [195,223]. Faulconbridge *et al.* [195] found a correlation between changes in ghrelin concentrations and changes in neural responses, which our data do not confirm. Discrepancies in neural activation and ghrelin concentrations could be related to a difference in the timing of the measurements (6 months post-surgery in the study by Faulconbridge *et al.*, [195]; 2 months

in the current study), as the effect of RYGB on ghrelin concentrations might change over time [224]. In addition, our participants were tested in the afternoon, after a three-hour fast, instead of in the morning after an overnight fast, which may influence ghrelin levels and neural responses.

Endocannabinoids have been suggested to mediate the orexigenic effects of ghrelin [190] and are implicated in reward anticipation [189,191-194]. Within the range of eCBs that we measured, we showed increased plasma anandamide concentrations after compared to before RYGB. The other six eCBs that we measured did not change. Previous studies assessing eCBs at alternate time points after RYGB included only two to four eCBs and suggest either decreased or unchanged anandamide concentrations, and unchanged concentrations of 2-AG, OEA and PEA [225-227]. Similar to ghrelin, eCB concentrations may change over time and future studies should monitor the progression of eCB concentrations at different time points after RYGB. Increased eCB concentrations have been suggested to be a cause of obesity [228] and may be related to long-term weight loss and weight-regain after RYGB.

It can be seen as limitation that blood samples for plasma eCB and ghrelin concentration determination were drawn after at least three hours of fasting, whereas the fMRI measurements were performed after a lunch meal. Concentrations after this meal may be more closely associated with the neural responses we observed in a satiated state.

A particular strength of this study was that participants were measured in a satiated state, to more realistically mimic overeating situations. However, this may have reduced reward related neural activation, and complicates direct comparisons to previous literature. Moreover, most other studies looked into pre- to post-surgery changes in the relative difference between high-energy and low-energy food cues [106,107,109], whereas we examined the changes in response to HFHS and LFLS cues relative to baseline, as well as compared to non-food cues, to be able to draw more specific conclusions.

To conclude, RYGB leads to alterations in activation of the frontoparietal network, involved in cognitive control during (food) cue processing. These neural changes are in line with the observed increase in preferences for low-energy, and decreased preferences for high-energy foods. The current results suggest that there is no relation between changes in ghrelin and endocannabinoid concentrations, and changes in neural responses to

food cues in RYGB patients. Using olfactory food cues in addition to pictures did not reveal new information, but highlights the importance of odours for the anticipation of food.

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Supplementary Materials

Supplementary Table 5.1. Composition of the standardized meal.

	Men		Women	
	Pre	**Post**	**Pre**	**Post**
Bread Roll *(Wheat bread (± 22 g/roll))*	4 pcs	2 pcs	3 pcs	1 pc
Margarine *(Low-fat)*	30 g	15 g	15 g	15 g
Cheese *(Full-fat semi-cured)*	40 g	20 g	40 g	20 g
Ham	40 g	20 g	20 g	–
Orange Juice	150 g	75 g	100 g	50 g
kcal total meal	570	174	421	107

Supplementary Table 5.2. Stimulus ratings for liking, wanting and intensity (100 units VAS), pre- and post-RYGB surgery

		Category	**Before surgery Mean ± *SE***	**After surgery Mean ± *SE***	**Significance**
Picture	Liking	*HFHS*	46 ± 5	30 ± 5	$p < .001$
		LFLS	39 ± 6	43 ± 6	$p = .246$
		NF	19 ± 4	14 ± 4	$p = .069$
	Wanting	*HFHS*	42 ± 5	26 ± 5	$p < .001$
		LFLS	35 ± 6	37 ± 6	$p = .498$
		NF	10 ± 3	8 ± 3	$p = .244$
Odour	Liking	*HFHS*	49 ± 7	29 ± 7	$p < .001$
		LFLS	39 ± 6	37 ± 6	$p = .798$
		NF	35 ± 7	23 ± 7	$p = .008$
	Wanting	*HFHS*	38 ± 6	24 ± 6	$p = .018$
		LFLS	33 ± 6	35 ± 6	$p = .804$
		NF	14 ± 4	9 ± 3	$p = .191$
	Intensity	*HFHS*	76 ± 5	76 ± 4	$p = .984$
		LFLS	60 ± 5	66 ± 5	$p = .147$
		NF	68 ± 5	73 ± 5	$p = .114$

HFHS: high-fat/high-sugar; *LFLS*: low-fat/low-sugar; *NF*: non-food; RYGB: Roux-en-Y Gastric Bypass; VAS: visual analogue scale

Chapter 6

Altered neural inhibition responses to food cues after Roux-en-Y Gastric Bypass

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Submitted for publication

Abstract

Background Roux-en-Y gastric bypass (RYGB) surgery is a highly effective weight-loss intervention that often reduces preference for and intake of high-energy foods. Research into the neural mechanisms behind this shift has mainly focused on reward processing of food cues. However, the ability to successfully control food intake and thereby weight-loss also depends on inhibitory control capacity. We investigated whether RYGB leads to alterations in neural inhibitory control in response to food cues.

Methods A food-specific go/no-go task with pictures of high-energy (desserts) and low-energy foods (vegetables), was used to assess neural inhibition responses before and after RYGB with functional magnetic resonance imaging. Data from 18 morbidly obese patients (15 females; age 41 ± 11 years; BMI 42 ± 4 kg/m^2 before; BMI 36 ± 4 kg/m^2 after) were analysed. Pre- and post-RYGB BOLD fMRI responses were compared for response inhibition towards high-energy and for response inhibition towards low-energy foods. Participants were tested in a satiated state.

Results Response inhibition for high-energy food was associated with increased activation of the right anterolateral prefrontal cortex (PFC), right medial PFC, dorsolateral PFC, right middle cingulate cortex and the right inferior frontal operculum, after compared to before surgery (involved in inhibitory control). Response inhibition for low-energy food elicited diminished post- compared to pre-surgery responses in the left superior temporal pole, right parahippocampal gyrus and right hypothalamus (involved in metabolic control). The dorsolateral PFC, middle cingulate cortex, inferior frontal operculum, parahippocampal gyrus and superior temporal pole have also been associated with reward processing.

Conclusion Neural changes indicate improved response inhibition towards high-energy food cues, altered influence of satiety during response inhibition toward low-energy food cues and a more positive attitude to both high-energy and low-energy food after RYGB. Alterations in neural circuits involved in inhibitory control, satiety signalling and reward processing may contribute to effective weight-loss after RYGB.

Introduction

Roux-en-Y gastric bypass (RYGB) patients show decreased preferences and consumption of high-energy foods, which are associated with long-term weight reduction [102,104-106,229]. The underlying mechanism of this decreased preference for high-energy foods is unclear. Most studies to date focused on altered reward processing, but changes in inhibitory control may also play an important role. It has been suggested that people with low inhibitory control are more prone to overeating and hence to developing overweight or obesity [32,74,230]. Suppression of automatic tendencies to choose highly rewarding energy-dense foods over low energy-dense foods could help to decrease caloric intake, which contributes to successful weight-loss.

How well we are able to control our impulses in part determines how much and what we consume. Decreased inhibitory control is assumed to increase the odds of eating in the absence of hunger, especially in a tempting and food-rich environment [79,231,232], and could eventually lead to weight gain. Overeating and obesity have been associated with higher impulsivity, both in self-reported and behavioural measures [30,233-237]. Furthermore, individuals that were unsuccessful in regulating their weight show decreased inhibitory control[238] while behavioural responses of successful weight-loss maintainers to high-energy foods indicate better inhibition [239]. The extent of inhibitory control seems to influence the ability to maintain weight-loss after intervention. RYGB surgery is widely viewed as the most effective method for long-term weight loss in morbidly obese individuals [240]. Previous studies into neural responsivity after RYGB surgery have mainly focused on (alterations in) reward processing during presentation of high-energy food items [106,107]. In order to better understand successful weight-loss regulation upon RYGB, it is important to consider changes in inhibitory control processes as well [241].

Previous studies showed that people who were attempting to lose weight displayed increased activation of the inferior frontal gyrus and anterior insula/frontal operculum in response to pictures of high-energy foods [182]. These areas are involved in inhibitory control. Also, successful weight loss maintainers show greater activation to food cues in prefrontal regions (superior-, middle frontal gyrus) associated with inhibitory control [82]. Batterink *et al.* [101] have introduced a food-specific go/no-go task to assess neural measures of response inhibition to food items. In their study, a higher BMI was related to less activation during no-go trials in frontal inhibi-

tory regions, including superior- and middle frontal gyrus, ventromedial- and medial prefrontal cortex, and orbitofrontal cortex. A higher BMI was also associated with more activation during no-go trials in the temporal operculum. Increased understanding of the (neuro)biological mechanisms involved in inhibitory control is necessary to improve the outcome of weight-loss interventions.

With this study we aimed to determine whether RYGB surgical intervention in morbidly obese patients results in altered neural activation underlying response inhibition, using a food specific go/no-go task. Participants were tested in a satiated state to better understand alterations in mechanisms of overeating. We hypothesized that participants would be better able to suppress responses to high-energy (dessert) items after RYGB surgery, as reflected in changes in neural responses related to inhibitory control, while behavioural and neural responses to low-energy (vegetable) items would remain similar.

Methods

OVERALL DESIGN

This study had a 2 × 2 × 2 within-subject design, including the factors time point (pre- and post-RYGB), stimulus (dessert/vegetable), and task-instruction (go/no-go).

PARTICIPANTS

Twenty morbidly obese individuals participated in the food-specific go/no-go task, pre- and post- RYGB surgery. All participants were enlisted to undergo RYGB surgery at Rijnstate hospital, Arnhem, the Netherlands. Requirements for the surgery were: Body Mass Index (BMI) of > 40 kg/m^2 or > 35 kg/m^2 with co-morbidity that was expected to improve after surgically-induced weight loss, long-lasting obesity (> 5 years), proven failed attempts to lose weight in a conventional way, intention to adhere to a postoperative follow-up programme. Individuals were not considered for surgery when they were pregnant or lactating, had psychiatric disorders, alcohol or drug dependency, life threatening conditions or when they were dependent on the care of others. Patients were screened at Rijnstate hospital. All participants were right-handed, non-smoking, and did not have conditions that conflicted with MR safety or would cause artefacts in the MR images (*e.g.,* claustrophobic, irremovable ferromagnetic objects in or on their body, pacemaker). Participants received financial compensation for their contribution. All participants provided written informed consent

before entering the study. The protocol was approved by the Medical Ethical Committee of Wageningen University (NL45837.081.13) and was executed in accordance with the ethical principles of the Declaration of Helsinki of 1975, as revised in 2013. The study was registered on clinicaltrials.gov as NCT02068001.

EXPERIMENTAL PROCEDURES

Participants visited the test facilities at three occasions. First, they were familiarized with the MRI test environment and the experimental task in a dummy MRI scanner at Wageningen University (training session). After the trainings session, actual measurements were performed in two identical test sessions. The first test session took place on average 3.3 (*SD* 1.8) weeks before, and the second test session took place on average 9.3 (*SD* 1.2) weeks after RYGB surgery. Participants were instructed to refrain from eating and drinking anything but water and weak tea in the three hours before the test sessions. Upon arrival at hospital Gelderse Vallei (Ede, the Netherlands), blood samples were taken for analysis of plasma levels of endocannabinoids and ghrelin (data reported in **Chapter 5**). Participants were tested in comfortably full state, to mimic a context of eating in the absence of hunger. We provided a standardized meal that was adapted to pre- or post-surgery conditions in order to match the hunger states of the participants before and after surgery. Participants first drank orange juice, and after a small break they consumed a standardized meal consisting of bread roll(s), cheese, ham and butter (see **Supplementary Table 6.1.**). Following meal consumption, participants waited for 15 minutes. In order to assess changes in general inhibition participants filled in the 24-item BIS/BAS questionnaire [242]. Measurements of brain reward responses to visual and olfactory food and non-food cues were collected (data reported in **Chapter 5**). At the end of this reward paradigm participants rated their appetite (hunger, fullness, prospective consumption, desire to eat, and thirst) on a 100 mm visual analogue scale (VAS). Then a structural MR image was collected. Finally, participants took part in two functional runs during which a food-specific go/no-go task was performed.

FMRI – GO/NO-GO TASK

The food-specific go/no-go paradigm was adapted from Batterink *et al.* [101]. Participants were instructed to press a button as quickly and accurately as possible in response to go trials (75% occurrence) and to refrain from responding to no-go trials (25%

occurrence). Two separate functional runs were performed, each consisting of 48 trials. One run contained go-vegetable items and no-go dessert items, the other run contained go-dessert items and no-go vegetable items (see **Figure 6.1.**). The order of the runs was counterbalanced between participants. During each trial a picture was presented for 500 ms, depicting either a low-energy vegetable (*i.e.* corn, peas, Brussels sprouts, radishes, carrot, broccoli, cauliflower, haricots, zucchini) or a high-energy dessert (*i.e.*, ice cream, cake, frozen yogurt, pudding, chocolate mousse, chocolates, cookies). Participants had 2000 ms to respond from stimulus onset. Trials were presented in pseudo-randomized order. Between trials a fixation cross was presented for a duration of 7-19 seconds. No-go trials would appear after 1, 2, or 3 go-trials. Reaction times were measured from the beginning of trial onset and collected with a fibre-optic response box system. Stimuli were presented visually using the Presentation software package (Version 9, Neurobehav-

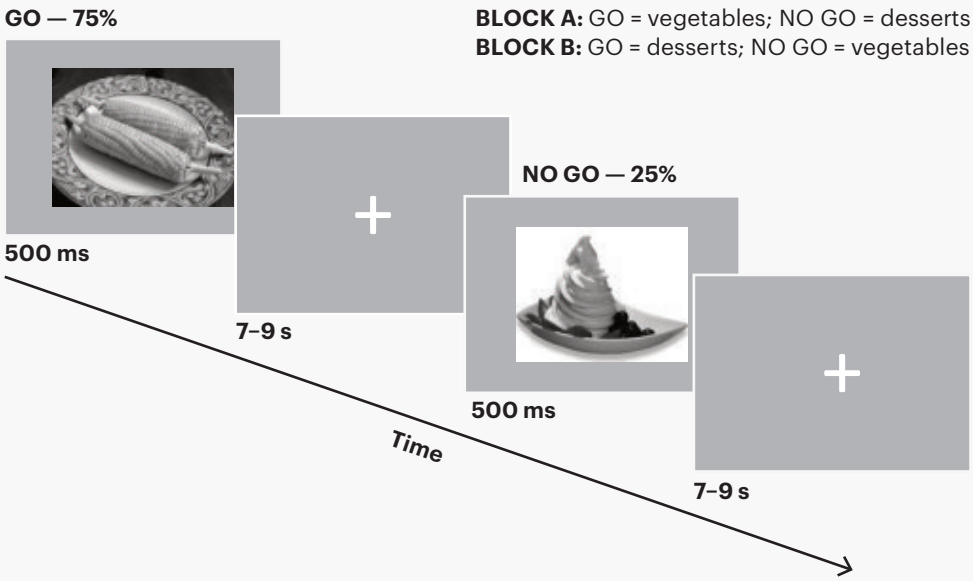

Figure 6.1. The food-specific go/no-go paradigm. In block A. participants were instructed to press a button in response to vegetable items (36 items) and withhold their response to dessert items (12 items). In block B. the instruction was reversed, participants had to press the button in response to dessert items (36 items) and withhold their response to vegetable items (12 items). The block order was counterbalanced between participants.

ioral Systems, Davis, CA) and were displayed using a video projector that illuminated a rear projection screen located at the end of the magnet bore. Subjects viewed the stimuli through an adjustable mirror attached to the head coil.

(F)MRI MEASUREMENTS

Each participant was scanned at approximately the same time of day, between 14:00-17:00 at hospital Gelderse Vallei (Ede, the Netherlands). Images were acquired with a 3-Tesla Siemens Magnetom Verio MRI scanner in combination with a 32-channel head coil. A high-resolution T_1-weighted anatomical MRI scan was acquired (MPRAGE: repetition time = 1900 ms, echo time = 2.26 ms, 9° flip angle, field of view = 256 × 256 mm, 192 sagittal slices, voxel size = 0.5 × 0.5 × 1 mm). Subsequently, 176 T_2^*-weighted gradient echo images with BOLD contrast (repetition time = 2240 ms, echo time = 25 ms, 90° flip angle, field of view = 192 × 192 mm, 45 axial slices, ascending order, voxel size 3 × 3 × 3 mm) were acquired for each of the two functional runs during which participants performed a food Go/No-Go task. The imaging volume was tilted at an oblique angle of 30° to the anterior-posterior commissure line to reduce signal dropout in the orbitofrontal and ventral temporal lobes [199]. Head movements were restricted by placing foam cushions next to the participants' head. In addition, adhesive tape was placed across the participants' forehead to provide feedback on head movements. Earplugs were provided for noise reduction.

DATA ANALYSES

Participant characteristics—Participant characteristics were analysed using SPSS in IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). Paired-samples *T*-tests were used to test differences in weight, BMI, hunger ratings and BIS/BAS-scores pre- and post-surgery.

Behavioural data go/no-go—Behavioural data of the go/no-go task were also analysed using SPSS. Mean commission error rates of the go/no-go task were calculated by dividing the total number of incorrect responses to no-go trials by the total number of no-go trials. Mean omission error rates were calculated by dividing the total number of non-responses to go-items by the total number of go-trials. Mean reaction times (ms) of responses to each type of trial (go-dessert, go-vegetable, no-go dessert, no-go vegetable) were calculated for each participant. Response times below 200 ms and

over 2000 ms were excluded. The low number of commission errors rendered the reaction time data for the no-go items unsuitable for statistical testing. Pre- to post-surgery differences in response time (ms) to go items were analysed by following a linear Mixed Effects Models procedure including stimulus type (go-dessert; go-vegetable) as fixed effects factor. Time point (pre- and post-gastric bypass surgery), stimulus (dessert/vegetable), and task-instruction (go/no-go) were included as repeated variables. A *p*-value of < .05 was considered statistically significant.

Table 6.1. Weight, BMI, Hunger ratings provided right before the go/no-go task and BIS/BAS scores before and after RYGB surgery.

		Before surgery Mean ± *SD*	**After surgery Mean ± *SD***	**Sign. Difference**
Weight (kg)		121 ± 15	105 ± 16	$p < .001$
BMI (kg/m²)		42 ± 4	36 ± 4	$p < .001$
Hunger	*100 mm VAS*	27 ± 31	14 ± 24	$p = .056$
Fullness		43 ± 29	72 ± 18	$p = .001$
Prospective consumption		37 ± 27	17 ± 21	$p = .002$
Desire to eat		44 ± 35	24 ± 26	$p = .009$
Thirst		76 ± 26	70 ± 28	$p = .349$
BAS Drive	*max 16*	11.6 ± 2.1	12.1 ± 2.2	$p = .132$
BAS Fun Seeking	*max 16*	10.8 ± 2.1	11.3 ± 2.3	$p = .166$
BAS Reward Responsiveness	*max 20*	17.8 ± 1.6	18.4 ± 1.6	$p = .045$
BIS	*max 28*	20.3 ± 4.4	19.8 ± 3.6	$p = .366$

fMRI data go/no-go—Whole brain functional images were pre-processed and analysed using the SPM12 software package (Wellcome Department of Imaging Neuroscience, London, United Kingdom) run within MATLAB 7.12.0 (R2011a, The Mathworks Inc). Functional images were slice timed, realigned and coregistered. The DARTEL framework was used to create a study-specific template and participant-specific deformation fields [201]. The images were then spatially normalized to the Montreal Neurological Institute (MNI) standard brain using the study-specific DARTEL template and the participant-specific deformation fields. Smoothing

was applied to the normalized images using an isotropic Gaussian kernel with a 6-mm full width at half maximum. Artefact Repair was applied using the ArtRepair toolbox in SPM12 (see: http://cibsr.stanford.edu/tools/human-brain-project/artrepair-software.html). Of the twenty datasets that were acquired, two datasets were excluded that contained movements more than 4 mm.

Subject level analyses: Each test session (pre-/post-surgery) was modelled separately. Four conditions were included per model: visual exposure to go dessert trials, no-go vegetable trials, go vegetable trials and no-go dessert trials. Motion-correction parameters were included in the model. For each subject four contrast images were calculated: $nogo\_dessert_{pre}$ vs rest, $nogo\_dessert_{post}$ vs rest, $nogo\_vegetable_{pre}$ vs rest and $nogo\_vegetable_{post}$ vs rest. Subsequently we subtracted the post-surgery contrast images from the pre-surgery contrast images using the SPM12 image calculation routine.

Group level analyses: Two one-sample *T*-tests were performed to test our hypotheses. In each test we looked at contrast images containing the difference between activations pre- and post-surgery ($nogo\_dessert_{pre}$-$nogo\_dessert_{post}$; $nogo\_vegetable_{pre}$-$nogo\_vegetable_{post}$). We report whole brain results, with a significance level of $p = .001$ (unc.) and a cluster extent threshold of $k = 8$ contiguous voxels. The MarsBar toolbox (http://marsbar.sourceforge.net/) run in Matlab 7.12.0 (R2011a; The Mathworks Inc., Natick, MA) was used to extract mean beta values from all significant clusters. These values were subsequently correlated with pre- to post- surgery changes in BMI, changes in body weight, and changes in feelings of hunger, fullness, prospective consumption and desire to eat. Correlation analyses were performed in SPSS using Pearson's correlation coefficient.

Results

RYGB EFFECTS - WEIGHT LOSS

The mean weight of our study population decreased from 121 ± 15 pre-RYGB to 105 ± 16 kg post-RYGB (mean ± *SD*), a mean weight loss of 17 ± 3 kg ($p < .001$). This weight change led to a decrease in BMI from 42 ± 4 to 36 ± 4 kg/m^2 ($p < .001$), with a mean decrease of 6 ± 1 kg/m^2.

HUNGER RATINGS

During the post-surgery test session, participants indicated less hunger before the go/no-go task (± 50 min after meal intake; $p =$

Table 6.2. Regions in which brain activation during no-go food items was significantly different pre- and post RYGB surgery

					Peak coordinates		
			cluster size	*Z*-score	x	y	z
Dessert							
$no\text{-}go_{pre} < no\text{-}go_{post}$	R	Middle Frontal Gyrus / Anterolateral PFC	30	4.42	45	54	6
	R	Medial Superior Frontal Gyrus / Medial PFC	23	4.02	12	60	27
	R	Inferior Frontal Gyrus (Tri) / Dorsolateral PFC	15	3.87	57	27	18
	R	Middle Cingulum (posterior part)	10	3.59	3	–27	33
	R	Inferior Frontal Operculum	10	3.53	51	9	24
Vegetable							
$no\text{-}go_{pre} > no\text{-}go_{post}$	R	Hypothalamus	10	3.65	3	3	-12
	L	Superior Temporal Pole	11	3.63	–36	12	–27
	R	Parahippocampal gyrus	9	3.34	18	–15	–21

.056), rated a higher fullness, a decreased prospective consumption and less desire to eat (all $p < .01$). Ratings for thirst were comparable between the two test sessions ($p = .349$; see **Table 6.1.**).

BEHAVIOURAL DATA

No-go items—Commission errors (incorrect responses during the no-go items) for no-go dessert items occurred at a mean rate of 8.5% (± 8.6) pre-surgery, and 8.8% (± 10.9) post-surgery. Commission errors for no-go vegetable items occurred around 16.8% (± 15.8) pre-surgery, and 14.8% (± 14.7) post-surgery.

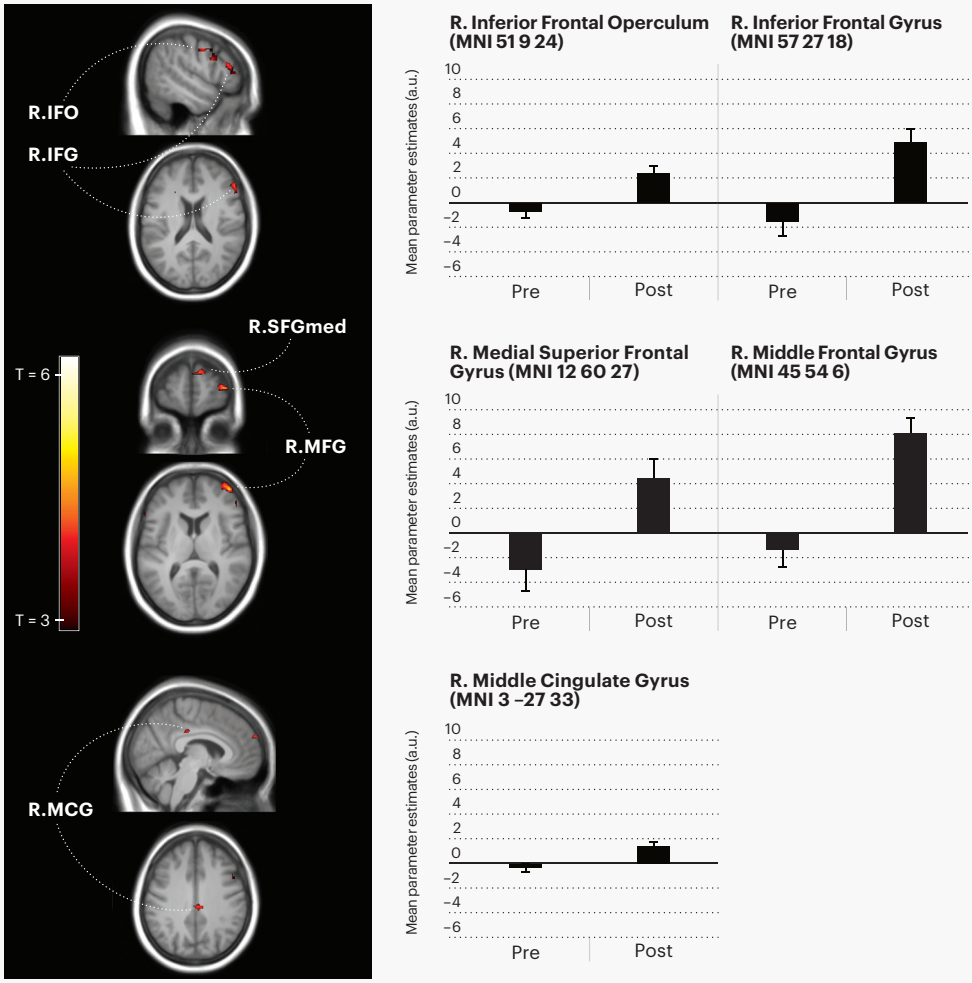

Figure 6.2. Regions in which brain activation during response inhibition to dessert items was significantly different pre- and post RYGB surgery. Brain images were thresholded at p = .005 for visualisation. **Upper:** The right inferior frontal gyrus (Tri; MNI: 57 27 18) was more activated after compared to before surgery and the right inferior frontal operculum (MNI: 51 9 24) showed more activation after compared to before surgery. **Middle:** The right middle frontal gyrus (MNI: 45 54 6) was more activated after than before surgery and the right medial superior frontal gyrus (MNI: 12 60 27) showed deactivation before surgery and activation after surgery. **Lower:** The right middle cingulate cortex (MNI 3 –27 33) was more activated after surgery.

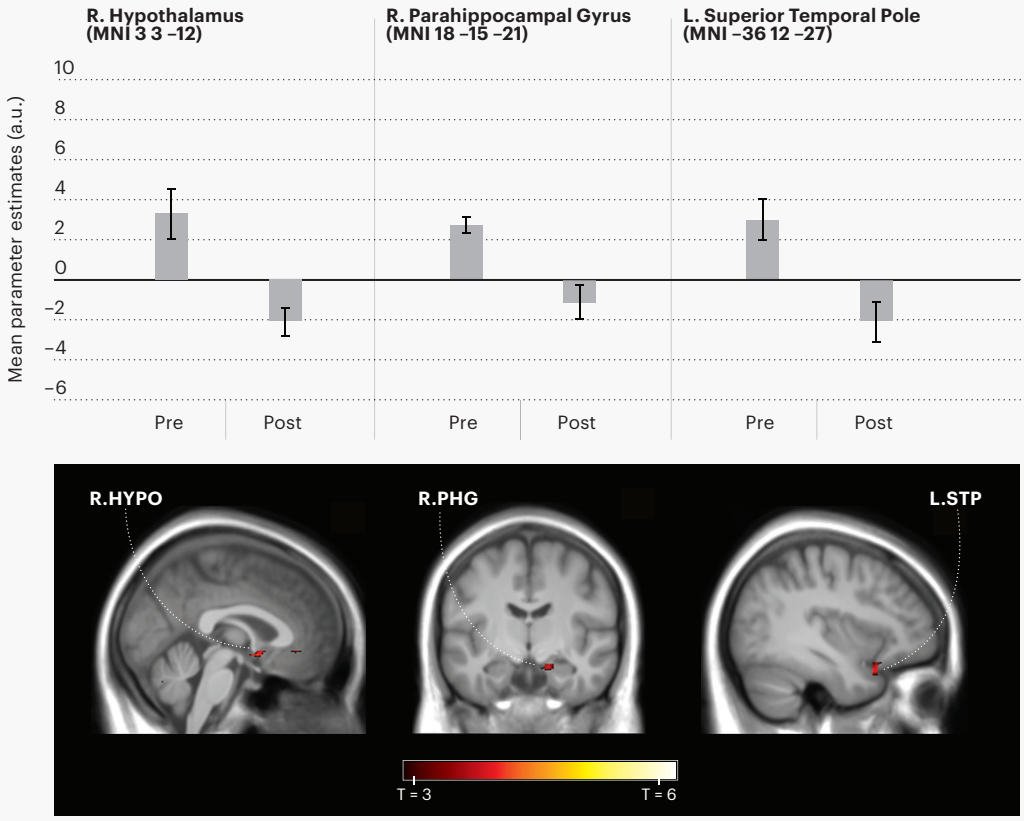

Figure 6.3. Regions in which brain activation during response inhibition to vegetable items was significantly reduced post- compared to pre-RYGB surgery. Brain images were thresholded at p = .005 for visualisation. **Left:** The right hypothalamus (MNI: 3 3 –12) was activated before surgery and deactivated after surgery. **Middle:** The right parahippocampal gyrus (MNI: 18 –15 –21) displayed activation before and deactivation after surgery. **Right:** The left superior temporal pole (MNI: –36 12 –27) showed activation before surgery and deactivation after surgery.

The go/no-go task included 12 no-go items per run. Commission errors to no-go dessert items occurred at 443 ± 87 ms (mean ± *SD*) pre-surgery and at 501 ± 132 post-surgery. Commission errors in response to no-go vegetable items had a mean reaction time of 526 ± 125 ms. After surgery, responses to no-go vegetable items occurred at 504 ± 143.

Go items—For the go dessert items, the omission error rate (non-responses during the go-trials) changed from 0.9% (± 1.7) before surgery to 1.7% (± 2.5) after surgery. The mean rate of omission errors for go vegetable items was 4.0% (± 2.7) before surgery and 3.4% (± 2.0) after surgery.

There were no significant differences between reaction times to go dessert items before (543 ± 90 ms; mean ± *SD*) versus after RYGB (567 ± 122 ms; $p = .395$), nor between reaction times to go vegetable items before (544 ± 138 ms) and after RYGB (538 ± 135 ms; $p = .395$).

FUNCTIONAL IMAGING DATA

No-go desserts—Comparisons between pre- and post-surgery fMRI BOLD responses for the no-go dessert trials revealed increased post-surgical activation of the right middle frontal gyrus (lateral part), the medial part of the right superior frontal gyrus, the right inferior frontal gyrus (pars triangularis), the right middle cingulum and the inferior frontal operculum (see **Table 6.2.** and **Figure 6.2.**). There were no regions in which no-go activation was significantly decreased post- compared to pre-surgery.

There were no significant correlations between pre- to post-surgery changes in neural responses during no-go dessert trials and changes in BMI or body weight, changes in feelings of hunger, fullness, prospective consumption and desire to eat (all $p > .05$).

No-go vegetables—Pre-surgical neural activation to no-go vegetable items was significantly higher in the right hypothalamus, left superior temporal pole and right parahippocampal gyrus, relative to post-surgery (see **Table 6.2.** and **Figure 6.3.**). There were no regions in which activation was significantly increased post- compared to pre-surgery.

Pre- to post-surgery changes in activation of the right parahippocampal gyrus during no-go vegetable items were correlated with changes in ratings of fullness provided right before the go/no-go task ($r = -0.625$, $p = .007$). No significant correlations were found between pre- to post-surgery changes in neural responses during no-go vegetable trials and BMI or body weight, and changes in feelings of hunger, prospective consumption and desire to eat (all $p > .05$).

Discussion

To our knowledge, this is the first study to investigate changes in neural inhibition to food cues after RYGB. We found pre- to

post-surgery increases in neural response to no-go high energy-dense food items in regions involved in inhibitory control (middle, medial superior- and inferior frontal gyrus). Further, neural activation in response to no-go low energy-dense food items was less pronounced in regions related to satiation (hypothalamus, parahippocampal gyrus, superior temporal pole) after surgery. Alterations in reward related activation were found for both no-go dessert and no-go vegetable trials (inferior frontal gyrus, middle cingulate gyrus, inferior frontal operculum, parahippocampal gyrus, superior temporal pole).

As expected, neural activation to no-go vegetable items did not change after surgery in regions involved in inhibitory control. During response inhibition towards desserts, however, we observed increased involvement of prefrontal regions (middle-, medial superior- and inferior frontal gyrus) after surgery. Previous research has linked increased activation in these regions to greater exertion and success of inhibitory control [85,98,99,243-246]. This suggests that activation in these frontal regions can serve as an indicator for response inhibition capacity. Interestingly, Lapenta *et al.* found that it is possible to induce changes in response inhibition processes by transcranial direct current stimulation (tDCS) of the dorsolateral prefrontal cortex (dlPFC) [247]. They showed that this type of neural stimulation leads to significant changes in neural markers of inhibitory control, and also to reduced craving and food intake. In our study, increased prefrontal cortex activation post-surgery could indicate an increase in neural inhibitory control in response to appetizing food items. In contrast to Batterink *et al.* [101] who found correlations between current BMI and prefrontal activation during inhibitory control, we did not find significant correlations between changes in prefrontal activation and changes in body weight or BMI. This is likely related to greater variation (from lean to obese) in current BMI in their study [101], versus limited variation in within-subject changes in BMI in the current study. The observed changes in neural processing after RYGB support an improved response inhibition towards high-energy food.

Post-surgical reductions in parahippocampal gyrus, superior temporal pole, and also hypothalamus activation during low-energy no-go items, but not during high-energy no-go items, could relate to metabolic signals of satiety. In the current study, participants were equally satiated directly after meal intake in both test sessions (see **Supplementary Table 6.2.**), but felt less hungry and more full post- compared to pre-surgery before starting the go/no-

go task. This could be related to accelerated digestion and absorption of nutrients after RYGB [248]. Moreover, a significant correlation was found between pre- to post-surgery changes in parahippocampal gyrus activation and changes in ratings of fullness. Previous studies found increased brain activation to high-energy food cues (visual, taste) in the parahippocampal gyrus and hypothalamus in a hungry compared to a satiated state [249-252], and related this to an increased salience of energy-rich products during hunger [253,254]. In light of this, the decrease in hypothalamic, parahippocampal and superior temporal pole activation during response inhibition after surgery suggests that the increase in feelings of fullness is related to a decrease in salience of low-energy products, but not high-energy products.

Besides increased activation in prefrontal regions of inhibitory control, we found increased activation in the inferior frontal gyrus, inferior frontal operculum and middle cingulate cortex during response inhibition towards high-energy food. Although, activation in these regions has been linked to selective attention and more effective response inhibition [246,255-257], these regions are also implicated in processing of reward value and taste evaluation in response to cue exposure during anticipation, consumption [86,140,258], and self-regulation [259-261]. Our results thus suggest greater engagement of these reward-related areas during response inhibition for high-energy products after surgery. Decreased post-surgery activation of the parahippocampal gyrus and superior temporal pole during response inhibition for low-energy food products could also be associated with changes in reward processing. Increased activation in the parahippocampal gyrus during exposure to taste and smell of food was associated with decreasing reward value in healthy [140] and obese subjects [184]. The observed reduction in parahippocampal gyrus deactivation during response inhibition to low-energy food cues thus could imply a more positive attitude towards these cues. However, we have no ratings of liking or wanting ratings for the food stimuli, so we can only speculate about a link between the decrease in inhibitory activation and higher preference for low-energy products. Nevertheless, the relative increase in preference for low-energy food found in RYGB patients [104,109,229], does support a more positive attitude towards vegetables after surgery.

As mentioned above, the regions in which we find increased activation in response to no-go dessert items after surgery have been linked to increased exertion of neural inhibitory control and also to more successful behavioural inhibition [85,98,99,243-246]. It

would be interesting to link these neural data to actual behavioural changes. However, the limited amount of no-go trials (n = 12) in the task we used, unfortunately rendered the behavioural data for this condition unsuitable for reliable statistical inferences about correlation to neural outcomes. Further, reaction times to go-dessert and go-vegetable items were not significantly different between the pre- and post-surgery test session. Thus, with the current data we cannot conclude whether the changes we find solely reflect increased exertion of neural inhibitory control or whether they have implications for actual behaviour. Future research including more extensive behavioural measures is needed to clarify the link between changes in neural and behavioural response inhibition in RYGB patients. However, the food specific go/no-go task does approach real-life decision-making processes better than the passive reward tasks that have been used in previous research. Because of limited statistical power due to the small sample size, we have used a relatively lenient threshold for the fMRI analyses. We are aware that this increases the risk of false positive results. Nonetheless, this research provides unique additional insight in the mechanisms underlying the effectivity of RYGB surgery. The within-subject design provides a solid method for testing RYGB related changes. Moreover, unlike most previous research, measurements in this study have been obtained in a satiated state, to better mimic a context of overeating that has a greater ecological relevance in obesity. Despite high effectiveness of RYGB on weight loss and promising results demonstrated in a 20 year follow-up study [102], weight regain after more than one year post-surgery is a recurring problem in a subset of patients [262]. Perhaps additional (cognitive) treatment focused on improving and maintaining response inhibition skills can reduce weight-regain after RYGB surgery.

Conclusion

After RYGB surgery, patients showed increased activation during a food specific go/no-go task to high-energy food cues in prefrontal brain regions implicated in inhibition. These neural changes after surgery indicate improved response inhibition towards high-energy food cues and increased influence of satiety on processing of low-energy food cues. We found altered neural responses during response inhibition towards both high- and low-energy food cues in reward-related areas, which indicate a more positive attitude towards these cues after RYGB. It is plausible that changes in the (re)activity of neural circuits involved in

inhibitory control, satiety and reward processing together underlie effective weight-loss by contributing to the shift in preference and intake from high- to low energy-dense foods observed after RYGB. Future research should aim to clarify the association between the neural changes we found and actual measures of eating behaviour and put effort into improving effectivity of weight-loss treatment.

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Supplementary information

Supplementary Table 6.1. Composition of the standardized meal.

	Men		Women	
	Pre	**Post**	**Pre**	**Post**
Bread Roll *(Wheat bread (± 22 g/roll))*	4 pcs	2 pcs	3 pcs	1 pcs
Margarine *(Low-fat)*	30 g	15 g	15 g	15 g
Cheese *(Full-fat semi-cured)*	40 g	20 g	40 g	20 g
Ham	40 g	20 g	20 g	–
Orange Juice	150 g	75 g	100 g	50 g
kcal total meal	570	174	421	107

Supplementary Table 6.2. Hunger ratings provided after meal intake and around 50 minutes before the go/no-go task commenced, before and after RYGB surgery.

		Pre-surgery Mean ± *SD*	**Post-surgery Mean ± *SD***	**Sign. Difference**
Hunger	*100 mm VAS*	11 ± 21	11 ± 24	p = .954
Fullness		74 ± 25	67 ± 35	p = .335
Prospective consumption		25 ± 26	8 ± 18	p = .051
Desire to eat		18 ± 19	12 ± 23	p = .435
Thirst		66 ± 27	53 ± 29	p = .049

Chapter 7

General discussion

Table 7.1. Overview of main findings

Chapter	Result and conclusion
How does odour exposure affect appetite, preference and intake of the food that is cued?	
2	—Exposure to odours (sweet/savoury) led to a higher increase in appetite for products similar in taste (resp. sweet: 17%, savoury: 19%) than for products with dissimilar taste (resp. savoury: –3%, sweet: –1%) or taste neutral products (resp. 7%, 2%) —Exposure to odours led to a higher increase in appetite for products similar in energy-density (resp. high: 18%; low: 14%) than for products dissimilar in energy-density (resp. low: –2%, high: 3%) *Food odours increased appetite for products that are similar, both in taste and energy density*
4	—There was no differences in food preferences and intake after ambient exposure to odours signalling high-energy food, low-energy food and non-food *Ambient odour exposure did not affect food preference and intake*
How does food intake affect olfactory food-cue reactivity?	
2, 4	—Sensory-specific appetite induced by odours was similar in hungry and satiated state —Both in a hungry and a satiated state, individuals did not show changes in food preferences and -intake after exposure to different odours. *Hunger state did not modulate effects of odour exposure on appetite, food preference and intake.*
3	—Sensory-specific satiety for a sweet product is associated with more negative amplitudes of early neural responses (< 150 ms) to food pictures that are congruent to the food eaten. —General satiety appears to lead to more negative amplitudes of late neural potentials (~300–700 ms) to food and non-food cues. *Food intake led to sensory-specific satiety, which contributed to top-down cognitive control over early processes of sensory perception in response to food cues*

How does weight status influence behavioural and neurobiological responses to food cues?	
4	Both normal-weight and overweight individuals did not show changes in food preferences and -intake after exposure to different ambient odours. *Effects of ambient odour exposure on eating behaviour were not influenced by weight status*
5	After compared to before RYGB surgery we observed: —Increased preference for high-fat/high-sugar food and decreased preference for low-fat/low-sugar food, which correlated with decreased superior parietal lobule responsivity to high-fat/high-sugar food odour and with a reduced difference in precuneus responsivity between high-fat/high-sugar and low-fat/low-sugar food pictures —Less deactivation of the precuneus to all cues (*z*-scores 3.43–3.92) —Less deactivation to low-fat/low-sugar food pictures ($z = 3.75$) and greater difference in activation between high-fat/high-sugar food and non-food odours in the anteroventral prefrontal cortex ($z = 3.92$) *RYGB led to a shift in preference from high- to low-energy foods, and to changes in top-down control orchestrated by the frontoparietal control network, rather than changes in activation of reward related brain regions.*
6	After compared to before RYGB surgery we observed: —Greater activation in the anterolateral-, medial- and dorsolateral prefrontal cortex, the middle cingulate cortex and inferior frontal operculum during response inhibition to high-energy sweet food pictures (*z*-scores 3.53–4.42) —Less activation in the hypothalamus, superior temporal pole and parahippocampal gyrus during response inhibition to low-energy savoury food pictures (*z*-scores 3.34–3.65) *RYGB led to increased neural inhibitory control towards high-energy food cues.*

Percentages change was not mentioned in the chapters, but were converted from the raw data and included in this table for clarity.

The overall aim of this thesis was to elucidate the role of orthonasal odours in (over)eating. Within this scope four experimental studies were carried out to determine the influence of food odour exposure on appetite (**Chapter 2**), food preferences and -intake (**Chapter 4**); to assess how food intake affects responses to olfactory food cues (**Chapter 2, 3, 4**); and to determine the effect of weight status on behaviour and on neurobiological processes of reward and inhibition in response to food cues (**Chapter 4, 5, 6**). This final chapter starts with an overview of the main findings, followed by a discussion and interpretation of these findings. Next to this, several methodological considerations are addressed and implications of the results are discussed. Finally, recommendations for future research are proposed.

Main findings

The main findings of the research described in this thesis are summarized in Table 7.1. In **Chapter 1** of this thesis three research questions were introduced that will be addressed in this section.

—1 How does food odour exposure affect appetite, preference and intake of the food that is cued?

The findings described in this thesis demonstrate that exposure to olfactory food cues increased appetite for products that are congruent to the cued food, both in taste and in energy density. For odours signalling sweet taste, savoury taste and high energy-density these effects did not only apply to the specific food product that was cued, but also to other products within the same category (**Chapter 2**). Ambient exposure to odours of high energy-dense food did not increase preference for high-energy food products more than low energy-dense food odours and non-food odours did. Moreover, food intake was similar after high energy-dense food, low energy-dense food and non-food (**Chapter 4**).

—2 How does food intake affect olfactory food-cue reactivity?

We found distinct effects of general and sensory-specific satiety on responses to odours. Odour exposure led to an increase in sensory-specific appetite, which was similar in hungry and satiated state (**Chapter 2**). Also, ambient odour exposure did not

influence food preference and -intake in both hungry and satiated state (**Chapter 4**). However, ad libitum food intake did lead to a general decrease in subjective ratings of appetite, liking and wanting for food cues (odours and pictures). Overall, this decrease was more pronounced for food cues that were most similar in taste (sweet/savoury) and energy-density (high/low) to the food eaten to satiety (**Chapter 3**): sensory-specific satiety. In addition, we found that food intake led to a more negative amplitude of neural event-related responses to food and non-food cues, measured with EEG. A more pronounced increase in negative amplitudes was found during processing of high-energy sweet food pictures after a high-energy sweet meal, which could be related to sensory-specific satiety.

—3 How does weight status influence behavioural and neurobiological responses to food cues?

There was no significant effect of ambient odour exposure on food preference and -intake in both normal-weight and overweight participants. This suggests that weight status does not play a modulatory role in olfactory food-cue reactivity (**Chapter 4**). On the other hand, Roux-en-Y Gastric Bypass (RYGB) weight-loss surgery did results in a shift in food preferences. After compared to before RYGB, patients demonstrated a decreased preference for high-fat/high-sugar foods and an increased preference for low-fat/low-sugar foods. This shift was correlated with a decrease in deactivation of the superior parietal lobule to high-fat/high-sugar food, and a reduced difference in precuneus responsivity between high-fat/high-sugar and low-fat/low-sugar food pictures. Moreover, regions within the frontoparietal control network (*i.e.* precuneus, anteroventral prefrontal cortex and superior parietal lobule) were less deactivated in response to high-fat/high-sugar food, low-fat/low-sugar food, but also to non-food odours and pictures. No pre- to post-surgery changes in activation of neural reward regions were found in response to the food and non-food cues (**Chapter 5**). However, increased activation of neural regions involved in inhibitory control (*i.e.,* the anterolateral-, medial- and dorsolateral prefrontal cortex) was uncovered during response inhibition to high-energy sweet food pictures, after compared to before RYGB. Further, we found decreased activation of neural regions involved in appetite regulation (*i.e.,* the hypothalamus, parahippocampal gyrus, and superior temporal pole) during re-

sponse inhibition to low-energy savoury food pictures, pre- compared to post-surgery (**Chapter 6**).

Discussion and interpretation

—1 How does food odour exposure affect appetite, preference and intake of the food that is cued?

Orthonasal olfactory signals in our environment are crucial in guiding attention to environmental hazards or rewards [42,53]. They can be considered as cues for availability of edible objects in our surrounding and where these objects can be found. Our results clearly demonstrate a role of olfactory cues in appetite regulation. Food odours stimulate appetite for food products that are directly available (**Chapter 2**). The odour-induced increase in appetite is most pronounced for the specific food that is smelled, but was also found to spill over (to a lesser extent) to foods that are similar in taste and in energy-density: sensory-specific appetite. Interestingly, the spill over effect to less specific but still congruent products was only present for odours signalling sweet, savoury and high-energy food products, but not for low-energy food products. This suggests that only odours that signal nutritional content (*e.g.,* carbohydrate, protein and fat) are potent in stimulating appetite for a broader category of food products. We found more pronounced specific appetite for olfactory signals of taste category, compared to energy-density category. Taste typically functions as a nutrient sensing system, in which sweet taste is linked to sugar content (and other carbohydrates) and savoury taste is associated with protein and salt content [126,132,263]. Energy-density could however be associated with all macronutrients, since they all provide energy. Similar to flavour-nutrient learning [23,264,265], learned associations between orthonasal odour qualities and rewarding metabolic consequences of nutrient ingestion can increase odour preference. Odour-induced appetite may be a mechanism for regulating macronutrient balance after depletion.

Food odours smelled from a glass bottle were found to increase specific appetite (**Chapter 2**), but exposure to ambient odours of low- and high-energy foods did not affect (sensory-specific) food preference and -intake (**Chapter 4**). The difference in odour exposure method could explain the discrepancy between results we found in **Chapter 2** and **Chapter 4**. Perhaps the role of olfactory cues in eating behaviours (*e.g.,* orientation, preference,

selection, and intake) is dependent on the distance of the odour source. Our surroundings carry a constant stream of olfactory information that is not constantly registered consciously [174]. It may be effective to spend more cognitive resources on processing food odours from proximal sources that likely indicate an immediate availability of food, rather than food odours from distant sources. Several studies have found that food odours in the surroundings increased odour-congruent food choice, when these odours were non-attended [112,121,122,266]. Other findings and our own findings (**Chapter 2**) indicate that conscious perception of food odours smelled from bottles can evoke increased sensory-specific appetite [119]. Attention to odours from distant sources, presented at a supra-threshold concentration, might be repressed through cognitive control to avoid distraction from environmental cues of foods that are more readily available.

Increased food intake in response to food odour exposure has been demonstrated before, but only in restrained eaters [66,67] and low-impulsive participants [123]. In contrast to this, ambient exposure to chocolate chip cookie odour decreased food intake in restrained compared to unrestrained eaters [267]. Food intake is thus influenced by personality traits and attitudes. In our study (**Chapter 4**), we did not find effects of odour exposure on food preference and intake. Differences in responses to odour exposure between the normal-weight and overweight participants may have been smaller because we matched overweight and normal-weight individuals on restrained eating [146]. Moreover, overall effects of ambient odour exposure on food intake could have been reduced. A recent study by Proserpio *et al.* [268] (in normal-weight, unrestrained eaters) did not reveal odour-induced specific appetite, but did find higher food intake after ambient exposure to a high-energy food odours relative to low-energy food odours and a no-odour control condition. However, they found similar amounts of food intake after ambient exposure to odours signalling different taste categories (sweet/savoury). The study in **Chapter 4** describes that energy-density signalled by ambient food odours did not impact food preference. Perhaps, signals of energy-density have an increased importance in determining expected-satiety before food intake [269], whereas taste related signals have a higher relevance in earlier processes preceding food selection. The odours selected for our studies and the study by Proserpio *et al.* [268] have associations to both taste and energy-density. In different phases of eating behaviour we may pay attention to different properties of food

cues. Together these results suggest a dynamic role of orthonasal food odours in eating behaviour, including nutrient signalling during orientation and selection, and energy-density signalling in food intake.

—2 How does food intake affect olfactory food-cue reactivity?

In our research, general satiety did not modulate odour-induced increases in specific appetite (**Chapter 2**). This indicates that exposure to palatable food odours may play a stimulating role in eating beyond satiety, and could hence be a risk factor in the development of overweight and obesity. However, general satiety did reduce general appetite. Bodily energy stores in adipose tissue are held constant over time by balancing energy intake with energy expenditure, a process referred to as homeostasis [270]. The effects of food intake on general appetite may be associated with repletion of energy stores in general, while odour-induced specific appetite may be involved in more subtle regulation of macronutrient balance. This regulation may be driven by the reward value of specific macronutrients, reflecting hedonic eating.

Next to effects of general satiety, appetite regulation is influenced by sensory-specific satiety. Rolls *et al.* [31] were the first to describe sensory-specific satiety, a phenomenon that refers to a larger decrease in pleasantness and intake of a food eaten to satiety than of a food not eaten. Our research (**Chapter 3**) demonstrates that eating a food with a specific flavour (sweet/savoury) reduces appetite, liking and wanting for cues of foods similar to the food consumed. In previous studies, sensory-specific satiety related reductions in neural responses to olfactory food cues in the orbitofrontal cortex [35,140,271], which indicates changes in evaluation processing. We found evidence for general and sensory-specific alterations in early neural responses to food pictures, which reflect changes in sensory perception and in processes of (hedonic) evaluation after food intake (**Chapter 3**). These results suggest that food intake leads to increased top-down control over cognitive processing of environmental cues. Previous findings also indicate that food intake modulates early and late neural processing of food cues [144,153]. Alterations in the early stages of information processing are thought to reflect changes in motivated attention; increased attention for state-relevant cues. After food intake, food cues become less relevant, because energy and nutrient levels have been restored. A need-related regulation of attention appears to

modulate sensory information processing in a top-down manner [144,209]. Changes related to food intake in later stages of information processing may reflect modulation of stimulus recognition, working memory representations and evaluation. Neural responses to odours showed a complex pattern of changes after food intake, possibly related to a low signal-to-noise ratio in electrophysiological responses to olfactory cues [272]. More research is needed to determine whether event-related neural responses to food odours also reflect modulation by sensory-specific satiety.

Overall, general satiety does not influence odour-induced specific appetite. This may be related hedonic eating. Modulation of sensory perception of meal-congruent food pictures by sensory-specific satiety indicates that cognitive control of attention is involved in specific appetite regulation.

—3 How does weight status influence behavioural and neurobiological responses to food cues?

What we choose to eat is largely driven by the sensory pleasure we experience in relation to food [90]. Increased body-weight in obesity is proposed to be the result of a heightened drive to eat palatable foods which remains high in the absence of hunger, also referred to as hedonic hunger or non-homeostatic eating [70,71,164,220,273,274]. Increased hedonic hunger was found to be reduced after gastric bypass surgery [275]. Also, preference for high-energy foods decreases, while preference for low-energy foods increases after RYGB surgery [103]. In line with these findings, we found a pre- to post-RYGB shift in food preferences from high- to low-energy foods (**Chapter 5**). In contrast to previous research findings [106,109,195], results in this thesis did not show changes in neural reward responses (*e.g.,* striatum, amygdala, orbitofrontal cortex, anterior cingulate cortex) to high and low energy-dense food cues (odours, pictures) after RYGB weight-loss surgery. However, these other studies performed their measurements in a hungry state, which was likely related to a higher salience of food cues and enhanced reward processing [79,276–278]. Excess food intake (overeating) in obese individuals is suggested to occur when responses to food cues overrule metabolic signals of satiety [164,220], which is referred to as hedonic eating. Therefore, we measured neural food-cue reactivity while our participants were in the absence of hunger. In this setting we found less deactivation of the precuneus, anteroventral prefrontal cortex and superior parietal lobule in response to food and

non-food cues (odour and picture), after compared to before RYGB. Decreased responsivity of the superior parietal lobule to a high-energy food odour and a reduced difference in precuneus responsivity between high- and low-energy food pictures were correlated to the shift in preferences from high- to low-energy food. These regions are all part of a frontoparietal network that orchestrates adaptive top-down control [206,219,279]. Increased feelings of satiety after RYGB [108] could affect cognitive control over processing of food cues. In our study, hunger feelings rated at the start of the passive viewing/smelling paradigm were similar pre- and post-RYGB. Hunger ratings at the end of this paradigm indicate increased satiety post- compared to pre-RYGB, which suggests accelerated satiation due to a smaller stomach and bypassing of the duodenum, and upper jejunum [248]. In **Chapter 3**, we observed that food intake and thus satiation led to more negative amplitudes of late event-related potentials to visual and olfactory food and non-food cues, which were thought to be related to modulation of motivated attention towards external sensory cues [154–157].

In previous research, a higher BMI was associated with increased behavioural and neural disinhibition towards palatable food cues [97,101]. After RYGB we found increased activation of anterolateral-, medial- and dorsolateral prefrontal cortex during response inhibition towards high-energy sweet food pictures, suggesting an increased exertion of inhibitory control (**Chapter 6**). In this research, we used a food-specific go/no-go task with food pictures. Neural response inhibition towards high-energy food odours should be examined in future research.

All in all, changes in neural mechanisms of cognitive control appear to be related to an increased capacity to inhibit responses to anticipated food reward in our food rich environment. This may facilitate weight-loss.

Methodological considerations

FOOD CUES

For this thesis, we were especially interested the role of odours in the anticipatory phase of eating, leading up to meal initiation. We therefore chose to research the impact of orthonasal odours on behavioural and neurobiological mechanisms of appetite control and food intake regulation. Retronasal food odours are cues that refer to the internal environment, *i.e.* when food has entered the mouth (during consumption), while orthonasal food odours signal objects in the external environment, before ingestion [280,281].

The food odours and pictures we used were carefully piloted and selected to represent palatable products from different taste categories (sweet/savoury), energy-density categories (high/low) and macronutrient content categories (high/low in fat/sugar). Sweet and savoury (salty, umami) taste are considered to be the dominant taste categories in our daily eating habits, and are therefore thought to be most relevant in appetite regulation [9]. Next to these two taste qualities olfactory signals representing fat taste could be included [127].

Besides selection based on representation of certain food categories, food cues were selected to be familiar and generally liked. However, inter-individual variation in liking for food products is high and cues from individually preferred food products may provide a stronger temptation to choose and consume the cued foods (for examples see [184,282]). Although food cue responsivity for the individuals' preferred food may be more pronounced, effects of the food cues selected for the research in this thesis are more generalizable.

In addition to food odours, non-food and no-odour control conditions were included. Differences in sensory-specific effects of odour exposure are relatively small between odours from different food categories. We have seen that using a baseline no-odour condition as a reference can help to separate sensory-specific effects from effects of general satiety (**Chapter 2**). Absence of such a no-odour baseline reference condition could explain the lack of differences in food preference and intake found after ambient exposure to high-energy food, low-energy food and non-food odours (**Chapter 4**). Although it is not substantiated by statistics, the results of **Chapter 2** suggest that appetite for all types of products (sweet, savoury, high-energy, low-energy) is decreased after non-food compared to no-odour exposure. Neural responses to non-food odours also varied as a function of metabolic state (**Chapter 3**) and weight status (**Chapter 5**). A non-food odour exposure condition must not be considered as a stable reference, but can serve to separate food-specific from general cue-induced effects.

HUNGER STATE

In studying modulatory effects of hunger state on food-cue reactivity we chose to instruct participants to eat a satiating meal of their own choosing at home (**Chapter 2 & 4**). In contrast to a standardized meal provided in the lab, this method will lead to a more ecologically valid reflection of food-cue reactivity in a

satiated state. Previous research and our own findings (**Chapter 3**) however indicate that sensory-specific satiety can affect evaluation as well as perception of sensory food cues. These alterations are likely to also impact appetizing effects of cue exposure. When investigating effects of hunger state on food-cue reactivity, the influence of sensory-specific satiety should be taken into consideration. Including a baseline condition (*e.g.,* no odour or non-food) in the study design provides the opportunity to eliminate possible effects of sensory-specific satiety that may interfere with effects of food cues on appetite

STUDY POPULATION

For three of the four studies we only included female participants. Further, inclusion criteria for age, restrained eating and BMI were instated to ensure similarity within the studied population. In **Chapter 4** we matched participants from the two BMI groups (overweight /normal-weight) on restrained eating and age. This was done to reduce the effects of these two factors in between-group comparisons. In all the studies, we ensured normal olfactory function in our participants since a decreased ability to detect odours would possibly diminish any effects of odours. These inclusion criteria did not lead to study populations that accurately reflect the general population. However, a more homogeneous study population reduces variation in outcome measures and increases the probability of finding variation that can be explained by the study interventions. Extrapolation of the findings discussed in this thesis to groups with other characteristics (*e.g.,* elderly or males) needs to be approached with caution. It is plausible that similar mechanisms of sensory stimulation of appetite based on food content are in place in the general population, but the strength of this effect may differ.

In the study described in **Chapter 5** and **Chapter 6**, we performed within-subjects comparisons to clarify the impact of RYGB surgery on neural processing. We focused on the RYGB patient population and can therefore not be certain whether the changes we found are unique to this specific surgical intervention, to surgical changes made in the gastro-intestinal tract more in general, or perhaps to the course of procedures around the surgery including psychological support. Our research should therefore be repeated for other surgical weight-loss procedures (*e.g.,* gastric banding, sleeve gastrectomy) and non-surgical weight-loss interventions.

EXPERIMENTAL DESIGN

In all of the studies we employed a within-subject crossover design. Confounding effects of between-subject variation is reduced in this type of design because participants serve as their own control. Test sessions performed on different days were planned at approximately the same time to minimize the influence of variation related to circadian rhythm [283,284]. The order of test conditions on different days were counterbalanced in order to rule out any influence of learning and familiarity over conditions. The impact of sensory-specific satiety on the (neural) evaluation of food cues was examined by comparing pre- and post-meal measures acquired in one test session (**Chapter 3**). The duration of the test session (± 3 h) may have led to boredom and reduced attention. Measurements during hunger and sensory-specific satiety (sweet/savoury) can be performed on separate days, but this leads to increased variation in the EEG signal related to application of the equipment and preprocessing. For the research of **Chapter 5** and **Chapter 6,** a training session was included to limit order effects from pre- to post-surgery.

Implications

FOOD PRODUCTS

Improving appetizing olfactory qualities of food products like fruits and vegetables could be useful in stimulating a healthy diet. For example, by developing varieties of strawberry and tomato containing volatile compounds that fit consumer preferences [285-287]. Moreover, supermarkets have started to educate their customers on storage conditions for fruits and vegetables, which may keep the quality of sensory attributes high. In previous research [285-288], the improvement of volatile compounds has focused on retronasal odours as a part of flavour. However, no, or only limited effects of retronasal odour exposure on intake have been found [289,290]. Our research (**Chapter 2**) indicates that orthonasal olfaction deserves attention in this field as it plays an important role in appetite regulation and perceived pleasantness of food.

Results of the research described in this thesis confirm an association between olfactory cues and nutritional content. We observed sensory-specific stimulation of appetite for categories of sweet, savoury and high energy-dense foods by orthonasal odours (**Chapter 2**). These categories are closely related to food content (*i.e.,* carbohydrates, protein, and fat, respectively). Many commercially available food products are packaged for better storage,

which limits the influence of orthonasal food odours during food selection. Reducing the use of packaging or adjusting packaging of foods to stimulate involvement of the nose in the selection process may influence food choice.

INDIVIDUAL

Orthonasal odours may also be applied to stimulate appetite for specific food products in individuals that have nutritional deficiencies. For example, protein intake in elderly individuals was found to be well below the average daily requirements [291]. This may compromise their physical well-being [292]. Exposure to orthonasal odours of protein-rich foods can be used before or during meal time to stimulate protein appetite and intake.

Recommendations for future research

PROXIMAL/DISTAL ODOUR SOURCES

Exposure to odours in the near vicinity induced specific appetite (**Chapter 2**), while odours that were emitted from a distant source did not affect food preference or intake (**Chapter 3**). In previous research a difference in effect of orthonasal odours on eating behaviour was suggested to relate to the way of smelling: active or passive. However, results revealed no effect of smelling method on odour-induced specific appetite [119]. Alternatively, a difference in impact of orthonasal odours in the surroundings and odours smelled from bottles could be related to the distance of the source that is emitting the odorant. Further, it has been suggested that orthonasal odours only influence food choice when they are not attended to [112,121,122,266], indicating that attention may influence how food odours regulate eating behaviour. We may pay more attention to food odours from proximal sources than to odours from more distal sources, because close proximity is associated with immediate availability. Exploring attentional bias (eye-tracking, visual probe task) and food choices during exposure to odours from sources with different proximities to the participant can provide insight into the role of orthonasal odours in appetite regulation.

ODOURS AND MACRONUTRIENT BALANCE

Our research findings suggest that orthonasal food odours signal macronutrient content and may thus be involved in maintaining macronutrient balance. Previous findings support a role for taste in the regulation of macronutrient status [293,294]. By examining effects of macronutrient status (*i.e.,* depleted vs balanced)

on appetizing effects of odours signalling either high protein, high carbohydrate or high fat content, we can determine whether orthonasal odours signalling the depleted macronutrient elicit a greater appetizing effects. This will provide information on the role of odours in macronutrient intake regulation.

COGNITIVE CONTROL OVER APPETITE REGULATION

Roux-en-Y gastric bypass is a surgical intervention that has proven to be effective in achieving weight-loss for many patients, also on the long term [102]. After RYGB we found changes in neural circuits involved in top-down control (**Chapter 5**) and inhibition (**Chapter 6**) over (food) cue responses. Longitudinal studies are required to establish whether the alterations we find two months after surgery are still present one or two years after surgery. Such results can help to find neural predictors for long-term weight-loss success.

In addition, cognitive behavioural weight-loss interventions should be developed that focus on improving the capacity to inhibit responses to fat and sugar-rich foods. Cognitive strategies are effective in modulating neural activation and might be effective in regulating appetite [100]. Training with a food-specific go/no-go task in which participants had to inhibit responses to palatable foods increased real-life inhibition towards palatable foods and decreased palatable food consumption [295]. Development of such a task for home-use would be an ideal tool for dieters. Next to this, implication of a behavioural intervention in addition to the RYGB protocol can be helpful in preventing weight regain observed in a subset of patients [262].

Main conclusions

1 How does food odour exposure affect appetite, preference and intake of the food that is cued? (**Chapter 2, 4**)

During the anticipatory phase of eating, olfactory food cue exposure increased specific appetite for foods similar in taste (sweet/savoury) and energy-density (high/low). Ambient odours that were dispersed into a room did not affect food preference and intake. This could be related to the relevance and impact of orthonasal odours in different phases of eating (orientation > preference > selection > intake) and/or variation in attention paid to the odours and the proximity to the odour source.

—2 How does food intake affect olfactory food-cue reactivity? (**Chapter 2, 3**)

Food intake affected general appetite, but not sensory-specific appetite induced by odour exposure. Food intake also influenced neural evaluation processes in response to pleasant cues in general (odours/pictures, food/non-food). Sensory-specific satiety appeared to modulate top-down control of attention specifically in response to cues similar to the food eaten to satiety.

—3 How does weight status influence behavioural and neurobiological responses to food cues? (**Chapter 4, 5, 6**)

Effects of ambient odour exposure on food preference and intake were not different between normal-weight and overweight participants. After weight-loss surgery (RYGB) we found altered neural responses to visual and olfactory food cues in the frontoparietal top-down control network. Also, pre- to post-surgery changes in neural responses to palatable food pictures indicate increased exertion of inhibitory control. Further, weight-loss led to alterations in neural responses to food odours and pictures, related to modulated attentional and metabolic control, and increased inhibitory control to high energy-dense foods.

THE ROLE OF ODOURS IN (OVER)EATING

To summarize, odours have a specific appetizing function in the anticipatory phase of eating. They are important in determining the taste quality and energy-density and may be involved in the selection of foods for macronutrient regulation. Orthonasal odours should be used to guide food selection towards a healthier eating pattern.

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Summary

The obesogenic environment we live in is characterized by an abundance of available foods. Every day, we are faced with signals (sight, smell) of palatable foods that that tempt us to eat. Via learned associations, food cues in the anticipatory phase of eating become predictors for taste, energy density and metabolic consequences after food intake. A well-orchestrated interplay between sensory and metabolic factors in the control of appetite and food intake is vital for maintaining a healthy weight in this environment. The capacity to balance food intake with energy expenditure is different for each individual. Eating behaviour of obese individuals is thought to be mainly driven by external food cues and less by internal signals of hunger and satiety. Next to this, a high sensitivity to the rewarding properties of food and an inability to control impulsive responses to food cues are proposed as risk factors for overeating. An increased understanding of the contribution of sensory signals of food and metabolic signals to eating behaviour is necessary to find tools for improving eating behaviour. The sense of smell is highly important in perceiving food flavours, locating food sources and determining food quality in terms of pleasantness and safety (toxicity, ripeness). It has been established that retronasal odours experienced via the back of the mouth during consumption play a role in satiation and satiety. With the research described in this thesis we aimed to elucidate role of orthonasal odours, perceived via the nose, in (over)eating to better understand how sensory and metabolic factors are involved in determining our eating pattern.

The following questions were addressed:

—How does food odour exposure affect appetite, preference and intake of the food that is cued? (**Chapter 2, 4**)
—How does food intake affect food-cue reactivity? (**Chapter 2, 3**)
—How does weight status influence behavioural and neurobiological responses to food cues? (**Chapter 4, 5, 6**)

In the first study (**Chapter 2**) we aimed to assess the appetizing effects of food odours signalling products with a certain taste (sweet/savoury) and energy density (high/low). Twenty-nine participants took part in two test sessions, once in a hungry and once

in a satiated state. We wanted to know whether the odours had different effects in these two conditions. In each test session six bottles were presented which contained either a food odour (4x), a non-food odour (1x) or an odourless solution (1x). After smelling an odour for three minutes, participants indicated their appetite for 15 different food products. This was repeated for each of the six odours. Our findings show that smelling a food odour increases the appetite for products that are similar to the odour, both in terms of taste (sweet/savoury) and energy-density (high/low). This effect was similar in a hungry and in a satiated state. Indicating a possible role of appetizing food odours in overeating.

Previous research found that food consumption led to a decrease in pleasantness that was more pronounced for sensory cues of the food that was just eaten relative to sensory cues of other foods, a phenomenon referred to as sensory-specific satiety. In the second study (**Chapter 3**) we used electroencephalography (EEG) to examine the influence of sensory-specific satiety on brain responses to viewing or smelling cues from different categories: high-energy/sweet, high-energy/savoury, low-energy/sweet, low-energy/savoury foods and non-food. Eating a sweet meal resulted in a more pronounced change in brain responses when the 20 participants viewed high-energy/sweet pictures, similar to the meal. These changes already occurred during the early phase of cue processing, which could be related to changes in cognitive control of attention to cues similar to the meal and/or decreased pleasantness (sensory-specific satiety). Consumption of a savoury meal did influence cue processing of congruent high-energy savoury odours, but not pictures. Next to modulation of neural processing by sensory-specific satiety, general effects of satiety were found on processing of food and non-food cues. This could be associated with a difference in the relevance of pleasant external cues between hunger and satiety.

We continued our research in a more realistic setting by studying whether odours signalling high-energy food, low-energy food or non-food that were dispersed into a room (ambient odours) affected food preference and intake (**Chapter 4**). Twenty-five normal-weight (BMI: 18.5–25 kg/m^2) and 25 overweight individuals (BMI > 27 kg/m^2) participated in six separate test sessions. They were exposed to the different odour categories in a state of hunger and a satiated state. This way we could determine the influence of

weight status and hunger state on odour induced effects. In this study, ambient exposure to odours from different categories of products did not have a different impact on food preference and intake. This was similar in participants with a different weight status and in hungry and satiated state.

Eating behaviour of obese individuals is proposed to be more driven by food reward and a decreased capacity to inhibit responses to palatable food cues. Roux-en-Y gastric bypass (RYGB) is a highly effective surgical weight-loss intervention, leading to long-term weight-loss success. Patients that underwent this type of surgery demonstrated a shift in preference away from high-fat/high-sugar and towards low-fat/low-sugar. We were interested in the underlying mechanisms of this shift, as these likely to contribute to the success of RYGB. Functional Magnetic Resonance Imaging (fMRI) was used to provide insight into differences in neural responses to food cues before and after RYGB.

In the first part of this study (**Chapter 5**) we investigated effects of RYGB on food preferences and on neural responses to pictures and odours of high-fat/high-sugar, low-fat/low-sugar foods and non-food items in 19 RYGB patients. The results of this study confirmed a shift in food preferences away from high-fat/high-sugar and towards low-fat/low-sugar food products. Changes in neural activation to high-energy food cues relative to low-energy food and non-food cues were found in the precuneus and anteroventral prefrontal cortex, regions that are part of a frontoparietal network involved in cognitive control. Changes in activation of the superior parietal lobule in response to high-fat/high-sugar odours and changes in the difference between activation to pictures of high-fat/high-sugar relative to low-fat/low-sugar foods were correlated with the shift in food preferences.

In the second part of this study (**Chapter 6**), we examined effects of RYGB on neural activation during inhibition of responses to food cues. Before and after surgery, 18 RYGB patients performed two versions of a food specific go/no-go task. During the go/no-go task they were presented with pictures of high-energy food (desserts) and low-energy food (vegetables). We used fMRI to measure neural activation during response inhibition and compared this between pre- and post-surgery test sessions. After surgery, neural activation during no-go high-energy food items was greater in brain regions related to inhibitory control (anterolateral-, medial- and dorsolateral prefrontal cortex, middle cingulate cortex and the in-

ferior frontal operculum), which indicates improved response inhibition towards high-energy food cues. We found decreased neural activation during no-go low-energy food items in regions involved in metabolic control (hypothalamus, parahippocampal gyrus, left superior temporal pole), which may be associated with decreased feelings of hunger after RYGB. Increased activation in the dorsolateral prefrontal cortex, middle cingulate cortex and inferior frontal operculum during no-go dessert items and decreased activation of the parahippocampal gyrus and superior temporal pole during no-go vegetable items are associated with a more positive attitude to these food cues. It is plausible that changes in the (re)activity of neural circuits involved in inhibitory control, metabolic control and reward processing for sensory food signals together underlie effective weight-loss by contributing to the shift in preference and intake from high- to low energy-dense foods, observed after RYGB.

Taken together, food odours stimulate appetite before food enters the mouth. They indicate the taste of the food and the energy-density, and may guide the selection of foods with specific macronutrient contents (*e.g.,* protein, carbohydrate, fat). Orthonasal odours should be used to guide food selection towards a healthier eating pattern.

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About the author

Curriculum vitae

Harriët Floor Aleida Zoon was born on April 11th, 1988 in Wageningen, the Netherlands. After completing secondary school at 'Pantarijn' in Wageningen, she started the Bachelor's programme 'Psychobiology' at the University of Amsterdam (UvA). She wrote her Bachelor's thesis entitled 'The role of glucocorticoids in emotional memory, and glucocorticoid treatment for post-traumatic stress disorder (PTSD)' and did a two month internship on effects of pharmacological interventions on conscious visual perception. After obtaining her Bachelor's degree in 2009, she enrolled in the Master's programme 'Neuroscience and Cognition'. As part of her Master's she did a nine month internship on the effect of testosterone on neurological mechanisms underlying processing of emotional facial expressions and she wrote her Master's thesis entitled 'Dissociating frontotemporal dementia from Alzheimer's disease using measures of executive functioning'. Another nine month internship within the international Study to Predict Optimized Treatment (iSPOT) in depression and ADHD resulted in a publication in the Journal of Clinical Neurophysiology. After receiving her Master's degree in 2011, Harriët was appointed as a PhD candidate at the chair of Sensory Science and Eating Behaviour, part of the Division of Human Nutrition of Wageningen University. Her research focused on the behavioural and neurobiological mechanisms of olfactory food-cue reactivity in normal-weight and overweight individuals. During her PhD project Harriët attended several (international) conferences and courses. She was a member of the PhD committee at the Division of Human Nutrition. Next to this, she was involved in teaching and supervising Bachelor's and Master's students.

List of publications

PUBLICATIONS IN PEER REVIEWED JOURNALS

Zoon HFA, De Graaf C, Boesveldt S. Food odours direct specific appetite. *Foods* 2016; **5**: 12.

Zoon HFA, He W, De Wijk RA, De Graaf C, Boesveldt S. Food preference and intake in response to ambient odours in overweight and normal-weight females. *Physiol Behav* 2014; **133**: 190–196.

Zoon HFA, Veth CPM, Arns M, Drinkenburg WHIM, Talloen W, Peeters PJ & Kenemans JL. EEG alpha power as an intermediate measure between brain-derived neurotrophic factor Val66Met and depression severity in patients with major depressive disorder. *J Clin Neurophysiol* 2013; **30**: 261–7

SUBMITTED PAPERS

Zoon HFA, De Bruijn SEM, Jager G, Smeets PAM, De Graaf C, Janssen IMC, Schijns W, Aarts EO, Boesveldt S. Altered neural responsivity to food cues in relation to food preferences, but not appetite-related hormone concentrations after RYGB-surgery. Submitted.

Zoon HFA, De Bruijn SEM, Jager G, Smeets PAM, De Graaf C, Janssen IMC, Schijns W, Deden L, Boesveldt S. Altered neural inhibition responses to food cues after Roux-en-Y Gastric Bypass. Submitted.

PAPERS IN PREPARATION FOR SUBMISSION

Zoon HFA, Ohla K, De Graaf C, Boesveldt S. Modulation of event-related potentials to food cues by sensory-specific satiety. In preparation for submission.

ABSTRACTS AND PRESENTATIONS

Zoon HFA, De Bruijn SEM, Jager G, Smeets PAM, De Graaf C, Boesveldt S. Effects of gastric bypass surgery on brain reward responses to food cues. Symposium 'A Flavor of Neuroscience', 2016, Groningen, the Netherlands. Oral presentation.

Zoon HFA. Proeven en de hersenen. Proeverij Voedselketen Friesland, 2016, Buitenpost, the Netherlands. Oral presentation.

Zoon HFA, Van Genderen L, De Graaf C, Boesveldt S. Food odours direct specific appetite. Annual meeting of the British Feeding and Drinking Group, 2015, Wageningen, the Netherlands. Oral presentation.

Zoon HFA, Van Genderen L, De Graaf C, Boesveldt S. Food odors direct specific appetite. Annual meeting of the Association for Chemosensory Science, 2015, Fort Meyers, FL, USA. Poster presentation.

Zoon HFA, Van Genderen L, De Bruijn SEM, Jager G, Smeets PAM, De Graaf C, Boesveldt S. Brain reward responses to olfactory food cues in obese participants – preliminary fMRI results. Pangborn sensory science

symposium, 2015, Gothenburg, SE. Poster presentation.

Zoon HFA, De Bruijn SEM, Weitkamp L, Jager G, De Graaf C, Boesveldt S. Measuring the Brain reward response to visual and olfactory food cues using fMRI – Pilot results. Netherlands Association for the Study of Obesity symposium, 2014, Oosterbeek, NL. Poster presentation.

Zoon HFA, De Bruijn SEM, Weitkamp L, Jager G, De Graaf C, Boesveldt S. fMRI Brain reward responses to visual and olfactory food cues – Pilot results. Annual Endo-Neuro-Psycho meeting, 2014, Lunteren, NL. Poster presentation.

Zoon HFA, He W, De Wijk RA, De Graaf C, Boesveldt S. Differences in food cue reactivity between normal weight and overweight individuals? Annual meeting of the Association for Chemosensory Science, 2013, Huntington Beach, CA, USA. Poster presentation.

Zoon HFA, He W, De Wijk RA, De Graaf C, Boesveldt S. Differences in food cue reactivity between normal weight and overweight individuals? Annual Endo-Neuro-Psycho meeting, 2013, Lunteren, NL. Oral presentation.

Zoon HFA, He W, De Wijk RA, De Graaf C, Boesveldt S. Food preference and intake in response to ambient odours in overweight and normal-weight females. NWO Nutritional Science Days, 2013, Deurne, NL. Oral presentation.

Overview of training activities

Discipline specific activities

Conferences

2016	Symposium 'A Flavor of Neuroscience' – University Medical Center Groningen	Groningen, NL
2015	Annual meeting of the British Feeding and Drinking Group	Wageningen, NL
	Annual meeting of the Association for Chemosensory Science	Fort Meyers, FL, USA
	Pangborn sensory science symposium	Gothenburg, SE
2014	Annual meeting Society of Applied Neuroscience	Utrecht, NL
	Netherlands Association for the Study of Obesity symposium	Oosterbeek, NL
	Annual Endo-Neuro-Psycho meeting	Lunteren, NL

2013	Annual meeting of the Association for Chemosensory Science	Huntington Beach, CA, USA
	Annual Endo-Neuro-Psycho meeting	Lunteren, NL
	NWO Nutritional Science Days	Deurne, NL
2012	Annual meeting of the British Feeding and Drinking Group	Brighton, UK
	Annual Endo-Neuro-Psycho meeting	Lunteren, NL
	Siemens Magnetic Resonance user day	Wageningen, NL
	MR Food: International conference on the application of magnetic resonance in food science	Wageningen, NL

Courses and training activities

2015	Tübingen International Summer School: Neuroscience and philosophy of taste	Tübingen, DE
2013	Summer School Human Olfaction	Dresden, DE
	Statistical Parametric Mapping (SPM) course	Utrecht, NL
2012	Regulation of Energy Intake: the role of product properties	Wageningen, NL

General courses

2015	Career orientation	Wageningen, NL
2014	Effective behaviour in your professional surroundings	Wageningen, NL
2013	Teaching and supervising thesis students	Wageningen, NL
	Basic statistics – Graduate school Production Ecology & Resource Conservation	Wageningen, NL
	Masterclass longitudinal data analysis (Mixed Models)	Wageningen, NL
2012	How to give and receive feedback	Wageningen, NL
	PhD week – VLAG Graduate School	Baarlo, NL

Optional courses and activities

2012-2015	Staff seminars & Chair group meetings	Wageningen, NL
2013	Wageningen University – PhD tour	Sydney & Melbourne, AU
2012	Preparation of research proposal	Wageningen, NL

Colophon

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Ceci n'est pas une banane.