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Satiation decreasing effect

on brain activation in insula and orbital frontal cortex

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Bachelor Thesis

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List of abbreviations

AIC:	Akaike information criterion
BDI:	Beck's Depression Inventory
BIS-11:	Barratt Impulsiveness Scale
BMI:	Body mass index
BOLD:	Blood oxygen level–dependent
DEBQ:	Dutch Eating Behavior Questionnaire
fMRI:	Functional magnetic resonance imaging
GLP-1:	Glucagon-like peptide-1
LME:	Linear mixed effect model
MNI:	Montreal Neurological Institute
OFC:	Orbital frontal cortex
PET:	Positron emission tomography
VAS:	Visual analogue scales

ABSTRACT

The reward value of food taste is represented in the orbital frontal cortex (OFC). The primary gustatory cortex is situated in the insula. When an individual is satiated, the neuronal response of OFC is decreased compared to a fasted condition. The effect of satiation on the neuronal response of insula is not clearly defined in the literature. By using a new approach to measure satiation, the decreasing effect of satiation on brain activity of the OFC and the insula throughout the duration of the experiment was investigated. 14 fasted healthy participants were scanned with functional magnetic resonance imaging while receiving chocolate milkshake at random intervals. The rating value of pleasantness and the blood oxygen level–dependent (BOLD) signals to the milkshake taste were recorded. The new method uses the cumulative mass of nutrients ingested during the experiment as the main parameter of satiation. Results showed no evidence that satiation has a decreasing effect on the pleasantness value of the milkshake and on BOLD signals in the OFC. Satiation was associated with a decreasing BOLD signal in the mid-insula. However, there is no evidence that the new method has reliably measured satiation. Therefore, the validity of the results is questionable.

ZUSAMMENFASSUNG

Der Belohnungswert von Geschmacksreizen wird im orbitofrontalen Cortex (OFC) verarbeitet. Der primäre gustatorische Cortex befindet sich in der Insula. Wenn ein Individuum gesättigt ist, ist die neuronale Reaktion im OFC im Vergleich zum Zustand des Fastens reduziert. Der Effekt der Sättigung auf die neuronale Antwort in der Insula ist in der Literatur jedoch nicht eindeutig definiert. Mit einem neuen Ansatz zur Messung der Sättigung wurde der senkende Effekt der Sättigung auf die Gehirnaktivität des OFC und der Insula während der Dauer des Experiments untersucht. 14 gesunde Probanden im Zustand des Fastens wurden mit funktioneller Magnetresonanztomographie gescannt, während sie in zufälligen Abständen Schokoladenmilchshake erhielten. Der Bewertungswert der Angenehmenheit und die vom Blutsauerstoffpegel abhängigen Signale (BOLD) für den Milchshake-Geschmack wurden aufgezeichnet. Die neue Methode verwendet die kumulative Masse der während des Experiments aufgenommenen Nährstoffe als Hauptparameter für die Bewertung der Sättigung. Es gibt keinen Beleg dafür, dass die Sättigung einen senkenden Effekt auf die bewertete Angenehmheit des Milchshakes und auf die BOLD-Signale im OFC hat. Die Sättigung war mit einem senkenden BOLD-Signal in der mittleren Insula verbunden. Es gibt jedoch keinen Beleg dafür, dass die neue Methode die Sättigung zuverlässig gemessen hat. Daher ist die Validität der Ergebnisse fraglich.

1 INTRODUCTION

In 2016, the problem of overweight affected 1.9 billion people and obesity more than 650 million people worldwide. Therefore, the World Health Organization considers obesity a very severe health problem in the developed world (Mishra *et al*., 2016). The consequences of obesity are cardiovascular diseases such as heart disease and stroke, diabetes, musculoskeletal disorders such as osteoarthritis, and cancer in the breast, ovaries, prostate, liver, gallbladder, kidney and colon. Obesity and overweight can be partly attributed to energy imbalance, which refers to a situation in which the calories consumed by an individual are superior to the calories expanded for a long period of time (Simmons *et al*., 2017). In this chronic state, the brain has lost its control to balance food intake with the energy requirements of the body. Environmental factors such as urbanization and the increase of sedentary work might have an impact on physical inactivity and thus on energy balance and ultimately overweight and obesity. Therefore, understanding the mechanism of eating behavior is a major concern.

1.1 Eating behavior

First, homeostatic eating behavior is described. When the internal metabolism or the external environment is changed, the body adapts to these modifications to cause appropriate behavior. Hunger is an important motivational driver for food-seeking behavior. Gut brain-interaction produces hunger information. Interoception refers to the mechanism of perception and integration of autonomic, hormonal, visceral and immunological homeostatic signals. Interoceptive signals related to food such as glucose, insulin levels and visceral signals of fullness allow the body to adopt appropriate allostatic visceromotor and behavioral responses to return to

homeostasis. Therefore, in a state of depleting energy in the case of hunger or weakness, the body compares actual energy needs to the energy in the previous steady state of equilibrium. This mechanism is modulated by multiple factors such as anticipated reward, habits, learning, and availability of food (Alonso-Alonso *et al.*, 2015). Subsequently, gut receptors and signals in the circulatory system send messages to the brain to initiate eating behavior.

However, there is a non-homeostatic system that interacts with the homeostatic mechanism and can override it. Non-homeostatic eating describes eating behavior that is unrelated to body physiological needs. Reward and pleasantness provided by food play a major role in this mechanism (Berthoud, 2006). One example of this mechanism is that a reward value is affected to food and can be modulated by hunger in such a way that the body changes its behavior by enhancing the motivational salience of food cues. The reward value of a food is enhanced because it has the potential to re-establish homeostasis (Cassidy & Tong, 2017).

The non-homeostatic system can override the homeostatic one in such a way that foods are similar to drugs. The increasing number of overweight people and easy access to palatable, energy-rich foods therefore suggest that overeating is due to a reward-dependent influence that cancel the homeostatic mechanism that keeps weight stable over time (Berthoud, 2006). Drugs and foods act on the same brain dopamine reward system, but in different ways. While drugs of abuse have a direct action on the brain dopamine network, the action of palatable foods on this network is more indirect since it involves more factors such as taste receptors and hormones. However, like drugs, palatable foods cause downregulation of dopamine D2 receptors and upregulation of D1 receptors in the dopaminergic system (Berthoud, 2006).

1.2 Food signal pathway

To understand eating behavior, an overview of the pathway of taste stimuli from the mouth to the brain through the reward-related region will be described. When food comes to the mouth, taste receptors in taste buds are activated. Signals from multiple bud receptors are transducted in a message. In mammals, this signal is carried by the cranial nerves VII, IX, and X, travels through the nucleus of the solitary tract, to the parabrachial nuclei of the pons, and to the medullary motor nuclei. These regions can identify the quality and the concentration of taste. The pons projects the message to the forebrain via three different pathways: in one pathway the message goes to the insula, in another one to the amygdala and in the last one to the hypothalamus. These three afferent regions belong to a distinct network receiving different types of information. The insula integrates somatosensory signals from the oral cavity and olfactory signals. It is also involved in interoception. The amygdala, part of the limbic system, processes auditory and visual cortical inputs. However, it also processes emotions like fear and pleasure and is involved in the desire to eat more. The hypothalamus, which is involved in homeostasis, processes interoceptive signals from location free of blood-brain barrier. After the message is processed in the primary gustatory cortex (insula), the signal is projected to the reward system: the ventral striatum, amygdala, hypothalamus, and orbitofrontal cortex (OFC). A reward value is then evaluated and represented in the OFC (Katz *et al*., 2011).

1.3 Food reward value

There are different types of subjective reward value: the outcome value and the goal value. The outcome value is the value affected to a product upon consumption. Typically, drinking a milkshake will have a higher outcome value than drinking water.

Cost or efforts to obtain the reward are not included in the calculation of the value. Outcome value of food is measured using subjective rating on an appropriate scale. However, since rating an outcome is not followed for the participant of a study by behavioral consequences, the accuracy of the rating may be questionable, since the participant is not properly motivated to perform the task. The goal value is related to a more abstract concept of a reward. It is typically the amount of money that the subject will be likely to pay to obtain a reward.

These types of reward value are represented in the ventral striatum and in the OFC. Brain activation for outcome value and goal value is more observed in the OFC than in the ventral striatum. Ventral striatum BOLD signal indicates prediction error processing related to reward rather than value processing (Peters & Büchel, 2010).

Furthermore, the reward value of a food can be differentiated in terms of 'liking' and 'wanting'. Liking refers to a conscious experience of pleasure when the food is consumed. However, sweet taste for example is not intrinsically pleasant. It has rather the potentiality to activate the psychological and neurobiological processes that induce subjective pleasure. This potentiality can be lost and thus the sweet taste can turn into an aversive taste. 'Wanting' refers to the concept of incentive salience. This term refers to an incentive motivation to move in order to consume food rewards. When the food becomes a stimulus for 'wanting', it retains attention, becomes attractive and the motivation to obtain it becomes enhanced (Berridge, 2009). Reward value can be usually measured on rating scale. Evaluation of 'liking' of taste are done by participants by answering questions such as 'how pleasant or unpleasant is the sweet you just ate?'. The answer would be reported on a rating scale from 1 ('awful') to 10 ('delicious'). The question asked for 'wanting' is 'how much would you like or not like to eat another piece of sweet'. The answer would be reported on a rating scale from 1 ('eating more would make me sick') to

10 (‘I really want another piece’) (Small, 2001). Such measures of liking and wanting involve a cognitive process by the participant while answering the question, which can alter the actual reward value (Berridge, 2009).

1.4 Two brain regions involved in eating behavior: The insula and the OFC

In the human brain, two regions - the OFC and the insula - play a key role in eating behavior by respectively evaluating reward value of food and sensing body signals. Therefore, the OFC and the insula are analyzed here in more detail.

The insula

The insular cortex belongs to a neural network involved in the neural control of appetite and homeostatic regulation of energy (Frank *et al*., 2013). Obese people show a greater insula activation response to gustatory stimuli compared to healthy people. Their homeostatic energy imbalance might be due to a default in integrating signals from interoceptive satiety (Avery *et al.*, 2017).

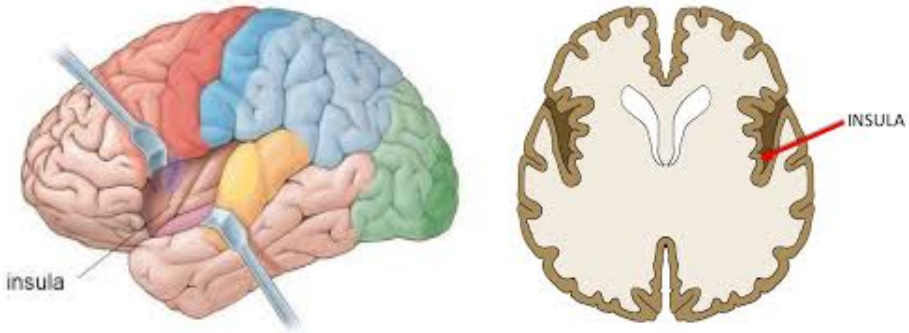


Figure 1.1: The insula
Brains are presented in lateral (left), coronal (right).

The insula is located deep within the lateral sulcus, which splits up the frontal and parietal lobes from the temporal lobe and at the bottom of the lateral cerebral fossa. The insula is composed of three principal parts with distinct cell structures or

cytoarchitectures: the anterior agranular, the mid-insula dysgranular, and the posterior granular part. The insula interacts with the anterior cingulate cortex, the dorsolateral prefrontal cortex, the amygdala, and the ventral striatum. It is involved in the integration of autonomic and visceral information with emotional, cognitive, and motivational signals. Then, the insula cortex projects food-related information to the OFC. The anterior insula and the neighboring frontal operculum make up the primary taste cortex, which processes all food-related stimuli. Moreover, the anterior insula is involved in the identity and intensity of taste. During the process of taste, brain activation in this region is correlated with the intensity and concentration of the taste flavor (Grabenhorst & Rolls, 2008). The mid-insula is involved in interoception and gustation (Namkung *et al.*, 2017). The posterior region collects afferent sensory input from the spinal cord and brainstem via the thalamus and also projections from parietal, occipital, and temporal association cortices. Therefore, this region of the insula is involved in somatosensory processes, vestibular and motor integration. However, this region is also activated for other stimulation and events such as cardiovascular arousal, the heartbeat-evoked potential, gastric distension and stimulation of the vagus nerve (Simmons *et al.*, 2017).

The OFC

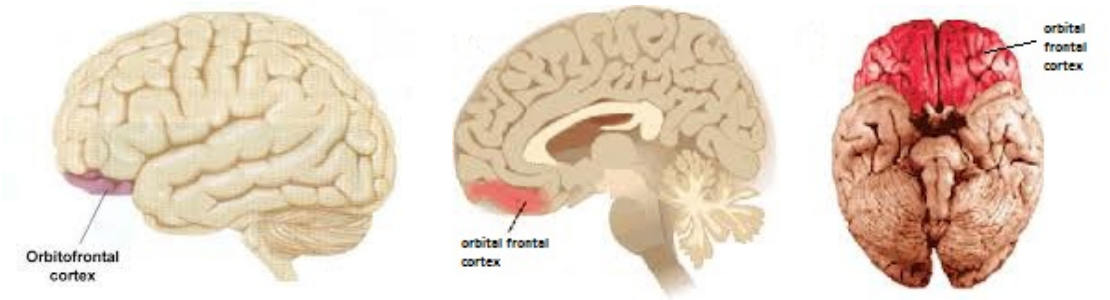


Figure 1.2: The OFC
Brains are presented in lateral (left), mid-sagittal (center) and inferior (right) section.

The OFC is located in the ventral region of the frontal lobes and is composed of a sensory network and a limbic network. The medial OFC is highly connected to the limbic part of the brain (hippocampus, perirhinal/parahippocampal cortices and amygdala) and the posterior midline structure. The lateral OFC receives mainly projections from the amygdala and sensory information such as visual, auditory, olfactory, gustatory, somatosensory and visceral signals. Processing in the dorsolateral and ventrolateral prefrontal cortex can affect the signal processing of the OFC in a top-down manner (Peters & Büchel, 2010). After integrating signals from the primary cortex and other sources of information related to reward, the OFC encodes the value of the reward. This value is retrieved in the working memory and latter forwarded to the lateral prefrontal cortex for preparing the appropriate behavior to obtain the reward. The medial prefrontal cortex calculates how the behavior toward the reward has led to a success or not. These different prefrontal parts of the brain operate in a way that behavior is in adequacy with the needs of the body (Wallis, 2007). The OFC and the anterior cingulate cortex are implicated in the reward value of the taste. Here, brain activation in this region is correlated with the pleasantness value of the taste (Grabenhorst & Rolls, 2008).

1.5 Satiation and satiety

To avoid overeating and obesity, eating behavior must be controlled. In the literature, the two terms satiation and satiety are employed interchangeably to define the process of satisfying hunger. However, there is a difference between these words. Satiation defines the point when the desire to eat after a meal is stopped. Hormones and stomach receptors send satiation signals to the brain and thus inhibit the desire to eat. Satiety is the somatic feeling of being full. This state prevents from eating for a period of time. When nutrients deplete in the body, satiety

declines. Thus the hunger triggers again. However, later stage of process of satiation can overlap on early stage of satiety (Benelam, 2009).

Physiological satiation

Two physiological mechanisms contribute to satiation: the gastric satiation and the intestinal satiation. In gastric satiation, the stomach is able to sense an increase in gastric volume and send this information to the brain via the vagus nerve. There is a suspicion that gastric distension provoked by foods or drinks is responsible for satiation. The nutrient content does not seem to play a major role. In an experiment where food ingested was drained out of the stomach via cannulas, animals did not stop eating. However, when the cannula were closed and allow food to stay in the stomach, animals came to satiation (Benelam, 2009). Nevertheless, gastric satiation signals need intestinal satiation signals to send a fullness message to the brain. Intestinal satiation signals are mediated by gut peptides secreted by cells in the small intestine and colon. In contrast to gastric satiation, the caloric content and the macronutrient composition of nutrients ingested play a role in intestinal satiation signaling. Macronutrients are detected by taste receptors in the small intestine. Intestinal secreted peptides such as cholecystokinin, glucagon-like peptide-1 (GLP-1), oxyntomodulin, peptide YY and neuropeptide W control appetite in the brain stem and hypothalamus (Benelam, 2009).

Measuring satiation in a laboratory

Satiation provided by food or drinks can be measured by quantifying the amount of nutrients eaten. Participants of an experiment are allowed to consume ad libitum the specific food tested until they reach satiation. The amount eaten is compared with a control food. Since satiation can be modulated by non-homeostatic factors as described above, satiation experiments are done in a laboratory setting where the environment is controlled (Benelam, 2009). Visual analog scales (VAS) are another

way of measuring satiation. Participants report to themselves their level of hunger, fullness and motivation to eat on particular scales. For the example of hunger, the scale is bounded at its ends by two opposite answers: 'Not at all hungry' vs. 'As hungry as I have ever felt'. Participants rate their hunger state on the scale by making a mark and the distance to the extreme end is measured. VAS can be monitored during the ingestion of the meal at defined intervals to record the satiation of the participants (Benelam, 2009) .

Hedonic satiety

Two hedonic processes are involved in satiety: sensory-specific satiety and alliesthesia. Sensory-specific satiety comes from olfactory and gustatory stimuli and defines the change in hedonic value of a specific food eaten or food flavor exposed during ingestion compared to uneaten food. Alliesthesia comes from postingestive preabsorptive stimuli and changes the hedonic value of the food ingested. Preabsorptive sensory-specific satiety and alliesthesia stimuli are produced when the food is not totally broken down by the gut and thus available in the blood. The pleasantness of the food eaten is also dependent on the internal state of the body. An individual will feel positive alliesthesia, which refers to pleasure toward food, when hungry. When just fed to satiety, the individual would feel negative alliesthesia, as a neutral or displeasure feeling (Simmons *et al.*, 2017).

Modulation of brain activity of the OFC and insula by satiety and satiation

Satiety can modulate brain activity, which can have an influence on the representation of the subjective value of the taste. There is a large consensus in the literature that satiety is associated with decreased brain activity in the OFC. Neuronal activation of the OFC of macaques has decreased to zero after having been fed to satiety. In humans fed to satiety, the same pattern is observed: a large decrease in activity of the OFC is reported. Satiety was also sensory-specific. A

large decrease in neuronal activity was observed when the subjects have been fed to satiety with a specific food but no decrease has been reported when they were exposed to another food that had not previously been eaten (Rolls, 2015). Moreover, subjects under calorie deprivation exposed to visual palatable food cues showed higher activation in the OFC (Stice *et al.*, 2013b). Moreover, pleasantness of food and brain activity in the OFC can be influenced by multiple factors other than just satiety state, such as the inter-individual difference in subjective evaluation of a specific food, the delay to receive food, the effort required to get the food, the selective attention given to pleasantness during ingestion (Rolls, 2015).

In the literature, the modulation of brain activity in the insula by satiety state is not clearly defined. Neuronal activity in the insula and frontal operculum of macaques has not been decreased to zero after macaques have been fed to satiety. In humans, no decrease in neuronal activity of insula has been reported after being fed to satiety (Rolls, 2015). However, other studies seem to contradict these results. Enhanced insula activation has been observed for participants exposed to beverage cues who have not drunk liquid for 7 hours. When subjects received milkshake, the left mid-insula BOLD signal showed a positive correlation with the numbers of hours that the subject has fasted (Stice *et al.*, 2013b). In a study using positron emission tomography (PET), hunger state of participants was associated with greater regional cerebral blood flow in insula, OFC and hypothalamus compared to sated condition (Tataranni *et al.*, 1999).

Usually, the satiation of participants is measured in a fasted condition and satiated condition using the ad libitum consumption meal and/or VAS scale. To the best of our knowledge, there are few studies evaluating the modulation of brain activity during the process of satiation. One study evaluates regional cerebral blood flow of participants who eat at regular intervals one square of chocolate. As

participants ate chocolate, PET scans were successively performed at each defined time point from a fasted state until satiation and finally beyond satiation. Until satiation, the regional cerebral blood flow of participants in the mid-insula decreased along with the reward value of chocolate (Small, 2001). However, caudolateral OFC activation increased with decreasing reward value. In another experiment on gastric satiation, water inflow in a gastric distension balloon produced significant BOLD signal change in the insula. Water inflow in lower balloon volume (250mL) was associated with an activation of sensorimotor cortices and the right insula while with larger balloon volume (500mL), water inflow was associated with an activation of the left posterior insula and left posterior amygdala (Wang *et al.*, 2008).

1.6 Aim of study

The insula and OFC play a key role in eating behavior. The insula is the primary gustatory cortex involved in taste of food while the OFC integrates information from the insula and represents the reward value of food in the brain. Satiation influences eating behavior by modulating brain activity in the OFC and insula. In this study, a new approach to the measure of satiation is adopted. Satiation is measured throughout the duration of the experiment. Using the new method, the aim of the study is to explore whether the process of satiation decreases brain activity in the OFC (neural correlates of reward value) and insula throughout the duration of the experiment. Therefore, after running a whole brain analysis, the study mainly focuses on these two regions: the insula and the OFC.

2 MATERIALS AND METHODS

2.1 Procedure

2.1.1 Participants

Fourteen healthy volunteers participated in the experiment, five of which were male and nine female. Their mean age was 30 ±8.45 years and their mean BMI was 22 ±2.79. Overweight people have a body mass index (BMI) higher than or equal to 25 and obese people a BMI higher than or equal to 30. The following inclusion criteria had to be met to be recruited: Participants must have fasted for at least four hours prior to the start of the experiment, be between eighteen and fifty-five years of age, and like sweets. The exclusion criteria were: smoking, pregnancy, weight over 180 kg, disliking of sweets or sweet tastes, vegan or regime diet, lactose intolerance, psychiatric, neurological, metabolic or eating disorders, Beck Depression Inventory score over 14, alcoholism or drug usage and psychopharmacological or metabolically active treatment. Participants were given an informed consent to sign.

2.1.2 Body measurement

The experiment started with body measurements of each participant. The blood glucose level of the participant was measured with a glucometer. A drop of blood was extracted at the tip of the finger of the participant, collected on a specific strip and engaged into the glucometer to obtain the glucose level value of the participant. This value allows control of the fasting of participant. Participant was asked to report its hunger level on a scale from 1 to 10 (10 = very hungry; 1 = not hungry). He was also asked to provide the time of his last meal knowing that he had to fast for at least 4 hours before the start of the experiment. Body height and weight were also determined for each participant. BMI and body composition were calculated via the

medical Body Composition Analyzer (mBCA) (seca mBCA 515, SECA GmbH, Hamburg, Germany).

2.1.3 Milkshake flavor

Four flavors of milkshake (chocolate, banana, strawberry, vanilla) (see recipes in Appendix 1) were tested for liking and wanting among ten randomly selected persons in the laboratory using a scale is from 1 to 10. The flavor with the highest average score of liking and wanting among the ten persons was chocolate. Consequently, all participants only tasted milkshake chocolate in the functional magnetic resonance imaging (fMRI) experiment.

2.1.4 Study protocol

Before entering the scanner, the experiment and the task were clearly explained to each participant. A 5 minutes exercise where the fMRI data and rating value were not recorded for the experimental evaluation of the results was used to train each participant. Inside the fMRI scanner, participant was given two types of rewards: either a monetary or a food reward (see figure 2.1). For the food reward, participant received drops of chocolate milkshake in two different concentrations ‘HIGH’ or ‘LOW’ (see recipe in appendix 1) and volumes (HIGH = 0.6mL; LOW = 0.2 mL). The chocolate milkshake and rinse solution were contained in 4 syringes (50mL) which were placed in a programmable automatic pump (Landgraf labor systeme HLL, SpritzenPumpe LA-100 art NR 106720100), which were in the scanner control room. The different liquids were carried independently from the syringe to the nozzle and finally to the mouth of the participant via a 3 meter transparent rubber tubing to the scanner room. Participant was asked to directly swallow the liquid in his mouth.

For the monetary reward, participant received coins corresponding to a value between 0 to 50 cents via a screen displayed to him. After each reward (food or monetary), a rinse tasteless volume of 0.5 mL was delivered to flush away the taste of the milkshake, it was also delivered after monetary rewards to keep food and money trials comparable. As control, a rinse tasteless solution volume of 0.3 mL was delivered instead of the milkshake and a 0 cents coin for monetary reward control.

2.1.5 Study design inside the fMRI scanner

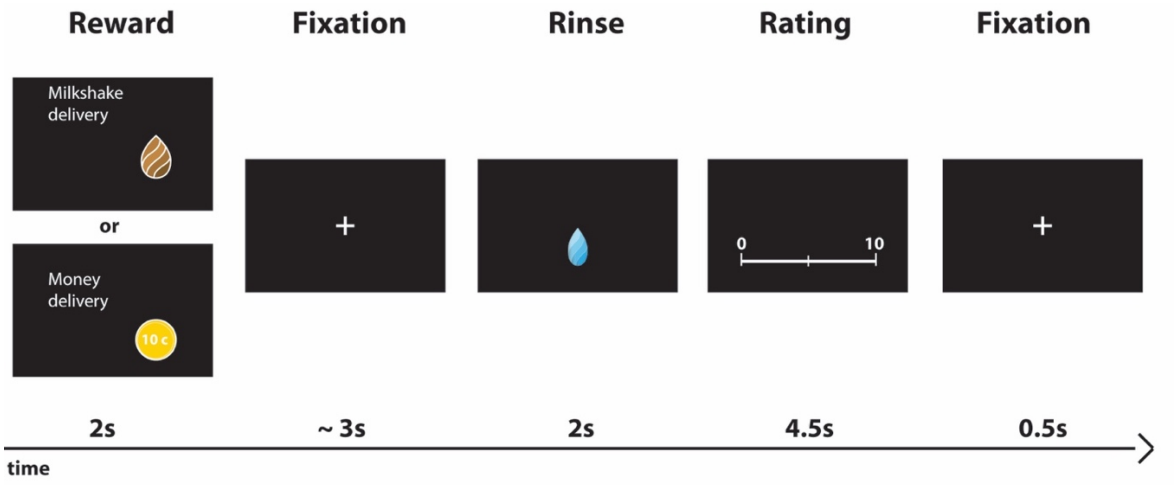


Figure 2.1: Time course of 1 trial
Reward: Event of reward received by the participant either milkshake reward or money reward. Fixation: Event which BOLD signal return to baseline. Duration of fixation was randomly jittered around 3 seconds to effectively retrieve the BOLD signal corresponding to the reward event. Rinse: the remaining milkshake in the mouth was washed away. Rating: Participant rated the reward received. Time was the duration of each event.

Ten different possible trial conditions were delivered: 1. high amount of highly concentrated milkshake; 2. low amount of highly concentrated milkshake; 3. high amount of low concentration milkshake; 4. low amount of low concentration milkshake; 5. medium amount of tasteless rinse solution (as a control for food

rewards); 6. winning €0.10; 7. winning €0.25; 8. winning €0.40; 9. winning €0.50; 10. no winning (€0).

These conditions were distributed to each participant in an event-related design. They were delivered randomly in two sets of 100 trials each with a 5 minutes break in between. During the break, syringes containing milkshake were shaken to keep the solution uniform and to avoid deposit of chocolate powder at the bottom. Each set lasted 20 minutes. After each trial, the participant has rated how much he liked the reward on a scale from 0 to 10 (0: not at all, 10: very much) (see figure 2.1).

2.1.6 Questionnaires

After the fMRI task, participants answered three questionnaires: the Barratt Impulsiveness Scale (BIS-11), Dutch Eating Behavior Questionnaire (DEBQ), and Beck's Depression Inventory (BDI).

2.2 Data analysis

For the data analysis part, the topic of this bachelor thesis focuses on the milkshake reward. The analysis of milkshake and money reward reception in the brain is part of a larger project at the doctoral level. Therefore, the analysis of the money reward rating value and its reception in the brain are beyond the scope of this thesis and will not be treated here. However, since a determined volume of tasteless rinse solution was delivered to participants after each reception of a monetary reward, this amount of liquid has to be considered in the analysis of satiation.

2.2.1 Satiation evaluation

To measure satiation throughout the duration of the experiment, an appropriate parameter representing the action of satiation, and an appropriate unit, had to be chosen. The unit volume does not reflect the different concentrations of the milkshake and the qualitative difference of the solution delivered (milkshake or rinse). Additionally, the unit kilocalorie could not be used to calculate the potential satiation effect of the rinse solution since the solution provides no energy measurable in kcal. Therefore, the units volume and kcal were discarded. The mass was used as a measurement unit for the amount of nutrients ingested by the participant. At each successive trial across 200 trials, a cumulative amount of mass of food ingested was calculated to construct the modulator of satiation. This parameter (orthogonalizedMass) was orthogonalized to trial.

Calculation of mass of nutrients ingested

From the recipe (see appendix 1), the density of the milkshake and rinse solution was calculated.

Highly concentrated milkshake:

(170g whole milk +30g cream +16g Kaba powder (chocolate)) /200mL(total volume)= 1.08g/mL

Low concentration milkshake:

(170g whole milk +30g cream +6g Kaba powder (chocolate))/200mL(total volume)= 1.06g/mL

Tasteless rinse solution

(500g distilled water+0.466g KCL+0.0525g NaHCO3)/500mL=1.00g/mL

The formula for the mass of nutrients ingested by the participant at each milkshake trial was:

Mass ingested = Volume delivered (HIGH :0.6mL or LOW:0.2 mL) * density of milkshake (HIGH = 1.08g/ml or LOW = 1.06g/ml) + (volume rinse solution * density of rinse solution)

As an example: Mass ingested = Volume delivered HIGH * density of milkshake HIGH +(volume rinse solution * density of rinse solution) = 0.6mL*1.08g/ml+(0.5mL*1g/mL) = 1.148 g

The formula for the mass ingested by the participant at each money trial was:

Volume rinse solution * density of rinse solution

2.2.2 Participant's rating

An appropriate statistical method had to be chosen to evaluate the overall ratings of the fourteen participants along trials. The underlying assumption on these data was that they were not independent. The set of ratings for each single participant was assumed to be correlated. Each participant had their own individual way of starting the ratings task and their own "regression" toward the ratings along trials. In statistical terms, the set of ratings of each individual had their own intercept and slope.

In the case of a linear regression, rating values of every participant across trials would have regressed with a constant slope among each other. Hence, a simple linear regression model with trials representing the independent variable and rating values the outcome dependent variable was discarded. A more appropriate linear mixed effect (LME) method was used to evaluate the effect of different parameters on the rating of the milkshake. This method has the advantage to account for individual differences among participants (individual slope and intercept for ratings of each participant). There is a fixed effect and random effect to construct

the LME model. The fixed effect is the explanatory variable for the outcome variable rating's value. Here, the main fixed effect one was the trial. The random effect (which is not related to mathematical randomness) is a grouping factor where data are gathered together and have their own slope and intercept. Here, the random effect was the participant. The set of rating values of each participant are grouped together. Different linear mixed effect models were tested using MATLAB R2014b.

> Formula entered in MATLAB: scaleValue = 1 + trial + (1 | participantID)

First, an LME model was performed with the subjective value as the dependent variable, the trial number as a fixed factor and the participant as a random factor. The scale value (the dependent variable): the column vector of the rating of the milkshake at each trial on a scale from 1 to 10. Trial (predictor variable) was the main fixed effect : column vector of trials number where the event was delivery of milkshake. The ParticipantID was the grouping categorical variable. Thus, a coefficient estimate was calculated to evaluate how the rating values of the milkshake regress over time (increase or decrease) for the whole group of 14 participants. The number 1 in the formula above corresponds to the calculation of the intercept by MATLAB.

> Formula entered in MATLAB: scaleValue = 1 + trial*glucoselevel + (1 | ParticipantID)

Moreover, the glucose level effect of the participant at the beginning of the experiment was tested since each participant had his own glucose level. The goal of this test was to control if there was an interaction between trial and glucose level, meaning, if trial and glucose level interacted with one another and affected the outcome variable scaleValue. The underlying question was: Did the change (regression slope) of rating values along trials depends of the glucose level of the

participant at the beginning of the task?

> Formula in matlab : scaleValue = 1 + trial + orthogonalizedMass + (1 | participantID)

The goal of the first formula (scaleValue = 1 + trial + (1 | participantID)) was only to describe the regression of the ratings along trials. Further analysis was required to test if the regression observed was associated with satiation along trials. The parameter adopted to control satiation was the cumulative mass of nutrients ingested by participants along trials. Therefore, another fixed factor (cumulative mass of nutrients ingested) was added to the first formula to test if satiation had an effect on ratings. If the first LME model is improved by this parameter, satiation has an effect on ratings.

The parameter cumulative mass of nutrients ingested progresses in time at the same rate as trials since at each trial an additional small amount of nutrients is ingested by the participant. There was an assumption that trial and cumulative mass of nutrients ingested are correlated in time. Hence, to reliably test the effect of the cumulative mass of nutrients ingested, this parameter had to be uncorrelated (orthogonalized) to trial number, otherwise its effect would be confounded with the effect of trial. orthogonalizedMass was the set of values representing the cumulative mass of nutrients ingested orthogonalized to trial.

For each LME model, an Akaike Information Criterion (AIC) was calculated. This parameter will help to compare between each different model solving for the same outcome variable scaleValue. It tests the performance of the model to fit the data without over-fitting it. The AIC with the lowest score will be the best model. The AIC score can be penalized and therefore increased if the model is too complex with too many variables and too over-fitted.

2.2.3 fMRI scan

In the fMRI scanner, a finger plethysmograph to measure heart rate and a breathing belt to record respiration were used to control physiologic functions of the participant inside the scanner.

A Magnetom Trio Prisma fit 3T whole-body scanner and a 64-channel head coil (Siemens AG Medical Solutions) were used to collect MRI data. For functional scans of the whole brain, a slice accelerated multiband echoplanar imaging sequence (Xu *et al.* 2013) was applied. The following parameters were used: repetition time (TR) = 810 ms, echo time (TE) = 30 ms; field of view (FOV) = 212x212x144 mm3; voxel size = 2x2x2 mm3; flip angle = 53°; 72 oblique transversal slices; multiband acceleration factor = 8. High-resolution T1-weighted images were acquired from a scan MRI session before the experiment and recorded in the institute's participant database. The following parameters were used : (MDEFT, TR = 1930 ms; TE = 5.80 ms; FOV = 256x256x160 mm3; voxel size = 1x1x1.25 mm3; 128 sagittal slices; or MPRAGE; TR = 2300 ms; TE = 2.32 ms; field of view = 256x256x192 mm3; voxel size = 0.9x0.9x0.9 mm3; 213 sagittal slices). The task in the scanner was run in two sessions with 1616 volumes per session. The first 10 scans as well as all scans after the end of the experiment were removed from the fMRI time series.

SPM12, version 7219, implemented in MATLAB R2014b was used for preprocessing the fMRI data and for first and second level analysis. A T1-weighted image was standardized to the Montreal Neurological Institute (MNI) reference space. After coregistration between anatomical T1 image and functional T2 image, deformation parameters were implemented to T2 images. A smoothing of 8mm full-width-half-maximum Gaussian kernel was applied to T2- functional images.

2.2.4 fMRI data 1st level analysis

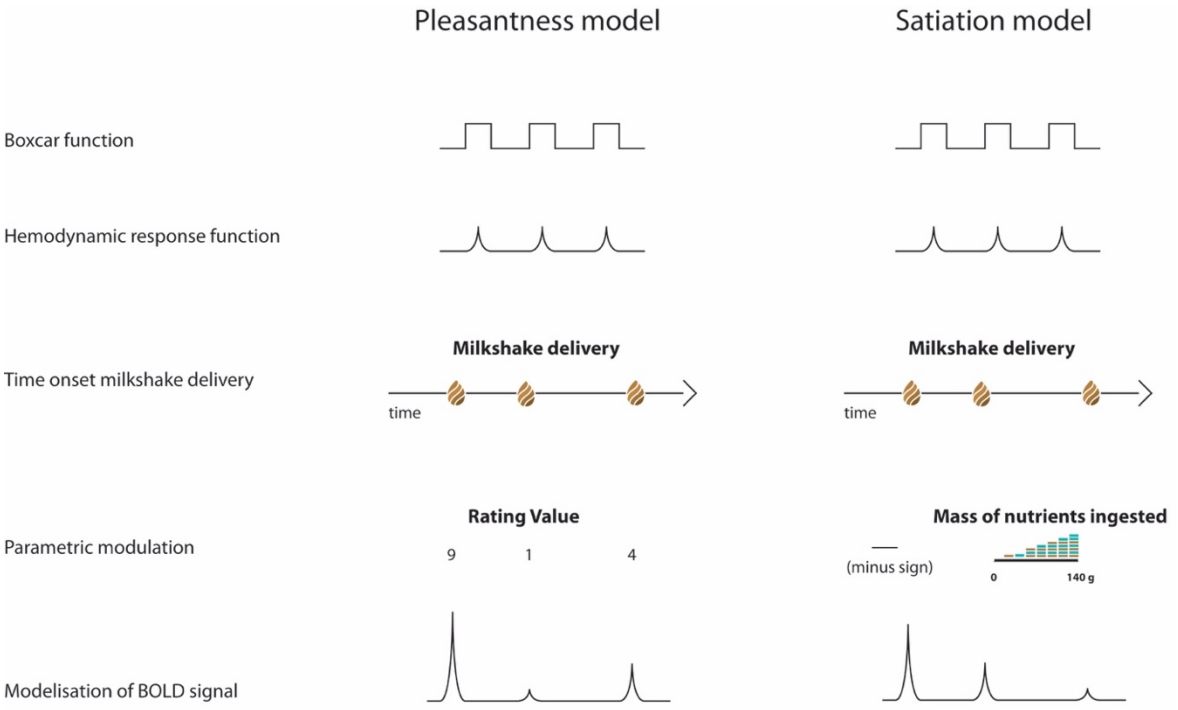


Figure 2.2: **1st level analysis, modelisation of BOLD signal for pleasantness model and satiation model at milkshake delivery time onset**
A boxcar reference model was convolved with the canonical hemodynamic response function and its time derivative. The resulting model was set at each time onset of milkshake delivery. For the pleasantness model, the model was modulated by the rating value of the milkshake given by the participant. For the satiation model, the model was modulated by the cumulative mass of nutrients ingested (along trials) affected by the minus sign. The final modelisation of the BOLD signal is tested statistically with the actual BOLD signal in the brain.

The first level model consisted of analyzing fMRI data at the single participant level. In the first level model, a boxcar reference model was convolved with the canonical hemodynamic response function and its time derivative (see figure 2.2). Different regressors were constructed in the model in order to fit the BOLD response in the brain detected by the fMRI scanner. Two models were tested for statistical inference (see table 2.1): the pleasantness model and the satiation model. The goal of these models was to statistically compare signals modelized with the actual BOLD signal in the brain. Therefore, significant activation in the brain can be localized.

In the pleasantness model, only significant activation responding to milkshake taste correlated with rating value are recorded.

In the satiation model, the model was modulated by the cumulative mass of nutrients ingested (along trials) affected by the minus sign. Therefore, only decreasing significant activation responding to milkshake taste correlated with cumulative mass of nutrients are recorded

2.2.5 fMRI data 2nd level analysis

Second level analysis consisted of analyzing fMRI data at the group level (14 participants). Two models, pleasantness and satiation, were tested for statistical inference (see table 2.1).

Table 2.1 : Regressors in 1st and 2nd level analysis

	Model	Regressors							
1st Level	Pleasantness	Shake	Money	Shake_control	Money_control	Shake_mod_value	Money_mod_value	Rinse	Rating scale
2nd Level	Pleasantness	Shake	Money	Shake_control	Money_control	Shake_mod_value	Money_mod_value		
Contrast chosen	Shake_mod_value								
1st Level	Satiation	Shake	Money	Shake_control	Money_control	Shake_mod_satiation	Money_mod_satiation	Rinse	Rating scale
2nd Level	Satiation					Shake_mod_satiation	Money_mod_satiation		
Contrast chosen	Shake_mod_satiation								

The first level consisted of analyzing fMRI data at the single participant level. 2nd level analysis consisted of analyzing fMRI data at the group level (14participants)
The regressors were constructed as follows.
Shake: Event of delivery of milkshake to participant
Money: Event of delivery of money reward (coins) to the participant
Shake_control: Event of delivery of milkshake control, which was a rinse tasteless solution
Money_control: Event of delivery of money control, which was a coin with an amount of 0 euros
Regressor correlated with modulators were constructed to fit the GLM.
Shake_mod_value: Event of milkshake was parametrically modulated with the modulator score rating value of milkshake given by the participant
Money_mod_value: Event of money was parametrically modulated with the modulator score rating value of money given by the participant
Shake_mod_satiation: Event of shake was parametrically modulated with the modulator cumulative masse of nutrients ingested by the participant. The regressor Shake with modulator satiation was tested for a negative correlation between brain activation and the cumulative masse of nutrients ingested by the participant. Meaning, while the masse of nutrients ingested by the participant increases in the body of the participant, the brain activation decreases in localized part of the brain. Therefore, the modulator cumulative masse of nutrients ingested was affected to a minus sign. The parameter Shake_mod_satiation will detect only the significant BOLD decreasing signal correlated with the cumulative masse of nutrients ingested by the participant
Money_mod_satiation: Event of shake was parametrically modulated with the modulator cumulative masse of nutrients ingested by the participant.
Rinse: Event of delivery of rinse tasteless solution
Rating Scale: Event of rating scale shown to participant on screen

For the two models, pleasantness and satiation, a threshold of p<0,001 uncorrected was chosen for statistical inference. Results of statistical inference are used with the MNI system. Coordinates are in the MNI format. The origin was at the anterior commissure. The x-axis was from the left to the right side of the brain. The y-axis was from posterior to anterior. The z-axis was from inferior to superior. To help precisely localize the region in which significant activated voxel are found, the tool MNI-Talairach converter of the Bioimage suite was used. The MNI coordinates of the significant activated voxels are entered in the tool to see in which brain region they are localized.

3 RESULTS

On average 140g of substance were ingested by each subject, only 34g on average were in fact chocolate milkshake, the rest were tasteless solution.

3.1 Subjective ratings value of milkshake

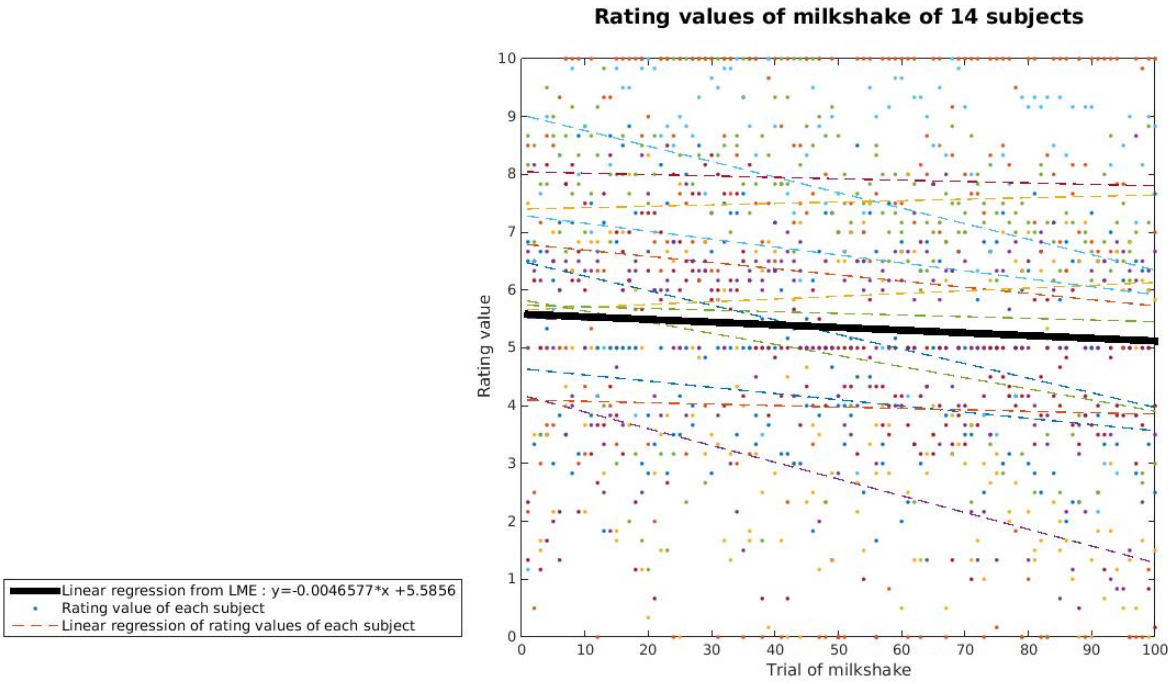


Figure 3.1: Rating values and linear regression for milkshake of 14 subjects.

On x-axis, trial of milkshake shows all event time point at which milkshake was delivered to the subject (participant) during the fMRI task. The y-axis represents the rating value given by the subject after tasting the milkshake. The black line is the linear regression resulting from the linear mixed effect model (LME) applied to results of 14 subjects (rating value in function of milkshake trial) (see Table 3.1). The formula of LME is scaleValue = 1 + trial + (1 | participantID). The dash colored line is the linear regression of each subject (participant).

Table 3.1: Coefficient estimates of linear mixed effect models

Formula of linear mixed effect models	Model fit statistics AIC	coefficient estimate	p value
scaleValue = 1 + trial + (1 | participantID)	6186.2	Trial -0.0046577 Intercept 5.5856	4.9606e-06 0
scaleValue = 1 + trial*glucoselevel + (1 | participantID)	6190	trial*glucoselevel -7.8234e-06	0.88109
scaleValue = 1 + trial + orthogonalizedMass + (1 | participantID)	6188.1	orthMass 0.016153	0.79679

Scale value consist of the outcome variable: column vector of the rating of milkshake at each trial on a scale from 1 to 10. Trial is the predictor variable: column vector of trials where the event was delivery of milkshake. ParticipantID is the categorical variable. GlucoseLevel is the glucose level of participants at the beginning of the experiment. orthogonalizedMass is the cumulative mass of nutrients ingested orthogonalized to trial.

To test the effect of the trial number on the subjective rating of the participants, an LME model was performed with the subjective value as the dependent variable, the trial number as a fixed factor and the participant as a random intercept and slope (scaleValue = 1 + trial + (1 | subjectID). In table 3.1, the results show a significant negative correlation across trials. The coefficient estimate for trial was -0.00466 ($p < 4.960e-06$) (see figure 3.1). Therefore, there is a significant decrease in the rating of the chocolate milkshake. As time passes (across 100 trials), the rating value decreased by 100*0.00466 =0.466 throughout the experiment. As an example, if the rating of the milkshake at the start of the experiment would be 10, the same milkshake at the end would be rated 10 - 0.466 = 9.534

In table 3.1, in the other LME model, no significant interaction was found between trial and glucose level, $p<0.88$.

The cumulative mass ingested set of values calculated was correlated to the set of value of trial ($r= 0.99$ $p < 0$). No significant effect was found between the mass ingested (orthogonalizedMass) and the rating value.

The main result is the significant decrease of the rating value by 0.00466 across trials, with no other correlation or interaction found to influence the rating value. The best model explaining the ratings value with the lowest AIC score was scaleValue = 1 + trial + (1 | participantID).

3.2 fMRI results

Table 3.2: Coordinates of significant voxels in relevant brain regions

Model	T statistic	P uncorrected	Coordinate of peak voxel			Brain region	
			x	y	z	Left side	Right side
pleasantness	3.49	0	-32	56	-4	OFC	
	3.93	0	20	58	-8		OFC
	3.27	0	-30	48	-18	OFC	
satiation	5.05	0	42	4	10		insula
	3.82	0	46	2	-6		insula

Coordinates are from the Montreal Neurological Institute. The origin is at the anterior commissure. The x-axis is from the left to the right side of the brain. The y-axis is from posterior to anterior. The z-axis is from inferior to superior. For the pleasantness model the T threshold is 3.198035. For the satiation model the T threshold is 3.434997.

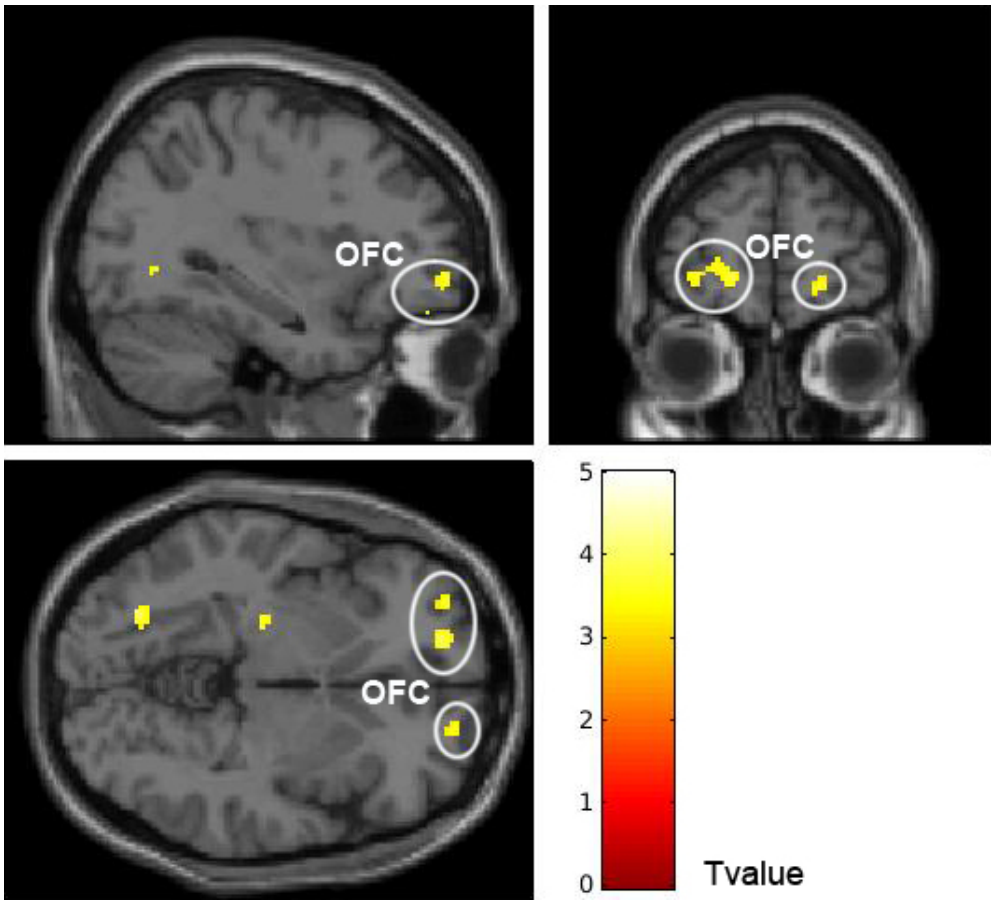


Figure 3.2 Significant activated voxels in the OFC in the pleasantness model
Brains are presented in sagittal (top-left), coronal (top-right) and horizontal section (bottom-left). 3 activated voxels are at MNI coordinates in the OFC (x=-32 y=56 z=-4), (x=20 y=58 z=-8) and (x=-30 y=48 z=-18)

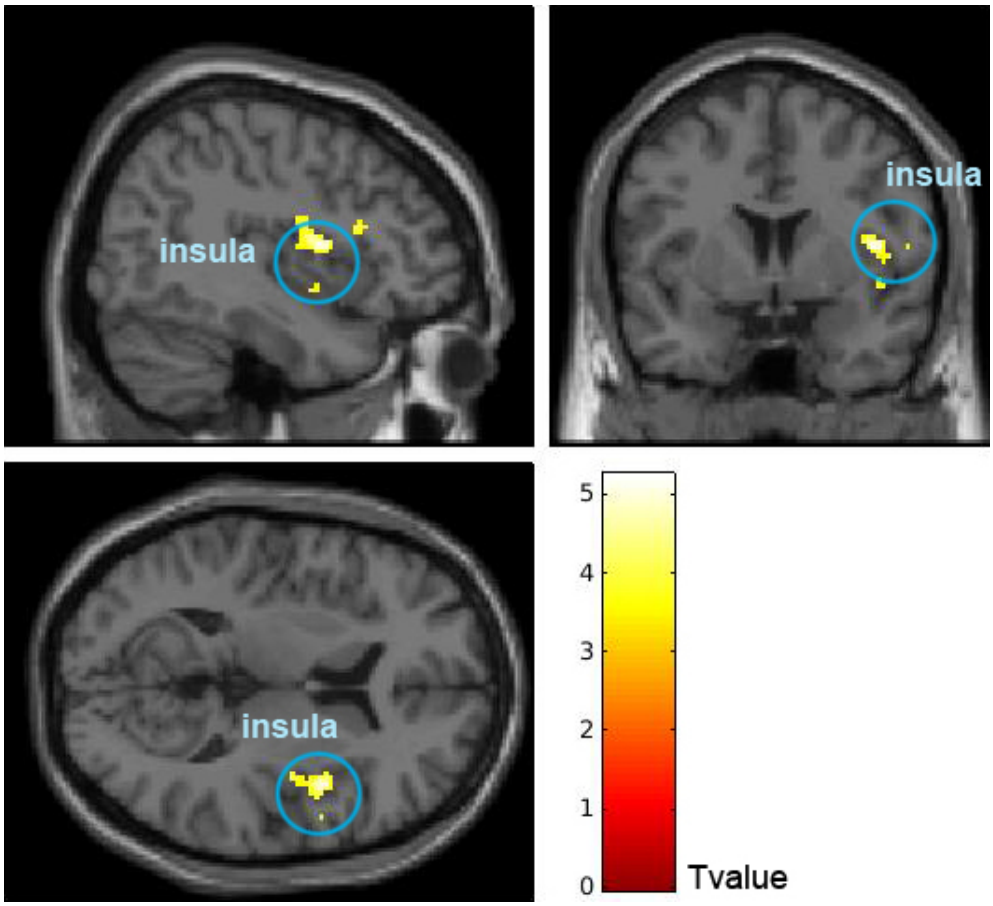


Figure 3.3 Significant activated voxels in insula in the satiation model

Brain are presented in sagittal (top-left), coronal (top-right) and horizontal section (bottom-left). 2 activated voxels are at MNI coordinates in the right insula (x=42 y=4 z=10) and (x=46 y=2 z=-6).

For the evaluation of the fMRI data, the level of significance below which the result is considered significant is p = 0.001 uncorrected. The regions of interest in the brain relevant for examining the results are the insula and the OFC. Therefore, during the whole brain analysis, the other statistically significant voxels in the region outside insula and orbital frontal cortex were ignored (see appendix 2 and 3 results of the whole brain analysis).

In the pleasantness model, where significant brain activation is tested for milkshake modulated by the rating value, three voxels are significantly activated above the T threshold = 3.198035 in the OFC region with p uncorrected < 0.001. Two voxels are in the left part of OFC and one in the right part. No significant activation is found in the insula (see table 3.2, figure 3.2).

In the satiation model, where significant decrease brain activation is tested for milkshake modulated by the mass of nutrients ingested, two significant voxels are significantly activated above the T threshold = 3.434997 in the right insula with p<0.001 uncorrected. No significant activation is found in the OFC region (see table 3.2, figure 3.2).

4 DISCUSSION

This experiment was conducted to evaluate the decreasing effect of satiation along the duration of the experiment on reward value (liking) of milkshake and its neural correlate in the OFC and the insula. However, the measure of satiation was done using a new approach. Therefore, the validity of the results is dependent of the reliability of this new method. The results show that there is not enough evidence that satiation has an effect on rating value and on OFC BOLD signal. However, satiation has a decreasing effect on insula BOLD signal. From inconclusive results, the question is to analyze wether satiation has indeed an effect but the new exploratory method measure of satiation has failed to measure this real effect. Therefore, further examination of the result is required.

4.1 Rating value analysis

On the behavioral level, the score rating of the chocolate milkshake significantly decreased by 0.46 points on a 10 points rating scale across 100 trials of milkshake. The same amount of milkshake with the same concentration rated 10 at the beginning of the experiment will then be rated 9.54 at the end. The significant decrease observed here confirms previous results found in literature. For example, in a study where participants were fed to satiety with a specific liquid food (tomato juice or chocolate milk) outside the fMRI scanner, a significant decrease was observed for the pleasantness rating of the liquid food eaten but not for the food not eaten (Kringelbach *et al.*, 2003).

However, the conclusions are contradictory since the experiment of Kringelbach showed that the significant decrease observed was associated with satiation state while in the present experiment, satiation was not associated with a

decrease in rating. The cumulative mass of nutrients ingested by the subject as a parameter of satiation has failed to show an effect of satiation on rating value.

Another parameter than satiation that might explain the significant decrease observed was analyzed. The glucose level, as measured at the start of the experiment, had no significant interaction with the trial number. There is no evidence that the glucose level at the start of the experiment has an effect on the slope of ratings value along trials.

Measure of satiation

From the contradictory conclusions mentioned above, there is a suspicion that the parameter cumulative mass of nutrients ingested in the statistical model of rating values may not be appropriate. The cumulative mass ingested set of values calculated is strongly correlated to the set of values of trial number. Orthogonalization of these two sets has not resulted in a significant correlation between milkshake rating and mass ingested in the linear mixed effect model. There was no effect for the regressor cumulative mass ingested above and beyond the trial number. Therefore, the regressor orthogonalized cumulative mass ingested has not improved the model explicating the rating value as it is confirmed by the comparison of AIC of the model with and without this added regressor. AIC of LME shake is inferior to the AIC of LME shake modulated by orthogonalized mass (see table 3.1).

It was assumed in this experiment that the cumulative mass of nutrients ingested was the main effect of satiation. Yet, satiation state can be explained by many other components such as the texture of food, the homeostatic state of the subject and the composition of the food ingested. In the evaluation of satiation, the calculation of the mass of milkshake ingested only takes into consideration the unit

mass and not the hedonic value that the subject affects to the liquid. Yet, the liking (rating value) of the milkshake measures the hedonic value of the milkshake.

All the components ingested (milk, cream, chocolate powder, rinse solution) do not have the same reward value. In 140g of substance ingested by the subject, only 34g were in fact chocolate milkshake, the rest was tasteless solution. In a recent study, increasing sugar concentration has resulted in higher BOLD signal in gustatory regions suggesting that sugar acts more effectively on reward system (Stice *et al.*, 2013a). Chocolate powder containing sugar compared to rinse solution should have a higher reward value and a differential effect on intestinal satiation. In intestinal satiation, the macronutrient composition of the meal has an effect on satiation. To show that sugar ingestion might have a satiating decreasing effect on rating value, a sugar parameter could be added. Hence, the statistical model would be scaleValue = 1 + trial +sugar + (1 | participantID). The difficulty is to find an adequate parameter which integrates the satiation effect of every component, which is ingested.

4.2 fMRI data analysis

The measure of satiation used in this experiment has failed to show the effect of satiation on the liking (rating value) of the milkshake. Now, this new method will be evaluated on the neural correlates of liking in the OFC. From the fMRI task, noticeable statistical inferences are further analysed.

OFC activation in the pleasantness model

In the second-level model of milkshake parametrically modulated by the rating value of the milkshake (pleasantness model), significant activation was found in the OFC but not in the insula. This finding confirms the results provided by the literature. In

the experiment of Kringelbach, the significant voxels activated and correlated with the pleasantness rating of the food are in the OFC (Kringelbach *et al.*, 2003).

In the pleasantness model, only BOLD signal that is correlated with rating value is significantly recorded in the OFC. A significant decrease of the rating value was reported along the experiment (see figure 3.1). Thus, there is a strong possibility that the BOLD signal activation in the OFC also decreased in intensity across trials. Therefore, the satiation model evaluates whether this BOLD signal decrease in the OFC is associated with satiation state.

OFC activation in the satiation model

In the satiation model, there were no significant activated voxels in the OFC. There is no evidence that satiation state has an effect on the OFC. This result contradicts the literature. In the paradigm of natural satiation by a meal consumed ad libitum, satiation was associated with decrease of BOLD activation in the OFC (Thomas *et al.*, 2015). Yet, in the literature it has been reported that the reward value of food is localized and correlated with BOLD signal in the OFC (Grabenhorst & Rolls, 2008). Therefore, in this experiment, there is no proof that satiation has an effect on reward value in the OFC. The decrease of the rating value and subsequently the decrease of the BOLD activation in the OFC cannot be explained by satiation state. This result contradicts the literature. In the paradigm of sensory specific satiety, a study showed that BOLD signal correlated with ratings of liquid food and significantly activated in OFC has decreased their BOLD activation intensity between fasted state and satiated state. The mean percent change in the OFC (compared to baseline) was higher in fasted state compared to a sated state (Kringelbach *et al.*, 2003).

Measure of satiation

Overall, the examination of the fMRI results suggests that the new method of measure of satiation is inappropriate to measure satiation accurately. There are

multiple causes that might explain this inappropriate method. First, the overall design of the experiment is not suitable to measure satiation correctly. There is a difference in a design of the experiment with the natural satiation experiment. In the experiment of Thomas *et al.* (2015), the state of satiation was controlled by allowing participants to consume a lunch ad libitum and controlling that they do not want to eat anymore, whereas in this experiment, the measure of satiation from fasted until fully sated condition has not been controlled along the entire process of satiation. There is no way of knowing whether the subject was totally sated at the end of the fMRI task. The attempt to measure satiation was along the process of satiation, mostly at the beginning.

Besides, there is a possibility that the attempt to measure the satiation effect on OFC was made under a wrong assumption. In the literature, it was reported that satiation has decreased BOLD activation in the OFC. However, to the best of our knowledge, the way satiation effectively acts on the OFC during the process of satiation has not been reported. The parameter cumulative mass of nutrients ingested to modulate the BOLD activation response to milkshake stimuli was constructed under the possible false assumption that BOLD activation in the OFC would decrease linearly from a fasted condition to a sated one. However, in the experiment by Thomas *et al.* (2015), OFC brain activation is evaluated and compared only in two conditions: a fasted condition and a fully sated condition at the end of the process of satiation. Therefore, it suggests that the potential effect of satiation on the OFC might appear later in the satiation process and not directly in the beginning of a food intake. Satiation might start to act on brain activity only when the cumulative mass of nutrients ingested by the participant reaches a certain threshold. In this experiment, that threshold might not have been reached.

As on the behavioral level, examination of the fMRI data, the method used did not provide evidence on an effect of satiation on neural correlates of reward value in the OFC. Other methods such as biomarkers of satiation at the peripheral physiology such as Glucagon like peptide 1 or visual analog scale have been largely used and replicated to reliably assess satiation in literature. The difficulty remains in the measure of satiation along the duration of an experiment from the beginning until its completion inside a fMRI scanner.

Insula activation in the pleasantness model

The insula plays a key role in eating behavior. It is the primary gustatory cortex and projects its information to the OFC. Therefore, a potential satiation effect on the insula can also have an impact on the reward value represented in the OFC. In the pleasantness model, non-significant brain activation has been found in the insula region, which is in accordance with the literature. As in the experiment of Grabenhorst & Rolls (2008), participants are instructed to rate the milkshake in terms of liking and not intensity. This task involves a selective attention to pleasantness of the milkshake, which evokes brain activation in the OFC but not in the insula. Selective attention to intensity would have evoked brain activation in the insula but not in the OFC (Grabenhorst & Rolls, 2008).

Insula activation in the satiation model

In the satiation model, the significant brain activation found is in the middle part of the insula (see figure 3.3). The modulator satiation (cumulative mass of nutrients ingested) is strongly correlated with trials along time. The minus sign of the modulator investigates the negative correlation between BOLD signal and trials (see table 2.1). Therefore, BOLD signal significantly recorded from the satiation model can be interpreted as a linear decrease of the BOLD signal along the duration of the trials. Thus, the linear decrease of the BOLD signal in the right insula may be the

result of the habituation or adaptation phenomenon. In one study, the habituation effect has been calculated as r = -0.59 of a percentage BOLD signal for a taste flavored no-fat control stimulus in the mid-insula. In another study designed with pseudorandom block, using a 10% sucrose solution as a taste stimulus, habituation was also observed (t=-5.20, p=0.0004) (Wagner *et al.*, 2006).

For the case of adaptation, an experiment showed a decrease of BOLD signal response to taste stimuli in dorsal mid insula following an interoception adaptation task which was attention to heartbeat sensation (Avery *et al.*, 2017). This result might be transferable to this experiment. Accordingly, the interoception adaptation event in the present experiment would be the repetitive identical interoception stimuli in the stomach induced by the ingestion of drops of milkshake at repeated intervals. This interoception adaptation event in the stomach might have induced the observed decrease in the mid insula. Therefore, the parameter cumulative mass of nutrients ingested adds a confound error, which is inappropriate to evaluate satiation effect on the brain.

4.3 Limitations

The results of this experiment have to be treated with great caution due to additional various limitations other than the satiation measure. To assess the reliability of the results, the experiment has to be replicated with the same procedure to verify whether the results are consistent.

The probability threshold ($p<0.001$ uncorrected) has not been corrected for multiple comparison on the whole brain analysis. Since the volume of the brain contains 233037 voxels, there is a chance that 233037/1000 = 233 voxels are false positive. There are multiple methods to correct for multiple comparisons by controlling the number of false positive such as family wise error rate correction and false discovery rate correction.

A chocolate flavor has been uniformly chosen for all participants. On the one hand, this method does not take into account individual preferences, which can have an effect on the liking value of the chocolate (a subject can evaluate higher his preferred flavor and thus lower chocolate flavor), on the other hand this unique choice has the advantage to control for variation in liking value that would be induced by including different flavors.

5 APPENDIX

APPENDIX 1

Recipes of liquids ingested by participants

Concentrated Milkshake for pre-test of flavor among 10 randomized persons in the laboratory :
- 170 g whole milk (109 kcal)
- 30 g cream (94 kcal)
- 16 g Kaba powder (chocolate or banana or strawberry or vanilla) / (22 kcal)
Total = 262 kcal
Volume = 200 mL

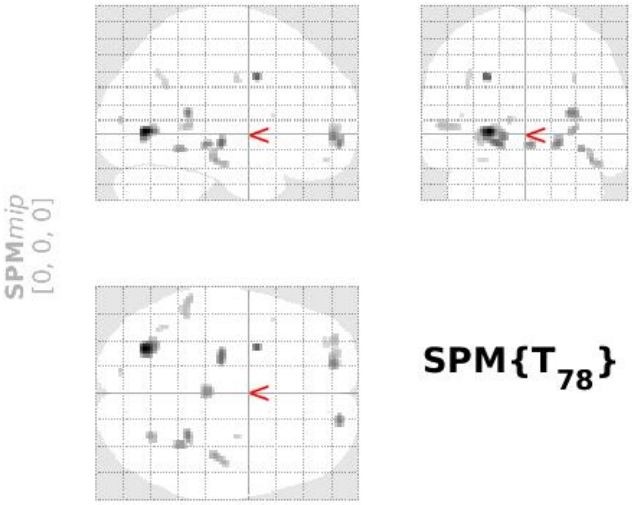
High Concentrated Milkshake for the fMRI experiment:
- 170 g whole milk (109 kcal)
- 30 g cream (94 kcal)
- 16 g Kaba powder (chocolate) / (22 kcal)
Total = 262 kcal
Volume = 200 mL

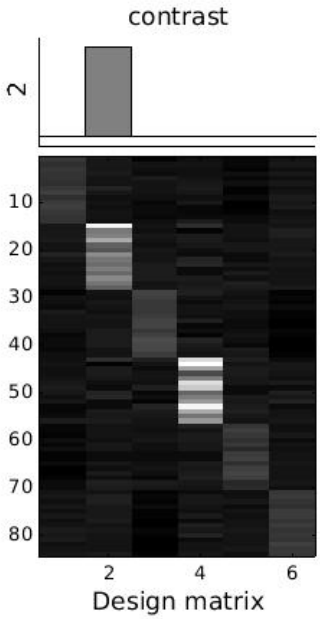
Low Concentrated Milkshake for the fMRI experiment:
- 170 g whole milk (109 kcal)
- 30 g cream (94 kcal)
- 6 g Kaba powder (chocolate) / (59 kcal)
Total = 225 kcal
Volume = 200 mL

Tasteless solution:
- 1000 ml distilled water (dH2O)
- 0.932 g KCl
- 0.105 g NaHCO3

APPENDIX 2

2nd level whole brain analysis of pleasantness model





SPMresults:.. satiety_regularallregMvalue
Height threshold T = 3.198035 {p<0.001 (unc.)}
Extent threshold k = 0 voxels

Statistics: ***p-values adjusted for search volume***

set-level		cluster-level				peak-level					mm	mm	mm
p	c	$p_{FWE\text{-}corr}$	$q_{FDR\text{-}corr}$	k_E	p_{uncorr}	$p_{FWE\text{-}corr}$	$q_{FDR\text{-}corr}$	T	$(Z_{=})$	p_{uncorr}			
0.061	16	0.499	0.546	87	0.067	0.067	0.142	4.97	4.62	0.000	-26	-70	0
		0.992	0.648	13	0.467	0.562	0.581	4.19	3.96	0.000	-26	4	34
		0.924	0.604	32	0.249	0.654	0.581	4.10	3.89	0.000	-20	-20	-8
		0.506	0.546	86	0.068	0.808	0.581	3.94	3.75	0.000	-16	54	-4
						0.995	0.783	3.49	3.35	0.000	-32	56	-4
		0.930	0.604	31	0.257	0.813	0.581	3.93	3.75	0.000	30	-42	12
		0.935	0.604	30	0.264	0.820	0.581	3.93	3.74	0.000	20	58	-8
		0.958	0.616	25	0.308	0.885	0.628	3.84	3.66	0.000	2	-30	-8
		0.992	0.648	13	0.467	0.946	0.728	3.73	3.56	0.000	36	-48	-12
		0.930	0.604	31	0.257	0.966	0.728	3.67	3.51	0.000	42	-24	-18
		0.987	0.648	16	0.417	0.972	0.728	3.65	3.49	0.000	30	-66	2
		0.900	0.604	36	0.223	0.995	0.783	3.48	3.35	0.000	-56	-42	4
		1.000	0.801	3	0.751	0.999	0.881	3.36	3.24	0.001	-12	-86	8
		0.999	0.801	4	0.707	0.999	0.881	3.35	3.23	0.001	30	-10	32
		0.993	0.648	12	0.486	1.000	0.892	3.32	3.20	0.001	-40	-58	30
		0.999	0.801	5	0.668	1.000	0.921	3.27	3.16	0.001	-30	48	-18
		1.000	0.871	1	0.871	1.000	0.975	3.21	3.10	0.001	-44	14	10

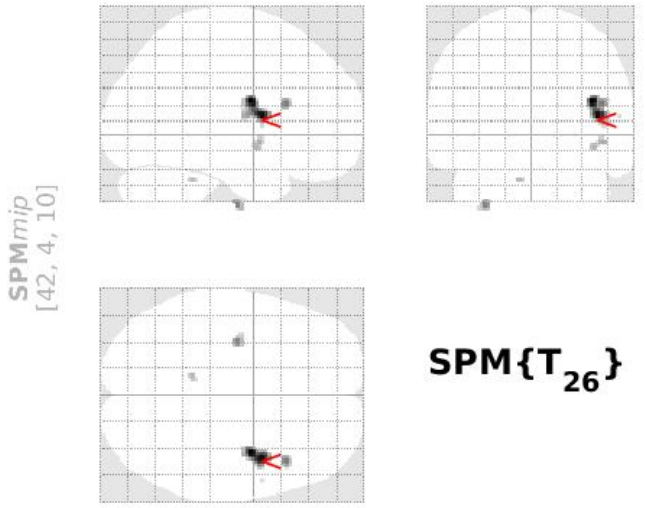
table shows 3 local maxima more than 8.0mm apart

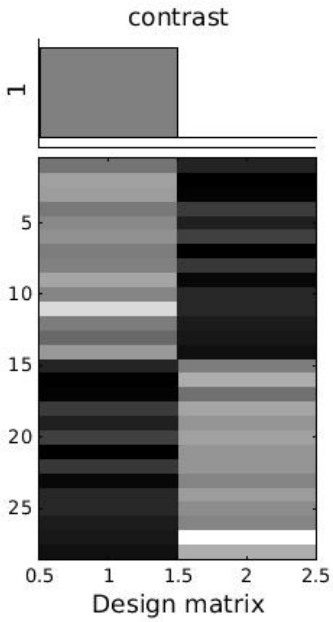
Height threshold: T = 3.20, p = 0.001 (1.000)
Extent threshold: k = 0 voxels
Expected voxels per cluster, <k> = 25.995
Expected number of clusters, <c> = 10.33
FWEp: 5.066, FDRp: Inf, FWEc: Inf, FDRc: Inf

Degrees of freedom = [1.0, 78.0]
FWHM = 12.7 12.5 12.2 mm mm mm; 6.3 6.3 6.1 {voxels}
Volume: 1864296 = 233037 voxels = 906.1 resels
Voxel size: 2.0 2.0 2.0 mm mm mm; (resel = 240.79 voxels)

APPENDIX 3

2nd level whole brain analysis of satiation model





Statistics: ***p-values adjusted for search volume***

set-level		cluster-level				peak-level					mm	mm	mm
p	c	$p_{FWE-corr}$	$q_{FDR-corr}$	k_E	p_{uncorr}	$p_{FWE-corr}$	$q_{FDR-corr}$	T	$(Z_{=})$	p_{uncorr}			
0.884	6	0.247	0.189	146	0.032	0.240	0.177	5.23	4.28	0.000	38	-4	20
						0.328	0.177	5.05	4.18	0.000	42	4	10
		0.969	0.658	21	0.387	0.889	0.531	4.20	3.64	0.000	44	20	18
		0.981	0.658	17	0.438	0.908	0.531	4.16	3.61	0.000	-32	-12	-46
		0.975	0.658	19	0.411	0.989	0.662	3.82	3.38	0.000	46	2	-6
		0.999	0.876	4	0.730	0.995	0.662	3.74	3.32	0.000	-10	-42	-30
		1.000	0.883	1	0.883	1.000	0.972	3.45	3.10	0.001	56	4	10

table shows 3 local maxima more than 8.0mm apart

Height threshold: T = 3.43, p = 0.001 (1.000)
Extent threshold: k = 0 voxels
Expected voxels per cluster, <k> = 30.186
Expected number of clusters, <c> = 8.99
FWEp: 6.029, FDRp: Inf, FWEc: Inf, FDRc: Inf

Degrees of freedom = [1.0, 26.0]
FWHM = 14.2 14.1 13.6 mm mm mm; 7.1 7.1 6.8 {voxels}
Volume: 1864296 = 233037 voxels = 642.2 resels
Voxel size: 2.0 2.0 2.0 mm mm mm; (resel = 339.75 voxels)

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