Functional MRI of food-induced brain responses

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Colophon

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Cover: A fascinating winding road whose ultimate end is unclear. Picture taken at the border of Nepal and Tibet in October 2005 by the author. Cover design by the author using Adobe® Indesign® CS2 Version 4.0.2.

Functional MRI of food-induced brain responses

Functionele MRI van voedsel-geïnduceerde responsen van de hersenen

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. W.H. Gispen, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 7 september 2006 des middags te 2.30 uur

door

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"You ought not to attempt to cure the eyes without the head, or the head without the body, so neither ought you to attempt to cure the body without the soul. This is the reason why the cure of many diseases is unknown to the physicians of Hellas, because they are ignorant of the whole which ought to be studied also, for the part can never be well unless the whole is well."

Socrates (470 – 399 B.C.) in Plato's *Charmides*

Contents

Preface

*"Je kan er wel naar streven de kapitein op je eigen
schip te zijn, maar zo nu en dan neemt het leven het
roer in handen. Gelukkig maar."*

As part of the TNO Board of Management year 2000 tender program "Biomerkers voor de verzadigende werking van (componenten van) voedingsmiddelen: een zaak van groot gewicht" TNO Nutrition and Food Research, located in Zeist, The Netherlands, started a project entitled "Biomarkers of satiety". The main objective of this project was "the development of the methodology for the measurement of biomarkers, that provide knowledge and can be used as valid and recognised standards, with respect to the effects of food ingredients on satiation and satiety." It consisted of two PhD-projects whose first goal was to find objective measures for the subjective feeling of satiety. Next, these measures were to be used for the identification and testing of the satiating effect of specific food components. One PhD-student was to look at "peripheral measures" of satiety like body temperature and blood hormones, and one at "central measures" like brain activation and cerebral blood flow. Ideally, central measures would be related to peripheral measures. These projects were a collaboration between TNO Nutrition and Food Research, the Nutrition Department of Wageningen University and the Department of Radiology and the Image Sciences Institute of the University Medical Center Utrecht. In 2001, Wendy Blom started working on the peripheral part and someone else on the central part. After about half a year though, my predecessor decided to quit and do other things. As a result, I got a call from my former supervisor, Marie-José Duchateau, in the beginning of March 2002. She had just visited a former PhD-student of hers, who was working as a postdoc in London. There, she had heard from his girlfriend, who had a friend that did a PhD-project at the Image Sciences Institute, that there was a PhD-position available there. I quickly looked up on the internet what on earth "fMRI" was, called professor Viergever and, eventually, started on the project in April 2002.

Chapter 1

Introduction

The research described in this thesis started out as a quest for 'central biomarkers of satiety'. To introduce this subject some definitions and background information are provided. In particular, the regulation of food intake is addressed. In addition, techniques that can be used to measure brain responses to food, in particular functional MRI, are introduced. Finally, an outline of the following chapters of this thesis is given.

• What is a central biomarker of satiety?

A central biomarker is a biomarker in the brain (part of the central nervous system). The term 'biomarker', which is short for 'biological marker', refers to "a distinctive biological or biologically derived indicator (as a biochemical metabolite in the body) of a process, event, or condition (as aging or exposure to a toxic substance)" (Source: Merriam-Webster's Medical Dictionary). Another definition of 'biomarker' is: "A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Frank and Hargreaves, 2003). According to the definition of De Graaf et al. (2004), a central biomarker of satiety is a physiological measure in the brain that relates to subjectively rated appetite, actual food intake, or both.

• Satiety, hunger and the regulation of food intake

Terminology

According to two dictionaries satiety is "the condition of being full or gratified beyond the point of satisfaction" or "the quality or state of being fed or gratified to or beyond capacity" (Sources: The American Heritage
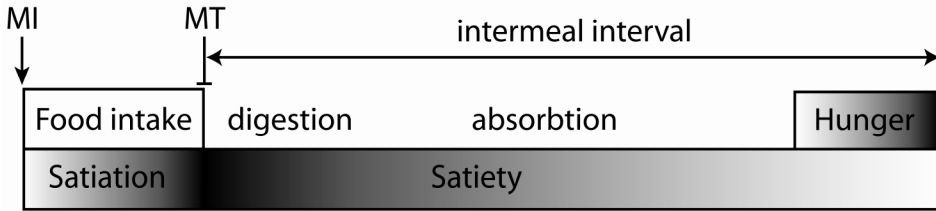
Figure 1.1. Schematic overview of one food intake cycle with related terminology. Changes in the degree of satiation, satiety and hunger are indicated by the gray level: black represents a high degree, white a low degree. MI, meal initiation; MT, meal termination.

Dictionary of the English Language, Fourth Edition and Merriam-Webster's Medical Dictionary).

Often, the term 'satiation' is used as a synonym for satiety. Here, however, the term 'satiety' refers to the state of being satiated that follows the process of satiation, as defined by John Blundell: "Satiety is associated with meal termination and the subsequent intermeal interval and refers to the inhibition of hunger and eating brought about by food consumption. It is preceded by satiation, the process which brings a period of eating (a meal) to an end" (Blundell, 1991; Blundell et al., 1996). The counterpart of satiety is hunger: the drive to find food and eat it. **Figure 1.1** shows a schematic overview of the above-mentioned terms.

Satiety can be subdivided into metabolic satiety and sensory satiety. Metabolic satiety relates to the physiological need for food (fuel, nutrients). Sensory satiety relates to the pleasantness of a particular food and is also termed selective satiety (Kringelbach, 2005) or sensory-specific satiety (Rolls et al., 1981). Metabolic and sensory satiety both play a role in the process of satiation: as more food is ingested, the feeling of fullness becomes stronger and the motivation to eat decreases (Rolls et al., 2000; Rolls and Roe, 2002). However, when a certain food is eaten, its pleasantness and the motivation to eat more of it decrease gradually, even though the stomach is not yet full (Rolls et al., 1983). In this case, one is still motivated to consume other foods, particularly those with different sensory characteristics; *i.e.*, one is not satiated per se, but satiated to the sensory properties of the specific food that was consumed.

A similar way of looking at satiety and hunger has been put forward by Berridge (1996) who introduced the terms 'wanting' and 'liking'. Wanting refers to the motivation to eat a food (appetite) and liking to the perceived pleasantness of that food (its palatability or hedonic value).

Obviously, the sensations of hunger and satiety are crucial components in the regulation of food intake.

Why is food intake regulated?

Our body cells need energy and nutrients for maintenance and growth and, ultimately, for survival. We eat to live, although nowadays in the western world many live to eat. The regulation of food intake on the short-term affects our body's energy balance and thereby our body weight on the longer term. Eating occurs in episodes (meals), which leaves time for other activities such as mating and reproduction. To be able to bridge the time between meals and to cope with food shortage, the body maintains energy stores in the form of glycogen in the liver and muscles and in the form of fat in adipose tissue. Ideally, in adults, energy intake equals energy expenditure and body weight does not change. However, the regulation of food intake is 'tuned' differently in different people; some maintain a constant weight very easily whereas others gain weight easily. Generally, there is a bias towards the defence of body weight: loosing weight is much harder than gaining it. In uncertain times it was crucial for survival to be able to store energy for periods of food deprivation. However, in modern western society with its easy availability of palatable and energy-rich foods this trait has become detrimental because it promotes obesity. This idea of a genotype that has harmful consequences in a food-rich environment has been termed the 'thrifty' gene hypothesis (Neel, 1962).

How is food intake regulated?

The regulation of food intake is the result of a complex feedback system that involves multiple hunger and satiety signals from peripheral organs, such as the gastrointestinal tract and adipose tissue, and from the brain. Many reviews have been written about this topic (Berthoud, 2002; Blundell and Gillett, 2001; Grill and Kaplan, 2002; Hellstrom et al., 2004; Park and Bloom, 2005; Schwartz et al., 2000). A small but important part of the brain involved in the regulation of food intake is the hypothalamus, which integrates many neuronal and hormonal signals related to satiety and energy stores (Hillebrand et al., 2002; Kalra et al., 1999; Williams et al., 2001). For a comprehensive review of the anatomy and functions of the hypothalamus the reader is referred to Saper (2004).

Through the years, several hypotheses have been put forward as to what governs the regulation of food intake. The general idea in all of these hypotheses is that there must be some internal 'quantity', which reflects the body's energy balance and which is regulated through a feedback system (after Hervey (1969b)).

In 1948, Brobeck proposed his thermostatic theory in a paper entitled "Food intake as a mechanism of temperature regulation" (Brobeck, 1948). He argued that thermoregulation is a major physiological concern of humans and that food provides a major contribution to maintaining body heat. Therefore, "at a high temperature where loss of heat is difficult, food intake should be low, lest by eating and assimilating food the body acquire more heat than it can dispose of". This temperature-dependent variation in energy needs should, in principle, be reflected in appetite. Brobeck claimed that "everyone knows ... that appetite fails in hot weather". Thus, the important factor in the regulation of food intake is not a food's energy value, but rather the amount of extra heat released in its assimilation (Strominger and Brobeck, 1953). Energy that becomes stored as fat does not control feeding; it is the direct heating effect of food intake that is monitored and that provides a regulatory mechanism. Interestingly, the hypothalamus is, among others, involved in thermoregulation (Cooper, 2002; Mack, 2004) as well as in the regulation of food intake.

Mayer (1952; 1953) put forward the glucostatic hypothesis for short-term regulation of food intake, in which the controlled variable was the difference between arterial and venous plasma concentrations of glucose. He reasoned that "… the regulation of food intake proceeds by relatively infrequent partaking of food (meals). It appears improbable that hypothalamic centers are sensitive to decrease of the body content of fat or protein – during the short interval between meals, this decrease is proportionally small. On the other hand, the body stores of carbohydrate are limited. The postprandial liver glycogen content is approximately 75 g – only 300 calories' worth. In the postabsorbtive period, in spite of gluconeogenesis (the synthesizing of glycogen from body proteins), glycogen stores become rapidly depleted. This synthesis of glycogen from proteins and the shifting of metabolic oxidation in non-nervous tissues from glucose to fat … tend to minimize the drop in blood glucose resulting from depletion of liver glycogen stores. Thus, minimum levels necessary for survival of the central nervous system are maintained. Only partaking of food, however, can restore full homeostasis of the central nervous system." (Mayer, 1953). He suggested that the hypothalamus contains glucoreceptors, which serve to monitor the availability of glucose for the brain. Later, the existence of glucose-sensitive neurons has indeed been established (Levin, 2001; Oomura, 1980).

Around the same time, Kennedy (1953) proposed the existence of a centrally acting negative feedback signal, produced by adipocytes (fat cells), which regulated body fat stores via alterations in food intake and energy expenditure. Thus, in Kennedy's socalled lipostatic theory the regulated

quantity is the amount of body fat. Hervey (1969a) agreed with this view and argued that "disturbances of body temperature or blood glucose are short-lived and rapidly corrected by specific regulatory mechanisms…" and that "…there are no evident means by which past disturbances can be integrated over an indefinite period." (Hervey, 1969b). In 1994, a 'fat signal' produced by adipocytes was actually uncovered and named 'leptin' after the Greek *leptos*, meaning 'thin' (Zhang et al., 1994). This has boosted research on this topic. Jequier and Tappy (1999) nicely describe a feedback loop involving leptin production in adipose tissue and detection of leptin concentrations in the blood by the hypothalamus, which in turn affects food intake behavior. However, the importance of leptin in the regulation of body weight in humans is still far from being understood. Leptin is not an acute satiety factor, since its plasma concentration does not change after eating. Plasma concentrations of leptin appear to represent a long-term integrative signal of the size of the adipose tissue mass; this signal is proportional to the body's energy stores and can be received and integrated at regulatory sites in the brain, such as the hypothalamus (after Jequier and Tappy (1999)).

In the lipostatic theory the regulation of body weight comes down to the regulation of adipose tissue weight, as this is the most variable constituent of body weight. However, Cabanac (1971) hypothesized, less specifically, that body weight is the regulated variable. He called this the 'ponderostat'. Recently, he proposed that the ponderostat works as a regulation of blood glucocorticoids with a hypothalamic set-point related to the Corticotropin Releasing Hormone (CRH) concentration (Cabanac, 2001).

Very recently, a 'glucoadipostatic' hypothesis was put forward by Mobbs et al. (2005). These authors suggest reformulation of the classic glucostatic hypothesis without reference to satiety (*i.e.*, short-term effects on food intake). Instead, they argue that, like leptin signaling, glucose signaling regulates long-term energy balance, in part by regulating metabolic rate but also by chronically regulating food intake. Energy balance is regulated by glucose signals that converge with leptin and insulin signals on neuroendocrine 'nutrient sensors'.

What can be gathered from this historical overview is that, now the details of the regulation of food intake on the short term as well as on the long term are step by step being filled in, the early views of researchers on food intake regulation all play a part of some significance. What is emerging is a very complex system of multiple neural and hormonal signals that interact and are being integrated.

Food and reward

Animal trainers, researchers and parents alike have always exploited the fact that particular foods are very rewarding. And of course, everybody knows from his or her own experience that foods can be quite rewarding. During eating there is sensory pleasure and after that the state, and comforting feeling, of satiety. This makes sense from an evolutionary perspective, since food is such a basic need, vital for survival. Foods rich in sugar and fat are particularly attractive, because they are energy-rich (Nesse and Berridge, 1997).

Cabanac (1971) noted that a stimulus is perceived as either pleasant or unpleasant, depending on a subject's internal state. This, he termed *alliesthesia*. Based on experiments with several types of stimuli, including taste and odor, he argued that a pleasant stimulus is experienced as such because it indicates something useful. He put forward that "food intake will be limited by the displeasure caused by peripheral stimuli". Cabanac's observations go well together with Berridge's (1996) terminology of 'wanting' (motivation, which reflects an internal state) and 'liking' (sensory hedonics; the pleasure experienced). In principle, I agree with Cabanac that something pleasurable is something useful. However, nowadays there is such a wide and easy availability of 'useful' energy-rich foods that many overeat. In the case of occasional overeating, the internal motivation to eat (wanting) is low, but this is overruled by sensory pleasure. In the case of frequent overeating, there appears to be a strong motivation to eat, in spite of the absence of a normal physiological need (*i.e.*, a craving). In this respect it is interesting that there is growing evidence that craving for foods and drugs have the same underlying processes (Pelchat, 2002). At the same time, evidence is mounting that the overconsumption of palatable food (*i.e.*, food rich in fat and sugar) has profound neuro-endocrine effects, that lead to activation of the reward system and increased food intake (Erlanson-Albertsson, 2005; Levine et al., 2003).

• The hypothalamus and the regulation of food intake

The hypothalamus is located in the diencephalon, the posterior part of the forebrain which connects the cerebral hemispheres with the mesencephalon (midbrain). It lies below the thalamus on either side of the third ventricle, at the base of the brain. The hypothalamus is small, about the size of a cherry, and comprises about $1/300^{th}$ of the brain's total weight. The position of the hypothalamus is shown in **Figures 1.2a** and **1.2b**. The hypothalamus is part of the limbic system which is a set of interconnected deep brain structures

involved in emotional responses, motivation and various autonomic functions. The main function of the hypothalamus is homeostasis or maintaining the body's continuous steady level in all facets. As such it coordinates many seasonal and circadian rhythms, complex patterns of neuroendocrine outputs, complex homeostatic mechanisms, and many important stereotyped behaviors. Among others, it is involved in blood pressure, heart rate, body temperature, sex drive, thirst and appetite. Therefore, the hypothalamus must respond to many different signals, some of which are generated externally and some internally. It is richly connected with many parts of the central nervous system, including the brainstem, the limbic forebrain (particularly the amygdala and the olfactory bulb), and the cerebral cortex. It is comprised of many histologically distinct nuclei (groups of neurons), involved in processing and integrating myriad neural and hormonal inputs and outputs. A detailed overview of these inputs and outputs relevant to food intake and body weight regulation is given by Berthoud (2002). Neural hypothalamic inputs (afferents) include olfactory,
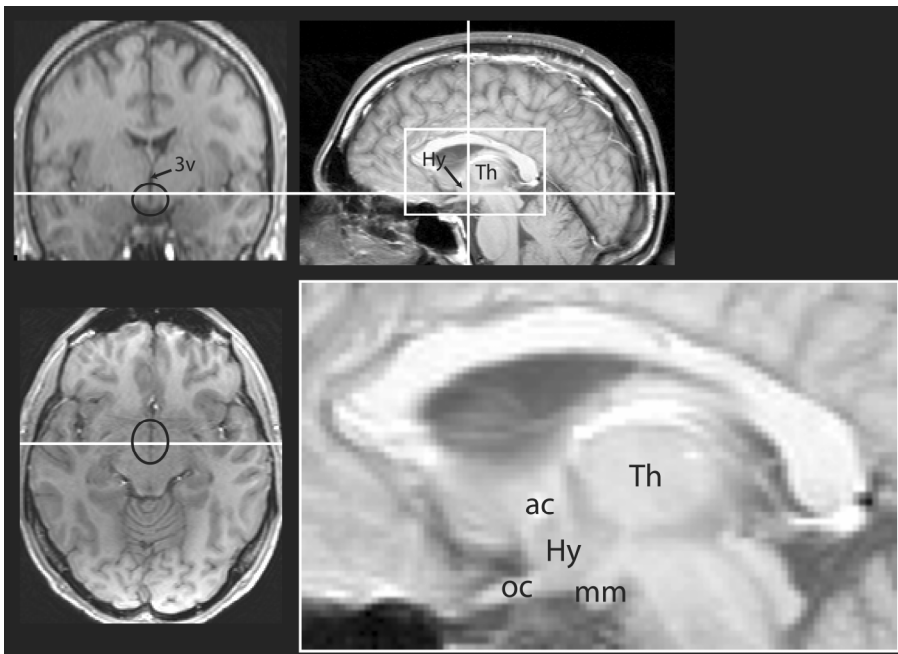
Figure 1.2. Anatomical MRI-images showing the position of the hypothalamus (Hy) in the brain. The area in the white rectangle is shown enlarged in the lower right corner. The white lines indicate the position of the axial (lower left) and coronal (top left) sections. The black ellipses indicate the approximate position of the hypothalamus. Top left: the hypothalamus lies around the floor of the third ventricle (3v). Lower right: The position of the hypothalamus in the midsagittal plane is given by some anatomical landmarks: it is below the thalamus (Th) and cornered by the anterior commissure (ac), optic chiasm (oc) and mamillary body (mm).

visceral, limbic and cortical inputs. Visceral inputs come mainly from the vagus nerve which receives information from mechano- and chemoreceptors in the gastro-intestinal tract. The vagus nerve projects to the hypothalamus, the amygdala and the thalamus via the brainstem (the nucleus of the solitary tract (NTS) and the parabrachial nuclei). Limbic inputs include those from the amygdala and the hippocampus. Cortical inputs come from the insular cortex (anterior insula), the prefrontal cortex and the primary olfactory cortex. Neural hypothalamic outputs (efferents) include those to brainstem sites (NTS and parabrachial nuclei), the amygdala, sensory areas of the thalamus, the putamen and the cerebral cortex (among others the anterior insula).

Already in the 1940s, hypothalamic lesion experiments were done in rats (Hetherington and Ranson, 1940; Hetherington and Ranson, 1942). Eventually, this led to the dual center hypothesis (Anand and Brobeck, 1951; Stellar, 1954) which states that the lateral hypothalamus (LH, 'hunger center') is responsible for hunger and subsequent feeding, while the ventromedial hypothalamus (VMH, 'satiety center') is responsible for satiation and satiety. Stimulation of the LH increases food intake, while its destruction attenuates feeding and causes weight loss. Stimulation of the VMH inhibits food intake, whereas a lesion in this region causes overeating and weight gain. Over the years, the dual center hypothesis has been abandoned as our knowledge of the neuroendocrinology of the regulation of food intake has grown. The current view is that discrete neuronal populations in the hypothalamus express specific neurotransmitters which mediate particular effects on food intake and/or energy expenditure and which are regulated by specific signals of nutritional state (Williams et al., 2001). Apart from the VMH and the LH, several other hypothalamic sites including the arcuate nucleus, paraventricular nucleus and dorsomedial nucleus have been implicated in food intake and body weight regulation. Neurons in these hypothalamic areas produce many orexigenic (appetite-stimulating) and anorectic (appetite-reducing) peptides. A non-exhaustive list of such peptides is given in **Table 1.1**. These peptides have been described in detail elsewhere (Hillebrand et al., 2002; Kalra et al., 1999; Schwartz et al., 2000; Williams et al., 2001). In addition, other signals such as serotonin, sex steroids, glucocorticoids and catecholamines (*e.g.*, adrenaline, noradrenaline and dopamine) also play significant roles in energy homeostasis. The hypothalamus is a primary site of integration of all of these signals.

Table 1.1
Hypothalamic peptides involved in energy homeostasis.

Orexigenic: stimulate food intake	Anorectic: inhibit food intake
Agouti-related protein (AgRP)	α-MSH (product of POMC)
Galanin	Bombesin
Ghrelin	Cocaine- and amphetamine-related transcript
Melanin-concentrating hormone (MCH)	Cholecystokinin (CCK)
Neuro-peptide Y (NPY)	Corticotropin releasing hormone (CRH)
Orexins / Hypocretins	Enterostatin
	Glucagon-like peptide-1 (GLP-1)
	Insulin
	Leptin
	Oxytocin
	Peptide YY$_{(3-36)}$ (PYY$_{(3-36)}$)
	Proopiomelanocortin (POMC)

• Why would one want to find biomarkers of satiety?

The epidemic of obesity is spreading the world (McLellan, 2002; Popkin and Doak, 1998). Detailed knowledge of the regulation of food intake can help in preventing and curing obesity. Biomarkers of satiety could be used to measure the satiating efficiency of foods. As such, they could serve as a basis for type A claims with respect to functional foods, *i.e.*, claims that a certain food or food ingredient enhances satiety, reduces appetite, or does both (Diplock et al., 1999). At the same time, knowledge of and insight into biomarkers of satiety will help to understand the physiological mechanisms behind the regulation of food intake and energy balance. Of course, this also works the other way around: an understanding of the physiology of food intake may yield biomarkers of satiety.

One area of interest is that of peripheral biomarkers, mainly the concentration of hormones in the blood. These could constitute objective measures of aspects of a person's degree of satiety. However, eventually, what matters is the decision of a person to start or stop eating. This decision comes about through a combination of (objective) physiological aspects of the degree of satiety with subjective feelings and beliefs about the act of eating a particular food at a particular time in a particular place. All these aspects underlying the decision to eat are integrated in the brain. Therefore, another area of interest is that of central biomarkers, *i.e.*, biomarkers in the

brain. Functional neuroimaging-techniques could provide objective measures of brain correlates of the subjective feeling of satiety.

• How could one measure (biomarkers of) satiety?

An overview of measures relating to food intake behavior can be found in Hill et al. (1995). Measures of satiety and possible biomarkers of satiety have been extensively reviewed in De Graaf et al. (2004).

Subjective measures

The common way of assessing feelings of satiety and hunger is by means of subjective ratings. These are usually obtained in the form of Visual Analog Scales (VAS) (Rogers and Blundell, 1979; Stubbs et al., 2000) or category scales. With VAS, subjects are simply asked to rate a particular sensation on an analog scale with two anchors, *e.g.* extremely hungry and not hungry at all. When used appropriately, subjective ratings have been shown to be reproducible and predictive of food intake (De Graaf, 1993; Flint et al., 2000; Stubbs et al., 2000). However, it should be realized that 'appetite' may not always be accessible to introspection (Berridge, 1996). In addition, people do not always eat when they are hungry, and they do not always refrain from eating when they are satiated (Mattes, 1990).

Objective measures

Obvious objective measures relating to food intake behavior are actual food intake (amount or calories consumed) and the intermeal interval, *i.e.*, the time between two eating episodes. These, however, are post hoc measures. Other objective measures can be classified as either measures of the peripheral physiology or measures of the central nervous system. The latter are the subject of this thesis.

Peripheral measures are stomach distention, body temperature and hormone concentrations in the blood (De Graaf et al., 2004). Interesting hormones include the gut peptides cholecystokinin, glucagon-like peptide 1, peptide YY_{3-36} and the gastric peptide ghrelin, which is involved in meal initiation, as described in the thesis of Blom (2005).

Central (brain) measures are measures that relate to brain activity in a particular part of the brain, *e.g.* the hypothalamus or part of the frontal cortex. Using electrodes, one can measure the spiking activity of single neurons or groups of neurons. These rather detailed measurements on neurons are done in laboratory animals. For humans, several neuroimaging techniques are employed to assess measures that relate to neuronal activity

in a certain brain region. Most commonly used are positron-emission tomography (PET) and functional magnetic resonance imaging (fMRI). The latter is used in this thesis. These neuroimaging techniques are introduced below.

• Neuroimaging techniques that could provide biomarkers of satiety

Positron-emission tomography (PET)

Technique

PET employs positron-emitting radioisotopes, which are incorporated in molecules used in the body's chemical processes. In ^{15}O PET, the radioisotope ^{15}O incorporated in water molecules is administered intravenously and distributed to tissues throughout the body. Because it readily crosses the blood-brain barrier, it can be used to measure cerebral blood flow (CBF). At the site of a brain activation, blood flow increases, which leads to greater uptake of the ^{15}O water tracer into brain tissue, which in turn results in an increase in the number of gamma rays detected at that site. Thus, with PET, the local hemodynamic changes accompanying neuronal activity can be measured (Attwell and Iadecola, 2002). Subtraction of an experimental image from a baseline image yields an image of the changes in regional CBF (rCBF). The spatial resolution of these images is 5 mm at best. Because the half-life of ^{15}O is ~2 min, it is in practice possible to acquire a PET image every 8–10 min. The main drawbacks of PET are that it uses radio-active tracers, which are costly and not easily produced, and that it has a low temporal resolution.

Application of PET in food intake research

PET has been used in a number of studies in which the state of extreme hunger (36 h fast) was compared with that of satiety. The first study of this kind was that of Tataranni et al. (1999). They reported satiation-induced differences in rCBF in healthy normal-weight men in the hypothalamus, the insular cortex (gustatory cortex) and the prefrontal cortex (including the orbito-frontal cortex). Moreover, changes in rCBF in the insular and orbitofrontal cortices correlated negatively with changes in the plasma insulin concentration. Changes in plasma free fatty acid (FFA) concentration correlated negatively with changes in rCBF in the anterior cingulate and positively with changes in rCBF in the dorsolateral prefrontal cortex. Subsequent studies showed differences in the effect of satiation

between lean and obese men (Gautier et al., 2000), between lean and obese women (Gautier et al., 2001) and between men and women (Del Parigi et al., 2002). In response to satiety, both obese men and women showed greater increases in rCBF in the prefrontal cortex and greater decreases in rCBF in the orbitofrontal and temporal cortices than their lean counterparts (Gautier et al., 2000; Gautier et al., 2001). Obese men showed a strong negative correlation between changes in FFAs and changes in rCBF in a region in the prefrontal cortex, whereas lean men showed a positive correlation (Gautier et al., 2000). Obese and lean women also showed different correlations between changes in the FFAs concentration and changes in rCBF in a region in the prefrontal cortex. In addition, they differed in their correlation between changes in plasma glucose and changes in rCBF in the same region (Gautier et al., 2001).

In another PET study, where subjects ate chocolate beyond satiety, different sets of brain stuctures were shown to be active during tasting chocolate at the start (high motivation, pleasant taste) and at the end of the experiment (low motivation, aversion) (Small et al., 2001). In the insula and parts of the orbitofrontal cortex the changes in reward value correlated with changes in rCBF.

Functional magnetic resonance imaging (fMRI)

The term 'functional MRI' usually refers to Blood Oxygen-Level Dependent fMRI (BOLD fMRI). However, recently another functional MRI technique, perfusion functional MRI, has gained interest as a tool for measuring brain function (Aguirre et al., 2005). This technique is addressed in the discussion (Chapter 7). In the work described in this thesis BOLD functional MRI has been used.

Technique

During MRI, a subject is placed in a strong magnetic field, which magnetizes the tissues. Then, radiofrequency pulses are applied to excite nuclei, usually protons (hydrogen atoms, chosen because they are abundant in biological tissues). On returning to a state of equilibrium, the protons emit radiowaves, which are detected by a receiver coil. The time course of this relaxation process differs among tissues, and that difference is the source of contrast in MRI. In functional MRI, the blood oxygen level–dependent (BOLD) signal is used as a measure for neuronal activity. BOLD fMRI makes use of the paramagnetic properties of endogenous deoxygenated hemoglobin as a source of contrast (Bandettini et al., 1992; Kwong et al., 1992; Ogawa et al., 1992). Deoxygenated hemoglobin locally

distorts the magnetic field and thus affects the relaxation process. At the site of brain activation, increased local blood flow leads to a decreased concentration of deoxygenated hemoglobin, which in turn attenuates the local distortion of the magnetic field and results in a small increase (1–5 %) in the fMRI signal. The spatial resolution of BOLD fMRI can be as high as 1 mm^3, depending on the strength of the main field and other scanner characteristics. However, being a vascular signal, the BOLD signal does not colocalize perfectly with the actual spot of neuronal activation. One whole-brain image can be acquired in less than 2 s. The high temporal resolution of fMRI makes it suitable for measuring brain responses that can serve as markers for satiation.

Application in food intake research

In a classical fMRI study usually a block design is employed, in which blocks (periods) of stimulus presentation or task performance are alternated with blocks of rest or another appropriate control condition. The repeated measurements obtained in this way improve the statistical power of the experiment. Block length is usually around 30 s. A prerequisite for the analysis of such fMRI data is that the stimulus timing is known (from that the timing of the local hemodynamic effects is estimated). This makes classical fMRI suitable for investigating, among others, the effects of visual, olfactory and gustatory stimuli. Such stimuli can be offered repeatedly and accurately timed, within minutes (see *e.g.* Chapter 6). However, induction of the state of satiety can be done only once. Moreover, it is unclear when exactly the effects of a satiating stimulus, *e.g.* a meal, occur in the brain. Complicating practical factors in any fMRI experiment, but especially in one that includes the administration of food stimuli, are that subjects are in a supine position and should not move their head. The latter causes severe artifacts in the data (see *e.g.* Chapter 2).

• **Rationale**

The objective of this research project was to find biomarkers of satiety in the brain using fMRI. More in particular, we aimed to evaluate the use of fMRI measurements as biomarkers of metabolic as well as of sensory-specific satiety.

The hypothalamus was a brain area of particular interest, because of its central role in the regulation of food intake. Matsuda et al. (1999) and Liu et al. (2000) showed that one long fMRI-scan can be used to measure fMRI signal changes in the hypothalamus in response to a glucose solution. In

effect, these studies provided an experimental design suited for the measurement of temporal changes in hypothalamic activity following a single treatment. We aimed to evaluate the use of such fMRI measurements of the hypothalamus as a biomarker of satiety. This has resulted in Chapters 2 – 5, in which the emphasis is on metabolic satiety effects.

A first step in evaluating the use of fMRI to obtain markers of sensory-specific satiety in the brain is described in Chapter 6, which focuses on effects of taste-specific satiety.

• Contents of this thesis

To begin with, we performed an experiment aimed at replicating and extending the results of Liu et al. (2000). Importantly, we examined the dose-dependency of the hypothalamic response by using two different doses of glucose. This study is described in **Chapter 2**. We found a prolonged, dose-dependent, decrease in the hypothalamic fMRI signal in response to glucose ingestion. This response started shortly after the onset of ingestion, before absorption of glucose in the blood stream. We hypothesized that the sweet taste of glucose could trigger the pre-absorptive part of this response. Therefore, we did a follow-up experiment in which the contributions of sweet taste and caloric content to the hypothalamic response to glucose ingestion were examined. In addition, concomitant changes in the blood concentrations of glucose and insulin were measured. This experiment is described in **Chapter 3**. We found that only glucose triggered a decrease in the hypothalamic fMRI signal, again starting before absorption of glucose. Sweet taste (aspartame) and energy-content (maltodextrin, a non-sweet carbohydrate) had no such effect. This made us hypothesize that there were glucose-specific pre-absorptive mechanisms, other than those mediated by sweet taste. Therefore, the effects of glucose ingestion and intravenous administration of glucose were compared in order to assess the contribution of pre-absorptive mechanisms to the hypothalamic response to glucose. In addition, concomitant changes in the blood concentrations of glucose, insulin were measured. This experiment is described in **Chapter 4**. In **Chapter 5**, the hypothalamic response to glucose ingestion of obese type 2 diabetics was compared with that of healthy controls.

In **Chapter 6**, we focused on sensory-specific rather than metabolic satiety. We investigated the effects of satiation for chocolate on the brain activity associated with tasting chocolate in men and women, using a classical fMRI block design.

Finally, in **Chapter 7**, the results of the experiments are discussed in relation to the ultimate goal of this research, which was to find biomarkers of satiety in the brain. The sense and non-sense of such biomarkers is discussed and ideas for future research are given.

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Chapter 2

Functional MRI of human hypothalamic responses following glucose ingestion

Summary

The hypothalamus is intimately involved in the regulation of food intake, integrating multiple neural and hormonal signals. Several hypothalamic nuclei contain glucose-sensitive neurons, which play a crucial role in energy homeostasis. Although a few functional magnetic resonance imaging (fMRI) studies have indicated that glucose con-sumption has some effect on the neuronal activity levels in the hypothalamus, this matter has not been investigated extensively yet. For instance, dose-dependency of the hypothalamic responses to glucose ingestion has not been addressed. We measured the effects of two different glucose loads on neuronal activity levels in the human hypothalamus using fMRI. After an overnight fast, the hypothalamus of 15 normal weight men was scanned continuously for 37 min. After 7 min, subjects ingested either water or a glucose solution containing 25 or 75 g of glucose. We observed a prolonged decrease of the fMRI signal in the hypothalamus, which started shortly after subjects began drinking the glucose solution and lasted for at least 30 min. Moreover, the observed response was dose-dependent: a larger glucose load resulted in a larger signal decrease. This effect was most pronounced in the upper anterior hypothalamus. In the upper posterior hypothalamus, the signal decrease was similar for both glucose loads. No effect was found in the lower hypothalamus. We suggest a possible relation between the observed hypothalamic response and changes in the blood insulin concentration.

Introduction

An important part of the brain involved in the regulation of food intake is the hypothalamus. Many of its nuclei contain glucose-sensitive neurons, which are thought to play an important role in long-term body weight regulation as well as in acute feeding behavior (Oomura, 1980; Williams et al., 2001). In recent years, functional magnetic resonance imaging (fMRI) has provided an indirect but non-invasive way to measure changes in neuronal activity levels in the brain of awake subjects. Rather than measuring neuronal spiking activity, this technique measures a vascular correlate of neuronal activity, the Blood Oxygen Level-Dependent signal (BOLD signal). The BOLD signal changes due to the changes in the local concentrations of oxygenated and deoxygenated hemoglobin that result from the hemodynamic changes associated with neuronal activity (Ogawa et al., 1992; Attwell and Iadecola, 2002).

The small size of the hypothalamus, its nuclei and the small signal changes in fMRI make it difficult and technically demanding to image the effects of food stimuli in this part of the brain. Still, a few fMRI studies have shown effects of glucose administration on neuronal activity levels in the hypothalamus. Transient changes of the BOLD signal in the hypothalamus after administration of glucose have been reported in both rats (Torii et al., 1997; Mahankali et al., 2000) and humans (Gao et al., 1998; Matsuda et al., 1999; Liu et al., 2000). Although the signal changes reported in these studies are not entirely consistent with each other, it is clear that glucose administration somehow results in a response in the hypothalamus. Likely, the rise in blood glucose concentration that follows the administration of glucose is an important factor mediating this response. These studies have demonstrated that it is feasible to measure long-term effects of a food stimulus in the human hypothalamus with BOLD fMRI. However, to enable the use of this method in future studies comparing the effects of food or drug stimuli in different groups of subjects, for example, comparing the effects of a glucose stimulus in obese and normal-weight subjects, reproducibility of the method and dose-dependency of the measured response need to be addressed. Therefore, the purpose of our study was twofold: first, to try and replicate the kind of BOLD measurements performed in earlier studies to examine the temporal profile of the hypothalamic response to a glucose load and second, to investigate whether the amount of glucose ingested affects the hypothalamic response.

Materials and methods

Subjects

Fifteen healthy normal weight male volunteers participated, mean age 21.9 (SD 3.1) years, BMI 21.5 (SD 1.9) kg/m^2. Subjects were recruited by an advertisement put up at various locations in the University Medical Center Utrecht. We used a Health and Lifestyle Questionnaire to assess general health and aspects of lifestyle relevant to the study. Exclusion criteria included: having a body mass index (BMI) lower than 19 kg/m^2 or higher than 25 kg/m^2; being under 18 or over 28 years of age at the study day; smoking; having a history of alcohol consumption or current alcohol consumption of more than 28 units per week; having a history of medical or surgical events that may significantly affect the study outcome, such as metabolic or endocrine disease or any gastrointestinal disorder; having irregular eating habits; slimming or following a medically prescribed diet; using medication (except aspirin/paracetamol); suffering from claustrophobia; having diabetes; having metal implants or metal objects on the body which cannot be removed (e.g., piercing, hearing aid, brace). Written informed consent was obtained from all subjects according to the Declaration of Helsinki and the study protocol was approved by the Medical Ethical Committee of the University Medical Center Utrecht, Utrecht, The Netherlands.

Experimental procedures

Subjects were instructed to fast overnight from 10:00 pm until the scan the next morning, which started between 9:30 and 10:00 am (no food or beverages, except water). They were randomly assigned to one of three experimental conditions: 300 mL of orange-flavored water in which 25 or 75 g of d-dextrose (Avebe Corporate, Veendam, The Netherlands) was dissolved (n= 5 and n= 6, respectively), or 300 mL plain tap water (n= 4). Magnetic resonance imaging was performed using a 1.5-T Philips Gyroscan ACS-NT system. Subjects were positioned supine with their head immobilized by a vacuum cushion designed for use in a MRI head-coil (Medical Intelligence, Schwabmünchen, Germany). During the functional scan, a 10-mm thick midsagittal slice was scanned using a $T_2{}^*$-weighted gradient-echo segmented echo-planar imaging (EPI) sequence (repetition time = 120 ms, echo time = 40 ms, flip angle = 30°, image matrix = 198 × 256, field of view = 208 × 208 mm, 12 signal averages per scan, 33 k-lines acquired per excitation pulse, adapted from Liu et al. (2000)). Images were reconstructed to 256 × 256 pixels. Subjects were scanned for 37 min (256

scans). After a baseline of 7.2 min (50 scans), subjects ingested one of the test solutions through a peroral tube. After the functional scan, a T_1-weighted anatomical scan was made of the same slice (repetition time = 600 ms, echo time = 18 ms, field of view = 230 × 230 mm).

Data preprocessing

All 256 functional images of each time series were motion corrected with in-house software, which uses the MIRIT mutual information registration routine (Maes et al., 1997). Images were aligned to the middle image. The anatomical image was also co-registered with this image.

Data analysis

Every subject's hypothalamus was manually segmented using the anatomical image and divided into four regions of interest by two orthogonal axes following predefined criteria (Matsuda et al., 1999): the upper anterior hypothalamus (UAH), lower anterior hypothalamus (LAH), upper posterior hypothalamus (UPH), and lower posterior hypothalamus (LPH). The anterior–posterior axis was defined by the line passing through the centers of the anterior commissure and the mammillary body. The upper–lower axis was determined by the line passing through the optic chiasm, perpendicular to the anterior–posterior axis (**Figure 2.1**). Also, a square reference area (10 × 10 pixels) of about the same size as the hypothalamus was delineated in the frontal cortex, anterior of the genu of the corpus callosum. At every time point, the mean gray value in the hypothalamus as a whole and in each region of interest was calculated. Next, these mean gray values were normalized to the mean of the 7.2-min baseline, yielding the percentage signal change from the mean baseline. For statistical analysis we pooled the normalized data of every group in time, resulting in 37 time slots of 1 min. Hereafter, we tested for every time slot whether the two glucose conditions and the water condition differed. Statistical testing was performed by a Student's t-test with a Bonferroni corrected threshold of P = 0.0001. This method is comparable to differential regression analysis (Cho et al., 2003).
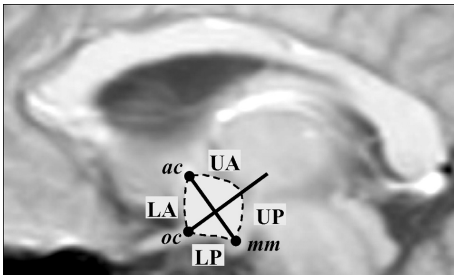

Figure 2.1. Segmentation and subdivision of the hypothalamus into four regions of interest (Matsuda et al., 1999). UA: upper anterior hypothalamus; UP: upper posterior hypothalamus; LA: lower anterior hypothalamus; LP: lower posterior hypothalamus; ac: anterior commissure; mm: mammillary body; oc: optic chiasm.

Results

The mean signal changes in the hypothalamus and the reference area, as a function of time, are shown in **Figure 2.2A**. At the start of drinking (t = 0 min), large signal drops occur for all treatments, in both the hypothalamus and the reference area. These result from artifacts caused by the drinking and last for about 3 min, obscuring possible fMRI signal changes. After that, both glucose treatments show a prolonged signal decrease (1 – 2.5 %), whereas the water treatment returns to baseline. Moreover, the 75-g glucose solution induces a larger decrease in signal than the 25-g glucose solution.
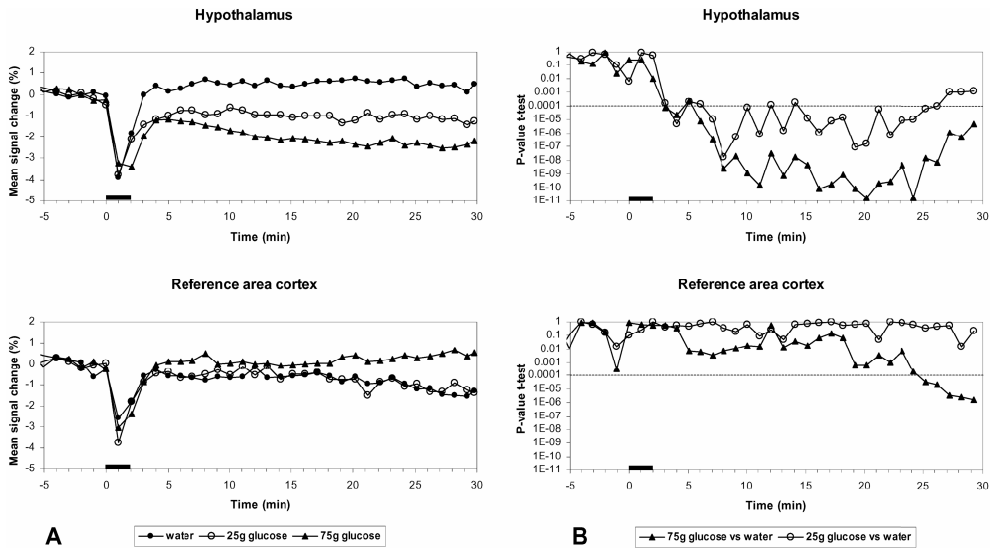

Figure 2.2. **A**: Mean signal change from the mean baseline in time for the hypothalamus as a whole and for a reference area of comparable size under three conditions. **B**: P-values of the Student's t-tests comparing the mean signal changes from the mean baseline of the two glucose conditions with that of the water condition for every 1 min time slot. The dashed line indicates the Bonferroni-corrected threshold of P = 0.0001. Time t = 0 min corresponds to the onset of drinking and the black bar indicates the approximate duration of drinking.

Figure 2.2B shows that, in the hypothalamus, the signal after ingestion of both glucose solutions is significantly lower than that after the ingestion of water, and that this effect persists for an extended period of time. In the 25-g glucose condition, the signal differs significantly from the water condition until about 25 min after the onset of drinking. In the 75-g glucose condition, the signal differs significantly from the water condition for the duration of the scan (30 min). In the reference area, there are no significant signal changes after drinking for any of the treatments until the end of the scan (t = 25 min), where the difference between the water and the 75-g

glucose solution becomes significant (Figure 2.2B). In Figure 2.2A, it can be seen that this is due to a slight decline in the signal of the water condition in the reference area, which is also apparent for the 25-g glucose condition. We attribute this drop in signal to scanner signal drift and consider it unrelated to the glucose content of the stimuli employed since both the water and the 25-g glucose condition show a similar signal decline.
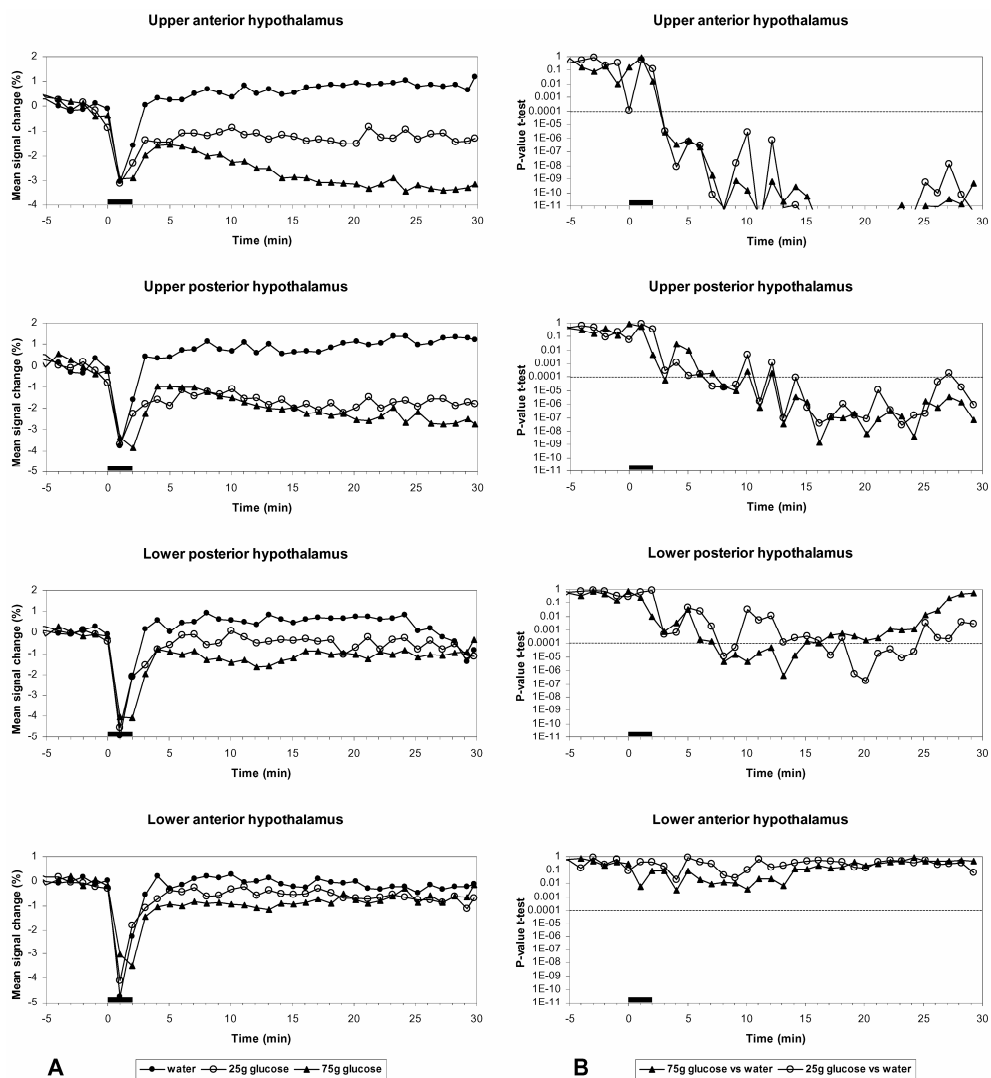
Figure 2.3. **A**: Mean signal change from the mean baseline in time for four subregions of the hypothalamus under three conditions. **B**: P-values of the Student's t-tests comparing the mean signal changes from the mean baseline of the two glucose conditions with that of the water condition for every 1 min time slot. The dashed line indicates the Bonferroni-corrected threshold of $P = 0.0001$. Time $t = 0$ min corresponds to the onset of drinking and the black bar indicates the approximate duration of drinking.

In addition to the hypothalamus as a whole, four sub-regions were studied (see Figure 2.1). **Figure 2.3A** shows the mean signal change as a function of time in these regions for the three conditions. The decrease in signal after glucose ingestion is present in the upper anterior hypothalamus (UAH) as well as in the upper posterior hypothalamus (UPH). In the UAH, this signal decrease is dose-dependent: around 1 % for the 25-g glucose condition and 2 − 3 % for the 75-g glucose condition. In the UPH, both treatments show a similar signal decrease of 1 − 2 %. In the lower posterior hypothal-amus (LPH), a small, but mostly not significant, signal decrease can be seen for the 75-g glucose condition. In the lower anterior hypothalamus (LAH), there are no significant signal changes for both glucose treatments. The water condition shows small signal increases (0.5 − 1 %) in the UPH, AUH and LPH, but not in the LAH and the reference area.

Discussion

The most important findings of this study are twofold. First, we observed a prolonged decrease in the fMRI signal in the hypothalamus following the ingestion of glucose. Second, this decrease was dose-dependent.

We are the first to report a prolonged decrease in the hypothalamic fMRI signal after glucose consumption. The time course of this response suggests that it is associated with the changes in blood insulin and glucose concentration. Although the exact onset of this signal decrease cannot be determined accurately due to image artifacts associated with drinking, it starts before the end of glucose ingestion. This is well before most of the glucose has entered the blood stream. Therefore, initially, the observed response cannot be solely associated with the rise in blood glucose. Possibly, it is the brain that triggers anticipatory changes in blood insulin, in response to the consumption of a glucose solution. (Rolls et al., 1976) showed that neurons in the lateral hypothalamic area of hungry monkeys decreased their firing rate at the sight of food, which shows that the hypothalamus can respond to a food stimulus, even before the actual onset of feeding. Early insulin secretion (0– 10 min after the onset of feeding), also called cephalic phase insulin release (CPIR), has been the subject of many studies (Berthoud et al., 1981; Bellisle et al., 1985; Lucas et al., 1987; Teff et al., 1991; Teff et al., 1993; Teff and Engelman, 1996). Although a relatively small amount of insulin is secreted, it is important in preventing post-prandial peaks in blood glucose (Kraegen et al., 1981; Harju and Nordback, 1987; Ahren and Holst, 2001). Some authors report the absence

of CPIR in humans in response to tasting, but not actually swallowing, sweetened liquids (Bruce et al., 1987; Teff et al., 1995). Still, others have shown that the sight and sweet taste of food can trigger CPIR (Rodin, 1985; Yamazaki and Sakaguchi, 1986). Moreover, several authors report that CPIR is mediated by the vagus nerve (Harju and Nordback, 1987; Ahren and Holst, 2001) (Yamazaki and Sakaguchi, 1986), which projects to the hypothalamus (Oomura, 1980; Storlien, 1985). In our experiment, subjects swallowed sweet colored glucose solutions which, however, they could not see during the scan. Apparently, swallowing 300 ml of a sweet solution when hungry was sufficient to trigger CPIR, or at least an anticipatory hypothalamic response, in our subjects.

After CPIR, normal post-prandial insulin secretion comes into play when blood glucose starts rising. Blood glucose peaks at about 30 min after ingestion of 75 g glucose in a standard 75 g oral glucose tolerance test (Kong et al., 1999; Yasuhara et al., 2003). The insulin concentration changes concomitantly (Kong et al., 1999). Our observation of a dose-dependent modulation of the fMRI signal in the hypothalamus, where a larger dose of glucose was associated with a larger and more prolonged signal decrease, fits the pattern of glucose-triggered insulin release.

In our design, the effects of taste and energy content of the stimuli on the hypothalamic response cannot be separated. In future research, taste effects could be ruled out by injecting solutions directly into the stomach, which would also bypass the problem of swallowing artifacts.

The effect of glucose administration on the hypothalamus has been studied previously in rats and humans (Torii et al., 1997; Matsuda et al., 1999; Liu et al., 2000; Mahankali et al., 2000). Matsuda et al. (1999) reported a large signal decrease (8 – 10 %) in the hypothalamus, starting 4 min after the onset of drinking and lasting about 10 min, whereas Liu et al. (2000) found a signal decrease of up to 4%, starting 5 min after the onset of drinking, reaching a maximum around 8 min and returning to about 1 % below baseline after 12 min. We found no pronounced effect of water intake. This corresponds with the finding of Liu et al. (2000), but contrasts with the finding of Matsuda et al. (1999) who report a signal decrease in part of the hypothalamus after the ingestion of water similar to that following glucose ingestion.

The decreases in fMRI signal we observe in the hypothalamus are possibly related to decreases in neuronal activity in the lateral hypothalamic area (LHA), which is known to contain glucose-sensitive neurons (Oomura, 1980). Moreover, decreased firing rates in response to glucose infusion have

been reported in this area in cats and rats (Brown and Melzack, 1969; Chhina et al., 1971; Oomura et al., 1974; Miller and Rabin, 1975).

We found a prolonged decrease of the fMRI signal in the hypothalamus. An important issue in the interpretation of this result is localization. The signal in fMRI represents local changes in blood oxygenation. This signal is obtained from several voxels (volume units of brain tissue), whose size sets the spatial scanning resolution. In our case, the voxels are $1 \times 1 \times 10$ mm. It is important to realize, however, that what we measure in fMRI is not the spiking activity of the multiple neurons present in a voxel, but rather the local changes in blood oxygenation and blood flow caused by a changing level of neural activity (Attwell and Iadecola, 2002). Because the fMRI signal relies on the hemodynamic changes associated with changes in neuronal activity, it does not co-localize perfectly with the neurons involved. Thus, localization of fMRI responses is not as accurate as that of the single cell or multi-unit electrical recordings made in animals, which have shown the modulation of neuronal activity in the ventromedial and lateral hypothalamus in response to glucose (Brown and Melzack, 1969; Oomura et al., 1969; Chhina et al., 1971; Miller and Rabin, 1975; Rabin and Miller, 1980).

We imaged a 10-mm midsagittal slice which includes all of the hypothalamus in the anterior–posterior direction and most, if not all of it, in the medio-lateral direction (Saper, 1990). This is the preferred orientation in this case because most movement associated with drinking comes from in-plane rotation and preventing side-ways motion of the head is relatively easy. The decrease in fMRI signal, as observed in the hypothalamus as a whole, is not present in all subdivisions (see Figure 2.1). Thus, this response associated with glucose ingestion is present only in part of the hypothalamus. It is tempting to try and identify one of the hypothalamic nuclei as the hot spot of signal change. However, this should be done with extreme caution for two reasons: First, because the fMRI signal does not directly, but indirectly, represent responses of groups of neurons and second, because every voxel contains parts of more than one nucleus. For example, in the ventral mid-tuberal region a typical voxel will, apart from 3rd ventricle CSF, contain cells belonging to both the ventro-medial nucleus (about 2 mm in cross-section) and the adjacent lateral hypothalamus (about 3 mm in cross-section) (Saper, 1990).

Regardless of its exact localization, the dose-dependent decrease we found in the UAH might provide a measure of satiation if this decrease relates to changes in the blood insulin concentration. Recently, it has been shown that the time-course of the satiating effect of carbohydrates relates to

their glycemic effect. High-glycemic carbohydrates, which cause a rapid rise of the blood glucose concentration, increase satiety and suppress food intake mostly in the short term (within 1 h), whereas low-glycemic carbohydrates, which cause a more gradual change in blood glucose concentration, increase satiety, and suppress food intake on the longer term (6 h) (Anderson et al., 2002; Anderson et al., 2003). The response we observed might be associated with this since it is associated with the glycemic changes induced by glucose ingestion. This, however, will require a more in-depth investigation of the correlation between the changes in hypothalamic fMRI signal, blood glucose and insulin concentrations, and the degree of satiation.

In conclusion, this is the first study showing a prolonged and dose-dependent decrease of the fMRI signal in the hypothalamus after glucose ingestion. The dose-dependency of the signal decrease was exclusively present in one subdivision of the hypothalamus: the upper anterior hypothalamus. The time course and dose-dependency of this response suggest a possible association with changes in the blood insulin concentration. This, however, will require further research.

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Chapter 3

Hypothalamic responses to sweet taste and calories

Summary

Evidence exists that beverages do not trigger appropriate anticipatory physiologic responses, such as cephalic phase insulin release. Therefore, it is of interest to elucidate the food properties necessary for triggering adaptive responses. Previously, we found a prolonged dose-dependent decrease in the hypothalamic functional magnetic resonance imaging signal after ingestion of a glucose solution. The aims of the present study were to measure the effects of sweet taste and energy content on the hypothalamic response to glucose ingestion and to measure the concomitant changes in blood glucose and insulin concentrations. Five healthy normal-weight men participated in a randomized crossover design trial. Subjects were scanned four times for 37 min on separate days with functional magnetic resonance imaging. After 7 min, they ingested one of the following four stimuli (300 mL of each): water (control), a glucose solution, an aspartame (sweet taste) solution, or a maltodextrin (nonsweet carbohydrate) solution. Glucose ingestion resulted in a prolonged and significant signal decrease in the upper hypothalamus ($P < 0.05$). Water, aspartame, and maltodextrin had no such effect. Glucose and maltodextrin ingestions resulted in similar increases in blood glucose and insulin concentrations. However, only glucose triggered an early rise in insulin concentrations. Aspartame did not trigger any insulin response. Our findings suggest that both sweet taste and energy content are required for a hypothalamic response. The combination of sweet taste and energy content could be crucial in triggering adaptive responses to sweetened beverages.

Introduction

Beverages are an increasingly important source of carbohydrate intake (DiMeglio and Mattes, 2000; Bray, 2004). Unfortunately, consumption of caloric beverages does not elicit appropriate dietary compensation and leads to excessive energy intake (de Castro, 1993; Mattes, 1996; DiMeglio and Mattes, 2000), which promotes obesity. A likely cause for this excessive energy intake is that beverages cause less sensory stimulation because they are swallowed and not chewed and therefore lack the olfactory and visual stimulation of normal foods. Sensory stimulation, such as the sight, smell, and taste of food, can trigger anticipatory physiologic responses, also called cephalic phase responses, which improve the digestion and absorption of nutrients (Rodin, 1985; Mattes, 1997; Teff, 2000). In view of the prevalent consumption of beverages, it is of interest to elucidate the properties of foods that are necessary for triggering these adaptive responses. Some evidence exists that liquids do not induce cephalic phase responses; for example, tasting sweet liquids does not elicit cephalic phase insulin release (CPIR) (Bruce et al., 1987; Teff et al., 1995), which is important for the prevention of peaks in blood glucose (Kraegen et al., 1981; Harju and Nordback, 1987; Ahren and Holst, 2001). In contrast, tasting food can trigger CPIR (Rodin, 1985; Teff et al., 1991) and so can the combination of sweet taste and the presentation of a meal (Bruce et al., 1987). Recently, we reported a prolonged dose-dependent decrease in the blood oxygen level dependent (BOLD) magnetic resonance imaging (MRI) signal in the hypothalamus a few minutes after the ingestion of a glucose solution (Smeets et al., 2005; Chapter 2). BOLD functional MRI (fMRI) measures changes in neuronal activity levels based on the associated changes in the local concentrations of oxygenated and deoxygenated hemoglobin (Kwong et al., 1992; Ogawa et al., 1992). We suggested that the initial part of this response could be triggered by the sweet taste of the glucose solution and could be associated with changes in the blood insulin concentration during the cephalic phase (ie, before glucose has entered the blood stream). The subsequent rise in blood glucose and insulin concentrations could relate to the additional hypothalamic response (Chapter 2). The aim of the present study was to investigate the separate effects of sweet taste and energy content on this hypothalamic response as well as on blood glucose and insulin concentrations.

Materials and methods

Subjects

Five healthy, normal-weight men participated in the present study (mean ± SEM age: 20.4 ± 2.5 y; mean ± SEM body mass index: 21.7 ± 1.1 kg/m^2). The subjects were recruited with advertisements that were put up at various locations in the University Medical Center Utrecht. We used a health and lifestyle question-naire to assess the general health and aspects of lifestyle of the subjects that were relevant to the study. Exclusion criteria were the following: body mass index < 19 or > 25 kg/m^2; < 18 or > 28 y of age on the study day; current smoker; a history of alcohol consumption or current alcohol consumption > 28 units/wk; a history of medical or surgical events that may have significantly affected the study outcome, such as metabolic or endocrine diseases or any gastrointestinal disorder; irregular eating habits; being on a self-imposed or medically prescribed diet; use of medication (except aspirin or paracetamol); claustrophobia; diabetes; and metal implants or metal objects on the body which cannot be removed (eg, a piercing, hearing aid, or brace). Written informed consent was obtained from all subjects according to the Declaration of Helsinki, and the study protocol was approved by the Medical Ethical Committee of the University Medical Center Utrecht, Utrecht, The Netherlands.

Experimental procedures

We used a randomized crossover design with four stimuli: water, water + glucose, water + aspartame, and water + maltodextrin (a nonsweet glucose dimer). Conditions were randomly assigned to the subjects by drawing lots the day before every visit. The sweetness of the aspartame solution and energy-content of the maltodextrin solution were matched to those of the glucose solution. The details and preparation of the stimuli were as follows: 300 mL tap water was used for water, the 75-g glucose solution was obtained by dissolving 82.5 g glucose monohydrate (D-dextrose; Avebe Corporate, Veendam, The Netherlands) in water to a volume of 300 mL, the equally sweet aspartame solution was obtained by dissolving 880 mg pure aspartame (Aspartamum; BUFA Corporate, Uitgeest, The Netherlands) in 300 mL water, and the equicaloric maltodextrin solution was obtained by dissolving 78.9 g Fantomalt (Nutricia, The Netherlands) in water to a volume of 300 mL (300 kcal). The amount of aspartame that was needed to match the sweetness of the 75-g glucose solution was estimated from the data of the PhD dissertations of De Graaf (1988) and Schifferstein (1992). A pilot test, in which five volunteers were asked to assess the sweetness of the

two solutions in a blind fashion, confirmed that this determination was correct. The subjects were blinded as much as possible; all stimuli looked identical and the subjects did not know in advance the stimulus they would receive. When asked afterward, the subjects were unable to discern between the glucose and aspartame solutions. The maltodextrin solution was unknown to the subjects, and most of the subjects reported a slight starch taste, which made them guess it was maltodextrin. Water was recognized by the subjects as such.

The subjects fasted (no food or beverages, except water) overnight from 10:00 pm and were scanned the next morning, starting between 09:30 and 10:00 am. The scans were performed on four separate days at least one week apart with a 1.5-T Philips Gyroscan ACS-NT system (Philips Medical Systems, Best, The Netherlands). The subjects were placed in a supine position and their heads were immobilized with a vacuum cushion that was designed for use in a MRI head coil (Medical Intelligence, Schwabmünchen, Germany). The functional scan consisted of a T_2^*-weighted gradient-echo, segmented echo-planar imaging sequence (repetition time = 120 ms, echo time = 40 ms, flip angle = 30 °, image matrix = 198 × 256, field of view = 208 × 208 mm, 12 signal averages/scan, 33 k-lines acquired/excitation pulse (Chapter 2) with which a 10-mm thick midsagittal slice was scanned. The images were reconstructed to 256 × 256 pixels. The subjects were scanned for 37 min (256 scans). After a reference period (baseline) of 7.2 min (50 scans), the subjects ingested one of the test solutions through a peroral tube. After the functional scan, a T_1-weighted anatomical scan was made of the same slice (repetition time = 600 ms, echo time = 18 ms, field of view = 230 × 230 mm).

Blood sampling and analysis

On every test day, 10 blood samples were drawn with 5-mL syringes in combination with a canula that was placed in an antecubital vein; one sample was taken before scanning and 9 samples were taken during scanning at -5 and -3 min (before stimulus ingestion) and at 1, 3, 5, 7, 10, 20, and 29 min after the onset of drinking. The samples were immediately injected into 2-mL K-Oxalate/Na-Fl tubes. The tubes were kept on ice until the experiment ended and were then centrifuged at 1730 × g for 10 min at 4 °C. Blood plasma was stored in aliquots at -80 °C until analyzed. Sample handling and analysis were performed by U-diagnostics Corporate (Utrecht, The Netherlands; an ISO 9002-certified laboratory). Glucose concentrations were measured with the glucose-oxidase method on a VITROS 250 Chemistry System (Johnson & Johnson Clinical Diagnostics, Rochester,

NY). Insulin concentrations were measured with an immunoassay (AxSYM insulin assay; Abbott, Abbott Park, IL).

Data preprocessing

fMRI data of the hypothalamus were preprocessed and analyzed as described previously (Chapter 2). All 256 functional images of each time series were motion-corrected with the Multimodality Image Registration using Information Theory software for image registration by maximization of mutual information (Maes et al., 1997). The images were aligned to the middle image and the anatomical T_1-weighted image was also coregistered with this image.

Data analysis

For every subject, the hypothalamus was manually segmented with the use of the anatomical image and divided into four regions by two orthogonal axes according to predefined criteria (20): the upper anterior hypothalamus (UAH), lower anterior hypothalamus, upper posterior hypothalamus (UPH), and lower posterior hypothalamus. The anterior–posterior axis was defined by the line passing through the centers of the anterior commissure and the mammillary body. The upper–lower axis was defined by the line passing through the optic chiasm, which is perpendicular to the anterior–posterior axis (**Figure 3.1**). The UAH and UPH were the regions of interest (ROIs) because these regions respond to glucose (Chapter 2). In addition, a square reference area (10×10 pixels) of about the same size as the hypothalamus was delineated in the frontal cortex, anterior of the genu of the corpus callosum. At every time point, the mean gray value in the hypothalamus as a whole and in each ROI was calculated. Next, these mean gray values were normalized to the mean of their 7-min baseline value, which yielded the percentage signal change from mean baseline. The global signal drift (scanner drift) was corrected by subtracting the mean signal in the reference area from those in the ROIs. We tested for an effect of every stimulus using differential regression analysis (21), in which the mean signal changes per minute were compared with the mean signal change during the 7-min reference period using Student's t-tests. Because drinking causes artifacts (Chapter 2), data from the first 2 min after the onset of drinking were excluded from the analysis. A Bonferroni-corrected threshold of $P = 0.0018$ was employed because we performed 28 t-tests per condition.
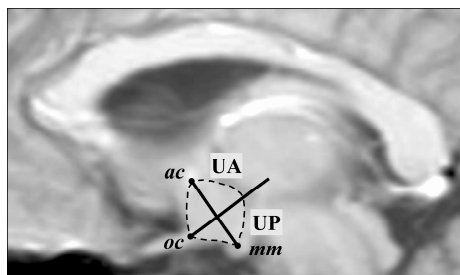
Figure 3.1. Segmentation and subdivision of the hypothalamus into two regions of interest (Matsuda et al., 1999). Ac, anterior commissure; mm, mammillary body; oc, optic chiasm; UA, upper anterior hypothalamus; UP, upper posterior hypothalamus.

To compare treatment effects, the area under the curves (AUCs) were calculated for every subject from 2 to 30 min after stimulus ingestion in the UAH and the UPH (193 time points). For the blood data, the AUCs above baseline (i.e., the first measurement) were calculated for every subject. The AUCs were calculated with trapezoidal integration. With the use of randomized block designs (analyses of variance), the AUCs of the fMRI data and the blood glucose and insulin data were tested for an overall effect of treatment. The AUCs of the fMRI data were also tested for an effect of ROI. If a treatment effect was observed, post hoc tests were performed to compare treatments. For the fMRI data, we used Dunnett's t-test to compare all other treatments with water (control condition) and Tukey's honestly significant difference test to compare the treatments pairwise. For the glucose and insulin data, we compared all treatments pairwise using Tukey's honestly significant difference test. Statistical analyses were done with SPSS statistical software (version 12.0.1; SPSS Inc, Chicago, IL). A P-value < 0.05 (two-sided and corrected for multiple comparisons when appropriate) was considered significant.

Results

Hypothalamic fMRI signal

The fMRI signal changes in the UAH and the UPH are shown in **Figure 3.2**. The P-values from the Student's t-tests that compared every time block with the reference period are shown below that. Glucose ingestion resulted in a prolonged significant decrease in signal compared with baseline in both ROIs; the decrease started 2–5 min after the onset of drinking and lasted for the remainder of the scan (≈30 min). Water, aspartame, and maltodextrin had no such effect on the hypothalamic signal. Maltodextrin showed some significant signal changes in the UPH, but these were not consistent. An analysis of variance of the AUCs uncovered a significant effect of treatment but no significant effect of ROI. The AUCs for all treatments in the upper hypothalamus are shown in **Figure 3.3**. The AUC of glucose was significantly different from that of water. Otherwise, the AUCs did not

significantly differ from each other. Thus, only glucose affected the hypothalamic fMRI response.
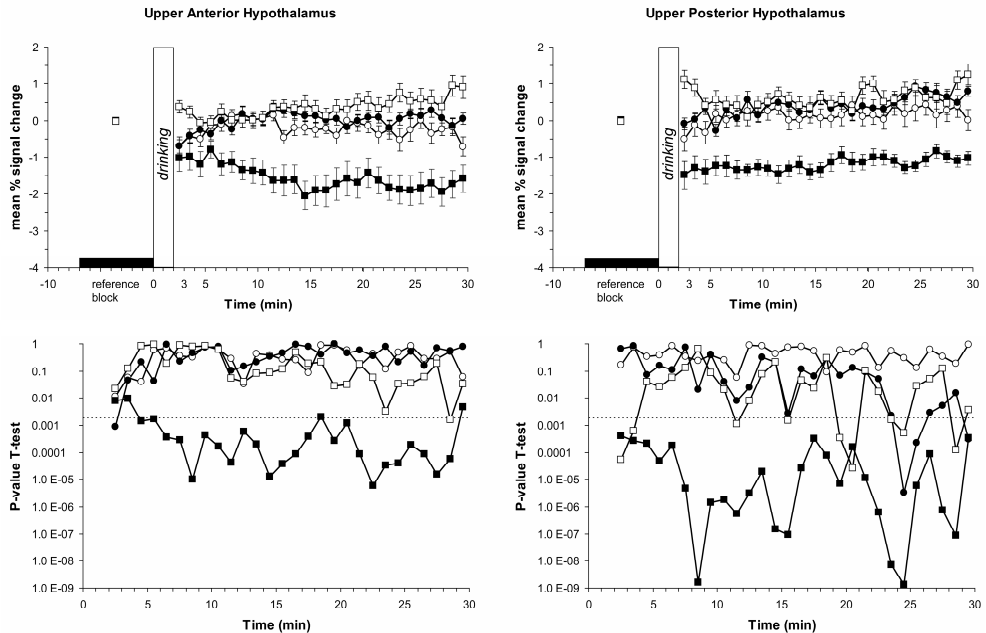

Figure 3.2. Mean (±SEM) fMRI responses in 5 men in the fasting state and after ingestion of four 300-mL test substances. ○, water; ■, water + 75 g glucose (G); ●, water + aspartame (A); □, water + maltodextrine (M). **Top**: signal changes from baseline in the upper anterior hypothalamus (left panel) and the upper posterior hypothalamus (right panel) during the 7-min reference period and in the 28 min after stimulus ingestion. The vertical bars labeled "drinking" indicate the approximate period of stimulus ingestion (2 min). **Bottom**: P-values of the Student's t-tests that compared the mean signal per minute with the mean signal during the reference period. Signal changes during the reference period are near zero. Mean (±SEM) reference in the upper anterior hypothalamus: -0.032 ± 0.050 (water), -0.003 ± 0.045 (G), -0.011 ± 0.065 (A), -0.033 ± 0.048 (M). Mean (±SEM) reference in the upper posterior hypothalamus: 0.025 ± 0.055 (water), -0.003 ± 0.046 (G), -0.009 ± 0.066 (A), -0.020 ± 0.069 (M). The dashed lines indicate the Bonferroni-corrected threshold of P = 0.0018.
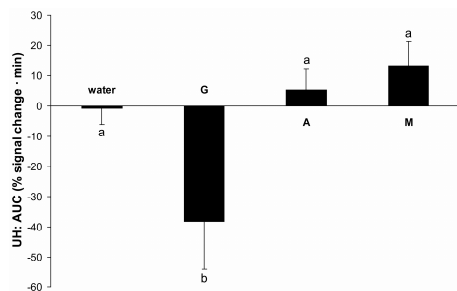
Figure 3. 3. Mean (±SEM) area under the curve (AUC) of the fMRI response in the upper hypothalamus (UH) of 5 men. The AUCs of the two hypothalamic regions of interest were combined because an analysis of variance showed no significant effect of the regions of interest on AUC. G, glucose; A, aspartame; M, maltodextrin. A significant effect of treatment was observed on the AUC, P = 0.005. Bars with different letters are significantly different, P < 0.05 (Dunnett's t-test in a comparison with water and Tukey's honestly significant difference in pairwise comparisons of treatments).

Blood measurements

Plasma concentrations and AUCs of glucose and insulin are shown in **Figure 3.4**. Energy intake (glucose and maltodextrin) resulted in increased glucose and insulin concentrations starting 5–10 min after the onset of ingestion. Similar to water, aspartame (ie, sweet taste) did not affect glucose and insulin concentrations. An early rise in insulin concentration was seen only after glucose ingestion, 5 min after the onset of drinking. At this time, no rise in plasma glucose is yet observed. Analyses of variance showed that significant effects of treatment on glucose and insulin concentrations existed. The AUCs of glucose and maltodextrin were not significantly different, but were significantly greater than those of water and aspartame.
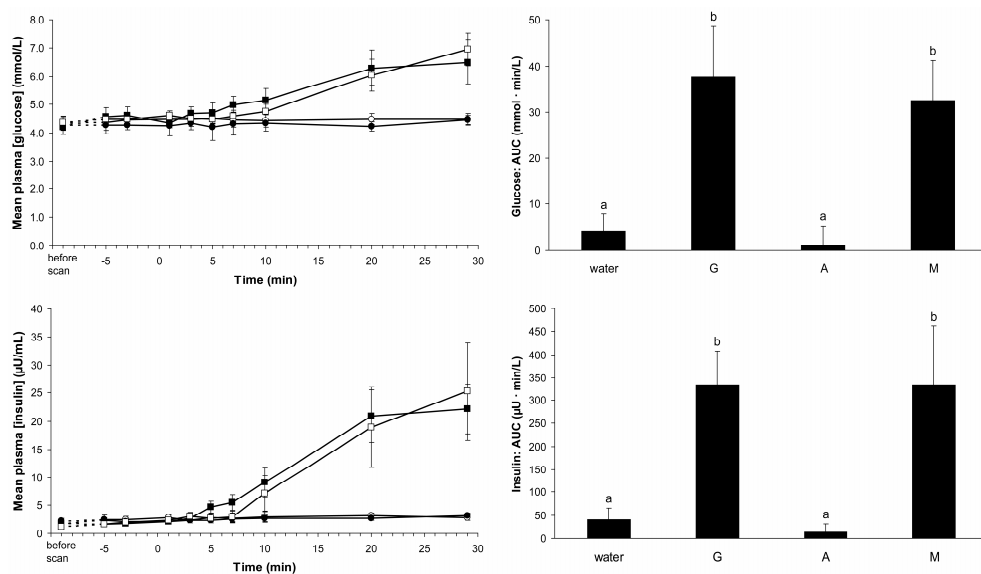
Figure 3.4. Mean (±SEM) responses of plasma glucose and insulin in 5 men in the fasting state (3 timepoints) and after ingestion of four 300-mL test substances (7 timepoints). AUC, area under the curve above baseline. **Left**: ○, water; ■, water + 75 g glucose (G); ●, water + aspartame (A); □, water + maltodextrine (M). The timepoint t = 0 min corresponds to the onset of ingestion. **Right**: Significant effect of treatment on the AUCs of glucose and insulin, $P < 0.05$ (ANOVA). Bars in the same panel with different letters are significantly different, $P < 0.05$ (Tukey's honestly significant difference).

Discussion

We investigated the effects of sweet taste and energy content on the hypothalamic response to glucose ingestion and on the concomitant changes in blood glucose and insulin concentrations. Glucose ingestion resulted in a

prolonged signal decrease in the upper hypothalamus. Water, aspartame, and maltodextrin had no such effect. Ingestion of either glucose or maltodextrin resulted in similar increases in blood glucose and insulin concentrations. However, only glucose triggered an early rise in plasma insulin. The sweet taste of aspartame did not trigger any insulin response.

The prolonged signal decrease we observed in the UAH and UPH after the ingestion of a 75-g glucose solution was similar to the response we had previously reported but with a smaller amplitude (Chapter 2). Both the aspartame and maltodextrin ingestions were not associated with significant changes in the hypothalamic signal. Apparently, neither sweet taste nor energy content was sufficient to trigger a response. This suggests that a hypothalamic response requires both sweet taste and energy content.

The aspartame and maltodextrin ingestions did not result in early changes in the insulin concentration. Our finding that swallowing an aspartame-sweetened liquid does not elicit CPIR adds to reports of the absence of CPIR in response to tasting (not swallowing) aspartame-sweetened liquids (Bruce et al., 1987; Teff et al., 1995). In the study by Teff et al., tasting saccharine as well as sucrose solutions also did not induce CPIR. Moreover, Bruce et al. showed that only the combination of sweet taste with the presentation of a meal induced CPIR. Thus, although one would expect the existence of conditioned adaptive responses after the ingestion of sweet stimuli, these studies suggest that, in general, sweet liquids do not induce CPIR, presumably because they do not cause sufficient sensory stimulation. CPIR was not observed in our study, and although this agrees with the findings of other studies, it could have been due to our small sample size (n = 5) and to the high variability in glucose and insulin concentrations, especially after the ingestion of carbohydrates. Interestingly, Grill et al. (1984) found a preeminent role of glucose in triggering CPIR in rats; other sugars (ie, sucrose and fructose) and a nonnutritive sweetener (sodium saccharine) did not elicit CPIR, whereas glucose did. They note that this is particularly striking, because glucose is neither the most intense nor the most palatable substance that was studied.

The maltodextrin solution, a nonsweet energy-rich liquid, did not elicit CPIR or a hypothalamic response. The absence of CPIR is not surprising given the sort of sensory stimulation it caused (a nonsweet watery taste). Also, the subjects were unfamiliar with this particular substance and thus were not conditioned to this combination of taste and energy content.

We had previously hypothesized that the early decrease in the hypothalamic signal, which was observed less than 5 min after glucose ingestion, was associated with CPIR (Chapter 2). Our insulin data showed

that only glucose was associated with an early rise in insulin concentration, and only glucose triggered a decrease in fMRI signal in the upper hypothalamus. This supports our hypothesis. In addition, we hypothesized that the additional decrease in fMRI signal was associated with a rise in insulin concentration. However, because maltodextrin causes an insulin response similar to that of glucose but no significant change in fMRI signal, this hypothesis is not supported. Taken together, our data show no direct relation between changes in the hypothalamic fMRI signal and the plasma insulin concentration. It is striking, however, that only glucose ingestion was associated with a hypothalamic response as well as an early insulin response. This raises the question whether this hypothalamic response is specifically triggered by glucose. Our present findings in humans and the findings of Grill et al. (1984) in rats suggest a particular sensitivity of the digestive system to glucose. It remains to be investigated whether other sweet carbohydrates, such as sucrose, induce a hypothalamic response.

We found a similar but smaller hypothalamic response after glucose ingestion than was previously reported (Chapter 2). A possible reason for this is the stress caused by the blood sampling; otherwise, the experimental setup of both studies was the same. Stress could influence BOLD measurements of the hypothalamus in two ways. First, stress can affect responses of the hypothalamus because its paraventricular nucleus plays a major role in generating adaptive autonomic, behavioral, and hormonal responses to stress (Sawchenko et al., 1996; Herman and Cullinan, 1997). Second, stress could affect the BOLD signal. Acute stress has been shown to cause changes in hematocrit (Patterson et al., 1995), which affects the source of the BOLD signal. It has been suggested that the stress caused by MRI is a source of variation in hematocrit and therefore of variation in the BOLD signal (Levin and Uftring, 2001); in our case, however, both experiments involved the same MRI protocol. Still, the added stress of the blood sampling procedures could have affected hematocrit. In addition, the effect of venipuncture on cortisol concentrations, a measure of stress, has been shown to be highly variable (Rose and Hurst, 1975; Meeran et al., 1993), which suggests that the effects of stress on the fMRI response will vary between subjects, too. Therefore, the assessment of stress in future MRI experiments is warranted, especially when the experiments involve blood sampling; stress should be measured with salivary cortisol measurements, which allow for an unstressed reference measurement, and with a stress questionnaire to assess the subjective stress of each person. This also suggests that future studies could benefit from a larger sample size, given the small effect size seen in the present study.

In conclusion, we found that sweet taste and energy content on their own did not trigger a prolonged decrease in hypothalamic signal similar to that observed after glucose ingestion. Also, the sweet taste of aspartame was not sufficient to trigger an early insulin response. An early rise in plasma insulin concentration was observed after glucose ingestion, but not after maltodextrin ingestion. It is of interest whether the fMRI signal decrease we observed in the hypothalamus is specifically triggered by glucose. Our findings suggest that the combination of taste (sweetness) and energy content might be important in triggering adaptive physiologic responses to liquid stimuli, such as soft drinks. Moreover, a matching combination of sweet taste and energy content could be necessary for an adequate regulation of energy intake.

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Chapter 4

Ingestion versus infusion: contribution of preabsorptive mechanisms to the hypothalamic response to glucose

Summary

The hypothalamus plays a central role in the regulation of food intake and is involved in the homeostasis of the plasma glucose concentration. Previously, a prolonged decrease in the hypothalamic functional magnetic resonance imaging signal after ingestion of a glucose solution has been reported. Here, we investigated the contributions of preabsorptive mechanisms to the hypothalamic response to glucose. Seven healthy normal-weight men participated in a randomized crossover design trial. After an overnight fast, subjects were scanned four times on separate days with functional magnetic resonance imaging. They received one of the following four treatments: ingestion of a glucose solution (300 mL), intravenous infusion of a glucose solution, ingestion of water (300 mL), infusion of saline. Glucose ingestion resulted in a prolonged signal decrease in the hypothalamus, whereas glucose infusion was associated with a smaller and transient signal decrease. In conclusion, we found that signals that originate between mouth and blood stream contribute substantially to the hypothalamic response to glucose.

Introduction

The hypothalamus plays a central role in the regulation of food intake, energy expenditure and body weight (Schwartz et al., 2000; Stanley et al., 2005). It is involved in the homeostasis of the plasma glucose concentration, among others by modulating insulin secretion and by influencing the rate of hepatic glucose production (Benzo, 1983; Guillod-Maximin et al., 2004; Rohner-Jeanrenaud et al., 1983; Shimazu et al., 1966; Storlien, 1985). Preabsorptive signals, which are defined here as hormonal and neural signals which are triggered prior to and during absorption (*i.e.*, before nutrients reach the blood stream), are important for adaptive responses to food intake. A striking example of this is the incretin effect: the insulin response to intravenous glucose administration is smaller than that to oral glucose (Perley and Kipnis, 1967). The reason for this is that oral glucose induces the release of the insulinotropic gut-peptides glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) (Edwards et al., 1999; Kreymann et al., 1987; Lavin et al., 1996; McIntyre et al., 1965; Vilsboll et al., 2003). The incretin effect improves postprandial glucoregulation.

Recently, a prolonged decrease in the blood oxygen level-dependent (BOLD) magnetic resonance imaging (MRI) signal in the hypothalamus a few minutes after the ingestion of a glucose solution was reported (Smeets et al., 2005b) (Chapter 2). BOLD functional MRI (fMRI) provides a non-invasive measure of changes in neuronal activity levels based on the associated changes in the local concentrations of oxygenated and deoxygenated hemoglobin (Kwong et al., 1992; Ogawa et al., 1992). The rapid onset of this response, before absorption of glucose into the blood stream, suggested a contribution of some pre- absorptive mechanism to the hypothalamic response to glucose (Smeets et al., 2005b). One trigger of a rapid response could be sweet taste. However, a follow-up study showed that sweet taste did not affect the hypothalamic fMRI signal (Smeets et al., 2005b) (Chapter 3). This suggests that other preabsorptive signals cause the early onset of the hypothalamic response to glucose.

The aim of this study was to investigate the contributions of preabsorptive mechanisms to the hypothalamic response to glucose. In addition, blood glucose and insulin concentrations and subjective hunger ratings were determined.

Materials and methods

Subjects

Seven healthy, normal-weight men participated (mean ± SD age: 23.1 ± 2.2 y; mean ± SD body mass index: 21.7 ± 0.8 kg/m^2). The subjects were recruited with advertisements that were put up at various locations in the University Medical Center Utrecht. We used a health and lifestyle questionnaire to assess the general health and aspects of lifestyle of the subjects that were relevant to the study. Exclusion criteria were the following: body mass index < 19 or > 25 kg/m^2; < 18 or > 28 y of age on the study day; current smoker; a history of alcohol consumption or current alcohol consumption > 28 units/wk; a history of medical or surgical events that may have significantly affected the study outcome, such as metabolic or endocrine diseases or any gastrointestinal disorder; irregular eating habits; being on a self-imposed or medically prescribed diet; use of medication (except aspirin or paracetamol); claustrophobia; diabetes; metal implants or metal objects on the body which cannot be removed (*e.g.*, a piercing, hearing aid, or brace). Written informed consent was obtained from all subjects according to the Declaration of Helsinki, and the study protocol was approved by the Medical Ethical Committee of the University Medical Center Utrecht, Utrecht, The Netherlands.

Experimental procedures

To compare ingestion and infusion of glucose we used a randomized crossover design with four treatments: oral ingestion of glucose (Gpo), intravenous infusion of glucose (Giv), oral ingestion of water (Wpo) and intravenous infusion of saline (Siv). For the oral treatments (Gpo and Wpo), subjects ingested 300-mL glucose solution (standard oral glucose tolerance test solution, 75 g glucose in 300 mL water, with sorbic acid added as a preservative) or tap water through a peroral tube. For the intravenous treatments (Giv and Siv), equal volumes of 40% glucose solution or saline (0.9 % NaCl) were infused into an antecubital vein through a canula over ~3 min at a flow rate of 0.50 mL/s using an infusion pump (Medrad Inc., Indianola, U.S.A.). Subjects received 0.5 g glucose per kg body weight with a maximum of 35 g as recommended by Bingley et al.(1992), *i.e.*, a maximum of 87.5 mL 40% glucose or saline. Six out of seven subjects had a body weight over 69 kg and thus received 35 g of glucose and one subject received 34 g of glucose. Conditions were randomly assigned to the subjects by drawing lots the day before every visit. As far as possible, subjects were

unaware of treatment order; they were unable to discern between glucose and saline infusion, but could tell apart glucose from water upon tasting it.

The subjects fasted (no food or beverages, except water) overnight from 22:00 h and were scanned the next morning, starting between 08:00 and 10:45 h. The scans were performed on four separate days, at least one week apart, with a 3.0-T Philips Achieva system (Philips Medical Systems, Best, The Netherlands) equipped with a SENSE-head coil. The subjects were placed in a supine position and their heads were immobilized with cushions. The functional scan consisted of a T_2^*-weighted gradient-echo, segmented echo-planar imaging sequence [repetition time = 120 ms, echo time = 30 ms, flip angle = 30°, image matrix = 256 × 231, field of view = 208 × 208 mm, 12 signal averages/scan, 33 k-lines acquired/excitation pulse] with which a 12-mm thick midsagittal slice was scanned. The images were reconstructed to 256 × 256 pixels. The subjects were scanned for 38 min (225 scans). After a reference period (baseline) of 8.4 min (50 scans), the subjects received one of the four treatments. After the functional scan, a T_1-weighted anatomical scan was made of the same slice (repetition time = 550 ms, echo time = 10 ms, field of view = 208 × 208 mm).

To assess the effect of treatment on their general motivation to eat, the subjects filled out a set of four visual analogue scales (VASs, range: 0 to 100 mm) before and after every scan (*i.e.*, 30 min after treatment) on which they reported their feelings of hunger, fullness, desire to eat, and prospective food consumption (Flint et al., 2000). For every subject, the scores of these four scales were averaged to obtain two single hunger scores, one for the fasted and one for the treated state.

Blood sampling and analysis

On every test day, six blood samples were drawn from a canula that was placed in an antecubital vein (this canula was also used for infusion). The time points were: before scanning (fasted) and during scanning at -3 min (before treatment) and at 15, 30, 45, and 60 min after the onset of treatment. Samples were collected in 4-mL serum separation tubes, for determination of serum glucose and insulin concentrations. The tubes were centrifuged at 1730 × g for 10 min. Blood serum was stored in aliquots at -80 °C until analysis. Sample handling and storage was done by U-diagnostics Corporate (Utrecht, The Netherlands; an ISO 9002-certified laboratory). After completion of the study, blood samples were transferred to the laboratory for Clinical Chemistry at Leiden University Medical Center, Leiden, The Netherlands. There, serum glucose concentrations were measured using a fully automated Hitachi Modular P800 system. Serum insulin concentrations

were measured by an immuno-radiometric assay (INS-IRMA; BioSource Europe S.A., Nivelles, Belgium).

Data preprocessing

fMRI data of the hypothalamus were preprocessed and analyzed as described previously (Smeets et al., 2005b). All 225 functional images of each time series were motion-corrected with the Multimodality Image Registration using Information Theory software for image registration by maximization of mutual information (Maes et al., 1997). The images were aligned to the middle image and the anatomical T_1-weighted image was also coregistered with this image.

Data analysis

For every subject, the hypothalamus was manually segmented with the use of the anatomical image according to predefined criteria (Matsuda et al., 1999). Anatomical landmarks were the anterior commissure, optic chiasm and the mammillary body. In addition, a square reference area (10 × 10 pixels) of about the same size as the hypothalamus was delineated in the thalamus. At every time point, the mean gray value in the hypothalamus was calculated. Next, these mean gray values were normalized to the mean of their 8.4-min baseline value, which yielded the percentage signal change from the mean baseline. The global signal drift (scanner drift) was corrected by subtracting the mean signal in the reference area from that in the hypothalamus. We tested for an effect of every stimulus using differential regression analysis (Cho et al., 2003), in which the mean signal changes per minute were compared with the mean signal change during the 8.4-min reference period using Student's t-tests. A Bonferroni-corrected threshold of $P = 0.0017$ was employed because we performed 30 t-tests per condition.

To compare treatment effects, the areas under the fMRI curves (fMRI AUCs) were calculated for every subject from $t = 2$ to $t = 30$ min after the onset of stimulus administration. Data from the first two min after the onset of drinking were excluded because drinking causes artifacts (Smeets et al., 2005b)(Chapter 2). Also, to allow a better assessment of time effects, fMRI AUCs were calculated for three smaller time blocks (tertiles): 7 – 14 min, 15 – 22 min and 23 – 30 min. The first tertile was chosen from 7 – 14 min because the recovery of the fMRI signal after stimulus ingestion took ~3 min and would otherwise have disturbed the comparison between treatments. For the blood data, the AUCs above baseline (*i.e.*, the first measurement) were calculated for every subject. The AUCs were calculated with a trapezoidal integration. With the use of randomized block designs

(analyses of variance), the AUCs were tested for effects of treatment (glucose) and the mode of administration. In addition, the interaction between treatment and mode of administration was assessed.

Because correlations between fasting blood glucose and insulin concentrations and the hypothalamic fMRI response have been found (Matsuda et al., 1999), the analyses of variance were also done with the fasting glucose and with the fasting insulin concentration as a covariate. Also, Pearson correlation coefficients were calculated between the fMRI AUCs and fasting blood glucose and insulin concentrations. In addition, to investigate whether the fMRI response of the hypothalamus relates to changes in subjective hunger ratings, Pearson correlation coefficients were calculated between the fMRI AUCs and the (changes in) hunger scores after treatment.

Mean hunger scores were calculated for every subject by averaging the VAS scores of general hunger, 100 minus fullness, general desire to eat, and prospective food consumption. The effect treatment on the mean hunger score was calculated by subtracting the score in the fasted state from that in the treated state. With the use of a randomized block design (analyses of variance), the change in hunger score was tested for effects of treatment (glucose), mode of administration and the interaction between these two.

Statistical analyses were done with SPSS statistical software (version 13.0; SPSS Inc, Chicago, IL). A P-value of 0.05 (two-sided) was considered significant.

Results

Hypothalamic fMRI signal

The fMRI signal changes in the hypothalamus are shown in the left part of **Figure 4.1**. Stimulus ingestion took 1.5 ± 0.5 min (mean $\pm$ SD) and caused artefacts in the data (strong signal decreases). After that, the signal rose over $3 - 4$ min. Intravenous stimulus administration did not cause such artefacts. The right part of Figure 1 shows the significance of the signal changes after treatment compared to baseline. Oral glucose resulted in a prolonged significant signal decrease ($1 - 2.5$ %), which lasted for the remainder of the scan. Intravenous glucose was associated with a significant transient signal decrease (-1.0 %) which started at 3 min (the end of glucose infusion) and ended at 18 min. Ingestion of water was associated with a small transient signal decrease of $0.5 - 1.0$ % from about $5 - 10$ min. Saline

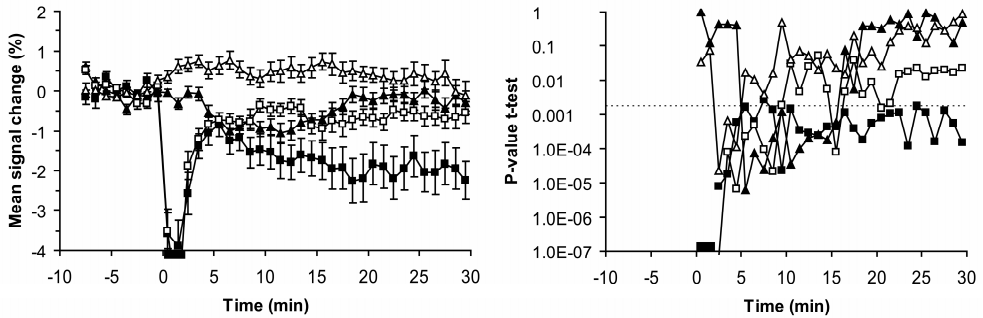infusion was associated with a small transient signal increase of ~0.5 % from 2 – 5 min.

Figure 4.1. Left: Mean ± SEM fMRI signal changes per minute in the hypothalamus under four treatments (n = 7). Right: P-values of the t-test comparing the mean signal per minute with the mean signal before treatment (the 8.4-min baseline). For clarity and because of artefacts in the data due to the drinking, the first two data points after stimulus ingestion have been omitted. The dotted line indicates the Bonferroni-corrected threshold of P = 0.0017. Legend: ■ oral glucose (Gpo), ▲ intravenous glucose (Giv), □ oral water (Wpo), △ intravenous saline (Siv). T = 0 min is the onset of treatment. The black bar indicates the approximate duration of drinking (2 min). Intravenous
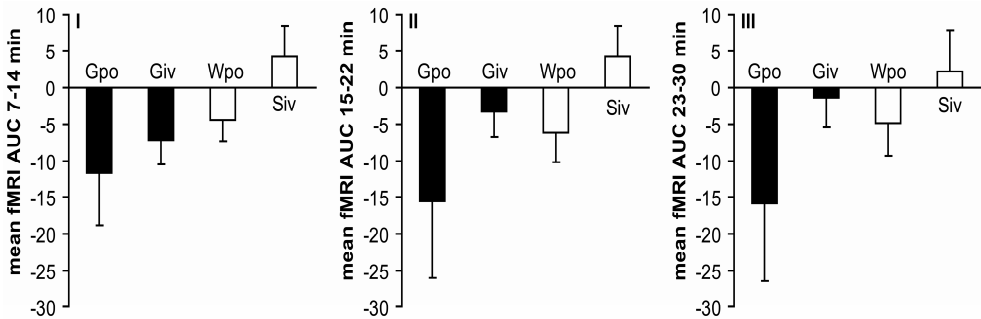
Figure 4.2. Mean ± SEM areas under the fMRI curves (AUC) for three 8-min periods after the onset of four treatments (n = 7): 7 – 14 min (I), 15 – 22 min (II) and 23 – 30 min (III). Gpo: oral glucose, Giv: intravenous glucose, Wpo: oral water, Siv: intravenous saline.

The mean fMRI AUCs in three 8-min periods after treatment are shown in **Figure 4.2**. The AUC after oral glucose, which is largest, stays the same over time. Intravenous glucose shows a declining mean AUC. AUCs for water and saline stay roughly the same over time. The initial analysis of variance showed no significant effects in the three periods. With fasting insulin concentrations added to the model as a covariate there was a near-significant effect of glucose in the first period (I) (P = 0.050) and no significant effect of the mode of administration. In the second period (II), there was no significant effect of treatment and a trend for an effect of the mode of administration (P = 0.053). In the third period (III), there was a

trend for an effect of the mode of administration (P = 0.075) and for an effect of fasting insulin on AUC (P = 0.10). With fasting glucose concentrations added to the model as a covariate there were no significant effects of glucose in the three periods. Thus, the fasting insulin concentration is a covariate of interest.

Blood measurements

Serum concentrations and AUCs of glucose and insulin are shown in **Figure 4.3**. Glucose ingestion caused a slow rise in glucose concentration up to ~7 mmol/L, along with a strong rise in insulin concentration. In contrast, glucose infusion resulted in rapid rises in glucose and insulin concentrations up to ~15 mmol/L and ~40 µU/mL, respectively, followed by decline. At 60 min, the glucose concentration was approximately back at baseline. Thus, ingestion of glucose was associated with much better
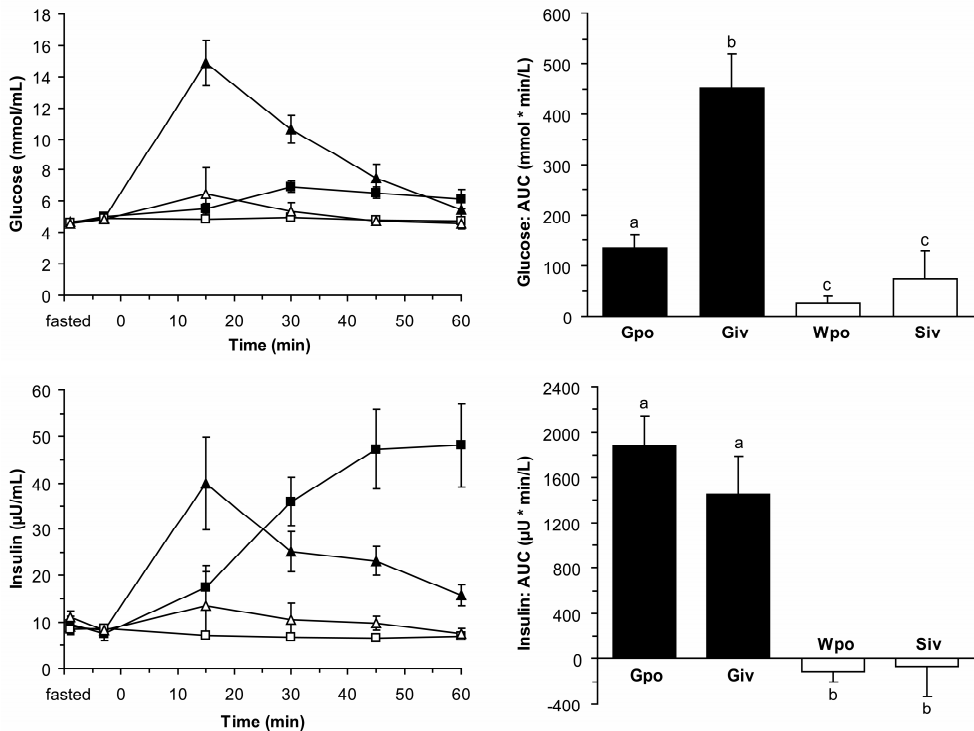
Figure 4.3. Left: Mean ± SEM glucose and insulin responses for the four treatments (*n* = 7). T = 0 min is the onset of treatment. Right: Mean ± SEM glucose and insulin areas under the curve above baseline (*i.e*, the fasting concentration). Effects of glucose administration and the mode of administration were compared between treatments with randomized block designs (analysis of variance). Bars with different letters are significantly different (P < 0.05). Legend: ■ oral glucose (Gpo), ▲ intravenous glucose (Giv), □ oral water (Wpo), △ intravenous saline (Siv).

glucoregulation than infusion of glucose. Administration of water or saline did not affect glucose and insulin concentrations much.

Analyses of variance showed significant effects of glucose administration on glucose and insulin AUCs. For glucose, there were also a significant effect of the mode of administration and a significant interaction. Thus, the AUC of glucose after glucose ingestion was smaller than that after glucose infusion and both were larger than the AUCs after control treatment. For insulin, there was no significant effect of the mode of administration and no significant interaction. Thus, glucose administration was associated with a larger insulin AUC than control administration, but there was no difference in AUC between ingestion and infusion of glucose.

Hunger scores

Mean hunger scores are shown in **Table 4.1**. Analysis of variance of the change in hunger score revealed a significant effect of treatment (glucose, $P < 0.05$) and no significant effect of the mode of administration. The interaction between these two factors did not reach significance ($P = 0.09$). Thus, glucose administration was associated with decreased hunger at 30 min after treatment.

Table 4.1. Mean ± SD hunger scores for four treatments ($n = 7$)[1].

Treatment	Hunger score fasted	Hunger score treated	Change (treated – fasted)
Oral glucose [2]	73 ± 15	60 ± 17	-13 ± 19[a]
Intravenous glucose	70 ± 11	68 ± 14	-1 ± 14[a]
Oral water [2]	68 ± 17	68 ± 17	0 ± 5
Intravenous saline	66 ± 11	68 ± 10	1 ± 8

[1] Hunger scores were recorded after an overnight fast (fasted) and 30 min after treatment (treated).
[2] N = 6 due to one missing value.
[a] Analysis of variance showed a significant effect of glucose ($P < 0.05$) and no significant effect of the mode of administration ($P = 0.09$).

Correlations

Pearson correlation coefficients for the correlation between the fMRI AUCs and fasting blood glucose and insulin concentrations and between the fMRI AUCs and the (changes in) hunger scores after treatment are shown in **Table 4.2**. Plots of significant correlations are shown in **Figure 4.4**. Fasting blood glucose concentrations did not correlate significantly with the fMRI AUC after treatment. Fasting insulin concentrations correlated strongly with

the fMRI AUC (signal decrease) in the hypothalamus after infusion (r = -0.90), but not after ingestion of glucose (r = -0.02). Higher fasting insulin concentrations were associated with a greater fMRI response (signal decrease) after glucose infusion (Figure 4, left graph).

The hunger score 30 min after oral treatment correlated strongly with the fMRI AUCs (Figure 4, right graph). For oral glucose, a higher hunger score was associated with a greater fMRI response (signal decrease). For water, a higher hunger score was associated with a smaller fMRI response (signal decrease). The change in hunger score did not correlate significantly with the fMRI AUCs.

Table 4.2. Pearson's correlation coefficients between the fMRI areas under the curve (AUCs)[1] after four treatments and fasting blood glucose and insulin concentrations and between the fMRI AUCs and (the changes in) hunger score (n = 7).

Treatment	Fasting blood [glucose]	Fasting blood [insulin]	Hunger score treated[2]	Change in Hunger score[2]
Oral glucose	-0.42	-0.02	-0.94**	-0.81
Intravenous glucose	-0.08	-0.90**	-0.12	-0.14
Oral water	0.15	-0.42	0.84*	0.32
Intravenous saline	0.10	0.12	0.21	0.17

[1] The fMRI AUCs after glucose administration are mostly negative (signal decrease) but can also be positive (signal increase).
[2] Hunger scores were recorded after an overnight fast and 30 min after treatment (treated). The change in hunger score was calculated as (score treated – score fasted) and can be negative (*i.e.*, less hunger after treatment) or positive (*i.e.*, more hunger after treatment). There were two missing values, one for oral glucose and one for oral water administration (n = 6).
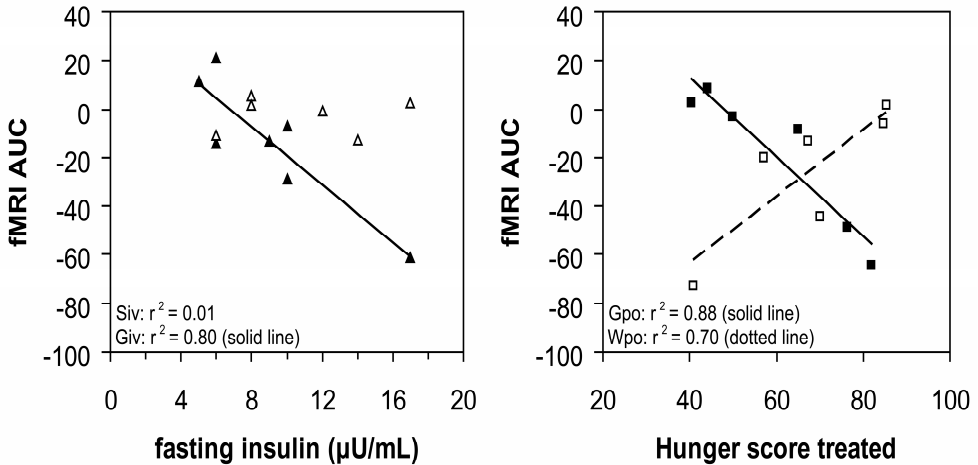* Significant correlation, P < 0.05
** Significant correlation, P < 0.01

Figure 4.4. Significant correlations with the fMRI area under the curve (fMRI AUC). See Table 4.2. Legend: ▲ intravenous glucose (Giv), △ intravenous saline (Siv), ■ oral glucose (Gpo), □ oral water (Wpo). Left: Correlation between the fMRI AUC after intravenous treatment and fasting insulin concentrations. Right: Correlation between the fMRI area under the curve (fMRI AUC) after oral treatment and the hunger score 30 min after treatment.

Discussion

We investigated the effects of oral glucose administration and intravenous infusion of glucose on the hypothalamic fMRI signal and on the blood concentrations of glucose and insulin and on subjective ratings of hunger. The most important finding was that glucose infusion was associated with a small and transient signal decrease in the hypothalamus, whereas glucose ingestion resulted in a greater and prolonged signal decrease. In the blood, infusion of glucose resulted in a greater increase in glucose concentration than ingestion.

The prolonged signal decrease we observed in the hypothalamus in response to glucose administration is similar to the responses to oral glucose we reported previously (Smeets et al., 2005a; Smeets et al., 2005b) (Chapter 2 and 3). Intravenous administration of glucose resulted in a shorter and attenuated hypothalamic response, which started at about 5 min after the onset of treatment and lasted for about 10 min, until 15 min. At 5 min the oral glucose fMRI signal has recovered from the drinking artefacts, but does not rise back to baseline. At 15 min after the onset of treatment, when the hypothalamic response to intravenous glucose ends, the blood insulin concentration starts declining. In the case of oral glucose, the blood insulin concentration is still rising at 30 min after ingestion, and the fMRI signal is still below baseline. These observations suggest that preabsorptive

mechanisms not only cause the early onset of the hypothalamic response, but are also crucial for the prolonged signal decrease after oral glucose. Moreover, they suggest that the hypothalamic response to glucose is associated with the changes in the blood insulin concentration.

The oral dose that was administered was 75 g of glucose, while the intravenous dose was 35 g. This lower dose might explain in part why the response to intravenous glucose was smaller than that to oral glucose. However, the changes in blood glucose concentration after intravenous glucose were much greater. Moreover, previously we found a smaller, but still prolonged, hypothalamic response to a lower oral dose of glucose (50 g) (Chapter 2). This suggests that the transient nature of the hypothalamic response to intravenous glucose is due to the absence of preabsorptive signals.

In contrast with our observations in previous studies (Smeets et al., 2005a; Smeets et al., 2005b) (Chapter 2 and 3), the response to oral glucose was not apparent immediately after the end of ingestion but 2 – 3 minutes later. The most probable reason for this is that the current study was conducted at a field strength of 3.0 Tesla (T), whereas previous studies were done at 1.5 T; higher field strength is associated with greater susceptibility artefacts due to movement. Apparently, small subject movements after stimulus ingestion result in artefacts at 3.0 T but not at 1.5 T. This is supported by the observation that the fMRI signal drops quickly after intravenous administration of glucose (which lacks drinking artefacts). In theory, the higher field strength should yield greater BOLD signal changes, but for our experimental setup this was not the case: hypothalamic signal changes were comparable to those found at 1.5 T (Smeets et al., 2005a; Smeets et al., 2005b) (Chapter 2 and 3).

During saline infusion, the fMRI signal rose ~ 0.5 %, after which it declined. In rats, blood pressure decreases due to blood withdrawal have been shown to cause BOLD signal decreases (Kalisch et al., 2001). Also, drug-induced changes in blood pressure have been shown to correlate with changes in BOLD signal intensity (Wang et al., 2006). Thus, blood pressure increases due to the volume of liquid infused into the blood stream (~ 90 mL) might have caused small BOLD signal increases in our experiment. In addition, the volume of liquid infused into the blood stream could have a dilution effect: a slightly lowered concentration of deoxyhemoglobin would result in a small fMRI signal increase. Both these mechanisms suggest that the fMRI signal decrease in response to intravenous glucose could be attenuated.

Water ingestion resulted in a small signal decrease. In accordance with that, Matsuda et al. (1999) have reported transient fMRI signal decreases after water ingestion in part of the hypothalamus. These findings are consistent with the role of the hypothalamus in the regulation of water balance (Denton et al., 1996; Hatton, 1983; McKinley et al., 1996).

Oral glucose was associated with much better glucoregulation than intravenous glucose, which is in accordance with the fact that preabsorptive mechanisms, such as the incretin effect, are crucial for glucose homeostasis (Perley and Kipnis, 1967).

Glucose administration was associated with decreased hunger. We found no significant difference between the effects of oral and intravenous administration of glucose on the hunger scores, however, there was a trend towards a greater decrease in hunger after oral glucose. This is in accord with the finding that oral glucose increases satiety more than intravenous glucose (Rezek, 1976), possibly due to the incretin effect.

Fasting insulin constitutes a long term negative feedback signal of energy intake and body adiposity (Havel, 2001; Schwartz et al., 2000) that acts via the hypothalamus and other brain regions (Baskin et al., 1999; Schwartz et al., 1999). We found that the fasting insulin concentration correlated strongly with the hypothalamic fMRI response to intravenous but not to oral glucose. When fasting insulin values were added to the analysis of variance of the fMRI AUCs as a covariate the results became more significant. This suggests that, in the absence of the incretin effect and other preabsorptive effects, fasting insulin is a long term signal that modulates the short term hypothalamic response to glucose. Interestingly, in patients with type 2 diabetes mellitus, higher fasting insulin concentrations were associated with a positive fMRI response to oral glucose (*i.e.*, a signal increase) (Chapter 5), whereas here, in healthy men, higher fasting insulin concentrations were associated with a greater negative fMRI response to intravenous glucose.

An interesting finding was the strong correlation between subjective hunger ratings and the fMRI response (signal decrease) after glucose and water ingestion. For water, a higher hunger score at 30 min after ingestion was associated with a smaller fMRI response (signal decrease). However, for water ingestion fasted and treated hunger scores were very similar (mean difference 0 ± 5). The correlation between fasted hunger score and fMRI AUC after water ingestion was also high ($r = 0.76$) but not significant (P = 0.082). For saline infusion, there was also no effect on hunger score (mean difference 1 ± 8). However, saline infusion did not result in a fMRI signal decrease as did water. Thus, subjects that were hungrier during the experiment showed a smaller fMRI response (signal decrease) after water

ingestion. This suggests that we measured an effect of thirst and raises the question how thirst relates to (subjective ratings of) hunger.

For oral glucose, a higher hunger score at 30 min was associated with a greater fMRI response (signal decrease) in the hypothalamus. A higher hunger score at 30 min was also associated with a smaller blood glucose AUC after oral glucose ($r = -0.90$, $P < 0.05$). The hypothalamus plays an important role in the regulation of the plasma glucose concentration and also influences the relative activity of the sympathetic and parasympathetic nervous systems (Perkins et al., 1981; Yoshimatsu et al., 1988). Hypoglycemia activates the sympathetic nervous system, a response which is inhibited by perfusion of the ventromedial hypothalamus with glucose (Borg et al., 1997). Therefore, it has been suggested that increased plasma glucose concentration causes inhibition of the sympathetic nervous system by direct inhibition of neural activity in the ventromedial hypothalamus (Matsuda et al., 1999). Our findings suggest a link between the blood glucose response after ingestion of a glucose solution, decreased neuronal activity in the hypothalamus and decreased hunger. This link is substantially weakened after infusion of glucose, which highlights the importance of preabsorptive signals.

In conclusion, our results confirm that signals that originate between mouth and blood stream contribute substantially to glucoregulation. In particular, we found that such preabsorptive signals contribute significantly to the hypothalamic response to glucose.

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Chapter 5

Hypothalamic functional MRI response after glucose ingestion is diminished in patients with type 2 diabetes mellitus.

Summary

Obesity is known to be one of the most important risk factors for development of type 2 diabetes. The hypothalamus plays an important role in glucose regulation. Previously, a prolonged decrease in the hypothalamic functional magnetic resonance imaging signal after ingestion of a glucose solution has been reported in healthy men. The aim of this study was to determine the hypothalamic response to glucose ingestion in patients with type 2 diabetes mellitus as compared to age-matched controls. 7 male patients with type 2 diabetes mellitus (body mass index 27.9 ± 2.0 kg/m^2) and 10 healthy (body mass index 26.1 ± 3.2 kg/m^2), age-matched men participated in a randomized, single-blind, case-control, observational study. Subjects were scanned twice on separate days, using functional magnetic resonance imaging. During the scan they ingested either a glucose solution (75 g in 300 mL water) or water (300 mL). Glucose ingestion resulted in a prolonged significant signal decrease in the upper hypothalamus ($P < 0.05$) in healthy men, but not in patients with type 2 diabetes. Furthermore, the fMRI areas under the curve after glucose ingestion were significantly correlated with fasting insulin and glucose concentrations in patients with diabetes. In conclusion, our findings suggest that the hypothalamic fMRI signal in response to glucose ingestion is impaired in patients with type 2 diabetes.

Introduction

The brain regulates energy homeostasis in response to signals from the gastrointestinal tract and adipose tissue. These signals inform the brain about the nutritional status. In order to remain constant body weight, appetite and energy balance are adjusted. The hypothalamus plays a central role in this regulation (Schwartz et al., 2000; Stanley et al., 2005). The hypothalamus contains glucose-responsive neurons and is involved in the regulation of glucose homeostasis (Benzo, 1983; Iguchi et al., 1981). Recently, a decrease in the blood oxygen level-dependent (BOLD) magnetic resonance imaging (MRI) signal was observed in the hypothalamus of healthy volunteers shortly after glucose ingestion (Smeets et al., 2005b)(Chapter 2). This signal decrease lasted for at least 30 minutes and was dose-dependent.

BOLD functional MRI (fMRI) provides a non-invasive measure of changes in neuronal activity levels based on the associated changes in the local concentrations of oxygenated and deoxygenated hemoglobin (Kwong et al., 1992; Ogawa et al., 1992). Earlier, an fMRI study provided evidence for distinct hypothalamic function in lean and obese humans (Matsuda et al., 1999): the fMRI signal changes in response to glucose ingestion were attenuated and delayed in obese subjects.

Obesity is known to be one of the most important risk factors for development of type 2 diabetes. Since the hypothalamus plays a role in glucose regulation, we hypothesized that the hypothalamic response to glucose might be disturbed in patients with type 2 diabetes. Therefore, the aim of this study was to investigate whether the hypothalamic response to glucose ingestion differed between patients with type 2 diabetes and healthy volunteers.

Materials and methods

Subjects
10 male patients with type 2 diabetes and 11 healthy men participated in this study, which was approved by the Medical Ethical Committee of the Leiden University Medical Center. Written informed consent was obtained from all subjects. Patients with type 2 diabetes were treated either with oral glucose-lowering medication or with diet alone and had to have a stable HbA1c (< 9.0%) for at least 3 months. Patients with poorly treated hypertension (>160/100 mmHg), chronic renal failure or leg ulcers were excluded from the study. The control group was matched for age, had fasting plasma glucose

(FPG) < 6 mmol/L and had no family history of type 2 diabetes. The use of medication known to alter glucose or lipid metabolism was prohibited. All subjects had to have a stable weight for at least 3 months, had to be non-smokers and were excluded in case of alcohol (>28 units/week) or drug abuse. All subjects underwent a medical screening including a physical examination and standard laboratory tests before inclusion in the study.

Experimental procedures

The subjects were studied after an overnight fast of at least 10 h. They were randomly assigned to one of the two experimental conditions: 300 mL glucose solution (75 g glucose) or 300 mL tap water. Subjects were studied on two separate days, at least one week apart. On every occasion magnetic resonance imaging was performed on a 3.0 Tesla scanner (Philips Achieva, Philips Medical Systems, Best, The Netherlands), equipped with a SENSE-head coil. The functional scan consisted of a T_2^*-weighted gradient-echo, segmented echo-planar imaging sequence (repetition time = 120 ms, echo time = 30 ms, flip angle = 30°, image matrix = 256 × 231, field of view = 208 × 208 mm, 12 signal averages/scan, 33 k-lines acquired/excitation pulse) with which a 12-mm thick midsagittal slice was scanned. The images were reconstructed to 256 × 256 pixels. The subjects were scanned for 38 min (225 scans). After a reference period (baseline) of 8.4 min (50 scans), the subjects ingested the test solution through a peroral tube. After the functional scan, a T_1-weighted anatomical scan was made of the same slice (repetition time = 550 ms, echo time = 10 ms, field of view = 208 × 208 mm).

Data preprocessing

fMRI data of the hypothalamus were pre-processed and analyzed as described previously (Smeets et al., 2005b). All 225 functional images of each time series were motion-corrected with the Multimodality Image Registration using Information Theory software for image registration by maximization of mutual information (Maes et al., 1997). The images were aligned to the middle image and the anatomical T_1-weighted image was also co-registered with this image.

Data analysis

For every subject, the hypothalamus was manually segmented with the use of the anatomical image and divided into four regions by two orthogonal axes according to predefined criteria (Matsuda et al., 1999): the upper anterior hypothalamus (UAH), lower anterior hypothalamus, upper posterior

hypothalamus (UPH), and lower posterior hypothalamus (**Figure 5.1**). The anterior–posterior axis was defined by the line passing through the centers of the anterior commissure and the mammillary body. The upper–lower axis was defined by the line passing through the optic chiasm, which is perpendicular to the anterior–posterior axis (Figure 5.1). In this study we used the UAH and the UPH as regions of interest (ROIs) as these regions are known to respond to glucose (Smeets et al., 2005a; Smeets et al., 2005b) (Chapters 2 and 3). In addition, a square reference area (10 × 10 pixels) of about the same size as the hypothalamus was delineated in the thalamus (Figure 5.1). At every time point, the mean grey value in the hypothalamus as a whole and in each ROI was calculated. Next, these mean grey values were normalized to the mean of their 8.4-min baseline value, which yielded the percentage signal change from mean baseline. The global signal drift (scanner drift) was corrected by subtracting the mean signal in the reference area from those in the ROIs.

For statistical comparison the data were pooled per minute, yielding 38 one-minute time slots. Differences between the two treatments were tested for by comparing the mean fMRI signal changes per minute between glucose and water ingestion with a Student's t-test. Also, the mean fMRI signal changes after glucose ingestion were compared for every one-minute time slot between healthy controls and patients with diabetes. This method is comparable to differential regression analysis (Cho et al., 2003). A Bonferroni-corrected threshold of $P = 0.0013$ was employed because we performed 38 t-tests per comparison.

The total fMRI response was assessed by calculating the areas under the fMRI curves (fMRI AUCs) with a trapezoidal integration. AUCs were
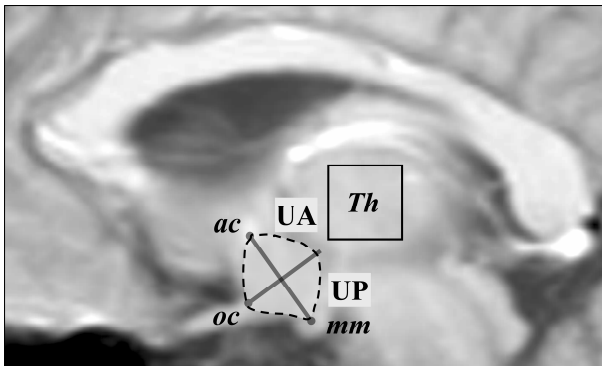

Figure 5.1. Segmentation and subdivision of the hypothalamus into two regions of interest (Matsuda et al., 1999). Abbreviations. ac: anterior commissure; mm: mammillary body; oc: optic chiasm; Th: thalamus; UA: upper anterior hypothalamus; UP: upper posterior hypothalamus.

calculated for every subject from t = 5 to t = 30 min after the onset of stimulus administration. Data from the first 5 min after the onset of drinking were excluded because drinking causes artifacts (Smeets et al., 2005b)(Chapter 2). Next, Pearson correlation coefficients were calculated between the fMRI AUCs and fasting blood glucose and insulin concentrations because correlations between fasting blood glucose and insulin concentrations and the hypothalamic fMRI response have been found (Matsuda et al., 1999).

Statistical analyses were done with SPSS statistical software (version 12.0; SPSS Inc, Chicago, IL). A P-value of 0.05 (two-sided and corrected for multiple comparisons when appropriate) was considered significant.

Assays

Serum glucose, creatinine, total cholesterol (TC), triglycerides (TG), alkaline fosfatase, ALAT, ASAT, γ-GT, and LDH were measured in the laboratory for Clinical Chemistry at Leiden University Medical Center, using a fully automated Hitachi Modular P800 system. HbA_{1c} was measured with high performance liquid chromatography (HPLC). Serum insulin was measured by an immuno-radiometric assay (INS-IRMA; BioSource Europe S.A., Nivelles, Belgium).

Results

10 male patients with type 2 diabetes and 11 healthy men were included in this study. Complete data sets were available for respectively 7 and 10 subjects. Data from three patients had to be excluded from the analysis; two patients moved their head out of the scan plane during the functional scans and one patient became unwell after swallowing air during the ingestion of the test solution. Of the healthy controls, data from one subject were excluded because of artefacts due to RF interference. **Table 5.1** shows clinical and biochemical characteristics of the two groups. There was no difference in the body mass index and body fat percentage between groups, while the weight-hip ratio was significantly higher in the diabetic group (P < 0.05).

Table 5.1. Subject characteristics[1].

Measure	Patients with type 2 diabetes	Healthy volunteers
Number of subjects	7	10
Age (years)	55.8 ± 3.6	52.3 ± 4.7
BMI (kg/m^2)	27.9 ± 2.0	26.1 ± 3.2
Waist-hip ratio	$0.95 \pm 0.05^{2*}$	0.87 ± 0.06
Fat percentage (%)	21.6 ± 3.4	18.2 ± 3.7
FPG (mmol/L)	$7.9 \pm 2.5^{*}$	4.8 ± 0.3
HbA$_{1c}$ (%)	6.3 ± 0.9	-
Fasting serum insulin (mU/L)	$13.5 \pm 10.0^{*}$	2.9 ± 1.4
Triglycerides (mmol/L)	1.3 ± 0.8	1.4 ± 0.5
Total cholesterol (mmol/L)	$4.1 \pm 0.55^{**}$	5.6 ± 1.1

[1] Data are presented as mean $\pm$ SD. Abbreviations: BMI: body mass index; FPG: fasting plasma glucose.
[2] N = 6 due to one missing value.
* Student's t-test P < 0.05
** Student's t-test P < 0.01

Hypothalamic fMRI signal

The average duration of stimulus ingestion was 5.5 ± 2.4 min (mean $\pm$ SD, both groups pooled). **Figure 5.2** shows the fMRI signal changes in the UAH (top) and the UPH (bottom) in the control group (left pane) and in patients with type 2 diabetes (right pane). At the start of the drinking (t = 0 min), large signal drops occur after ingestion of water and glucose solution in both groups. These signal changes result from artefacts caused by drinking and last for about 5 min. After that, a prolonged decrease in the fMRI signal was observed in the UAH and the UPH in the control group. In contrast, in patients with diabetes the fMRI signal returned to baseline shortly after glucose ingestion in both ROIs.
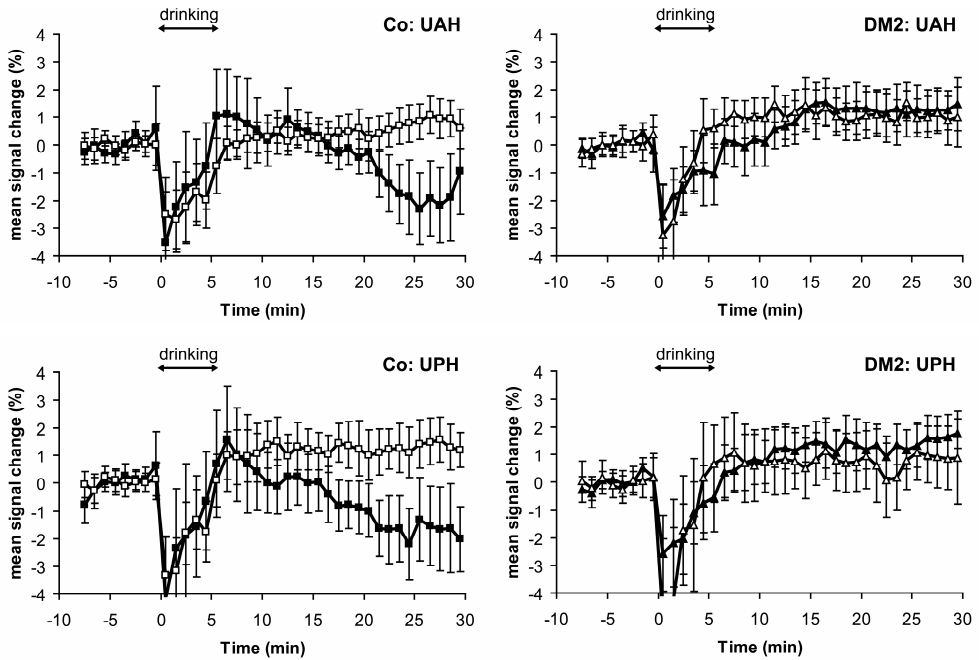
Figure 5.2. Mean (± SEM) fMRI responses in 7 patients with type 2 diabetes (DM2) and 10 healthy controls (Co) before and after ingestion of 300 mL glucose solution (75 g glucose, black symbols) or water (open symbols). UAH, upper anterior hypothalamus (top); UPH, upper posterior hypothalamus (bottom). Left: Control group: □, water; ■, glucose solution. Right: Patients with type 2 diabetes: Δ, water; ▲, glucose solution. T = 0 min is the onset of drinking. The horizontal arrows labelled "drinking" indicate the approximate duration of stimulus ingestion (~5 min).

Figure 5.3 shows the P-values of the Student's t-tests comparing the fMRI signal changes after glucose ingestion with those after water ingestion, in both ROIs. Exclusively in the control group there was a significant difference between the two treatments. In patients with diabetes no difference was observed.
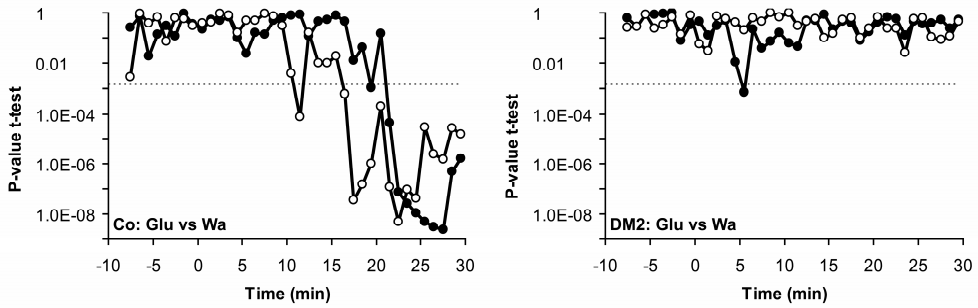
Figure 5.3. P-values of the Student's t-test comparing the mean fMRI signal changes per minute between the glucose condition (Glu; 75 g glucose in 300 mL water) and the water condition (Wa; 300 mL water) in the upper anterior hypothalamus (●) and the upper posterior hypothalamus (○) of healthy controls (Co, n=10) and patients with type 2 diabetes (DM2, n=7). T = 0 min is the onset of stimulus ingestion. The dotted line indicates the Bonferroni-corrected threshold of P = 0.0013.
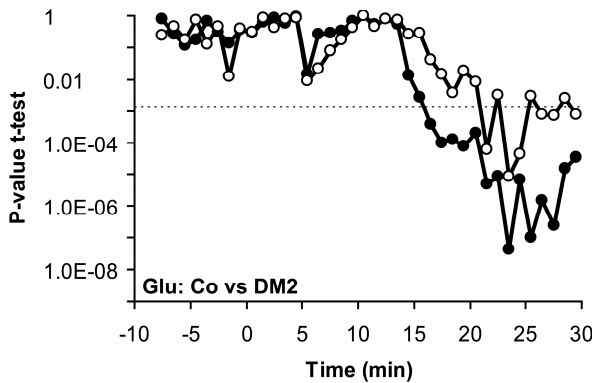
Figure 5.4. P-values of the Student's *t*-test comparing the mean fMRI signal changes per minute after glucose ingestion (Glu) between healthy controls (Co, n=10) and patients with type 2 diabetes (DM2, n=7) in the upper anterior hypothalamus (●) and the upper posterior hypothalamus (○). T = 0 min is the onset of stimulus ingestion. The dotted line indicates the Bonferroni-corrected threshold of P = 0.0013.

Figure 5.4 shows the P-values of the Student's t-tests comparing the fMRI signal changes after glucose ingestion between the two groups. The hypothalamic response to glucose in the control group differed significantly from that observed in diabetic patients from ~15 minutes after the onset of stimulus ingestion (in the UAH).

Table 5.2 shows that there is a significant correlation between the fMRI AUC in the UAH ($r = 0.86$, $P < 0.05$) and the UPH ($r = 0.83$, $P < 0.05$) after glucose ingestion and the fasting insulin concentration (at screening) in patients with diabetes. In addition, there is a significant correlation between the fMRI AUC in the UPH ($r = 0.87$, $P < 0.05$) and the fasting glucose

concentration (at screening) in patients with diabetes. These significant correlations found in patients with diabetes are shown in F**igure 5.5**. There were no significant correlations observed between these parameters in the control group.

Table 5.2. Pearson correlation coefficients between the fMRI area under the curve and fasting insulin and glucose concentrations after water and glucose ingestion[1].

Region	Fasting serum insulin		Fasting plasma glucose	
	Controls	DM2	Controls	DM2
UAH	0.30	0.86[*]	0.12	0.54
UPH	0.18	0.83[*]	0.14	0.87[*]

[1] Abbreviations: Controls, healthy age-matched control subjects (n=10); DM2, patients with type 2 diabetes (n=7); UAH, upper anterior hypothalamus; UPH, upper posterior hypothalamus
[*] $P < 0.05$
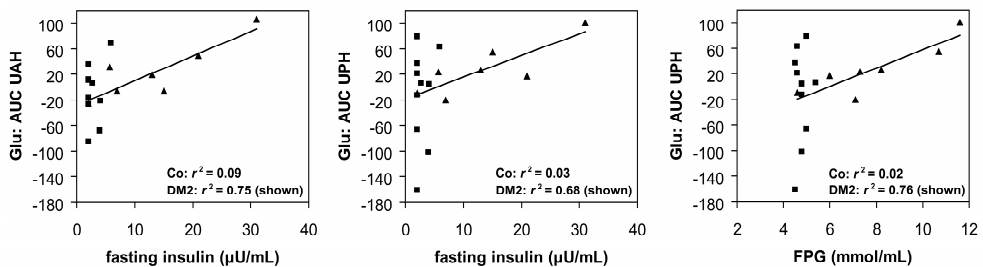
Figure 5.5. Scatter plots showing the relation between fasting insulin and glucose (FPG) concentration at screening and the fMRI area under the curve (fMRI AUC), after glucose ingestion (Glu). Legend: ■ Controls (Co) , ▲ Patients with type 2 diabetes (DM2). UAH, Upper anterior hypothalamus. UPH, Upper posterior hypothalamus.

Discussion

We here show that the hypothalamic response to glucose ingestion is impaired in patients with type 2 diabetes. The prolonged signal decrease that was observed in the UAH and the UPH after ingestion of glucose in non-diabetic healthy men was not seen in patients with diabetes.

Since the fMRI signal is thought to represent changes in neuronal activity, the inhibition observed in the hypothalamus after glucose ingestion in healthy subjects is absent in patients with diabetes. Recently, a fMRI study provided in vivo evidence for distinct hypothalamic function in lean and obese humans (Matsuda et al., 1999). In obese subjects, the inhibitory

fMRI signal in response to glucose ingestion, was attenuated and delayed. In more recent studies, a prolonged decrease in the fMRI signal was observed in the hypothalamus in healthy volunteers after ingestion of glucose (Smeets et al., 2005a; Smeets et al., 2005b)(Chapters 2 and 3). These data are in line with our findings in the control group, in which we found a prolonged decrease in the fMRI signal after glucose ingestion. However, the fMRI curves in our study show a delayed response compared with the curves in the previous studies. In our control group the fMRI signal shortly returned to baseline after glucose ingestion before declining, while in the other studies the decrease of the fMRI signal was present directly after drinking ended (Chapters 2 and 3) or at least did not return to baseline (Chapter 4). A possible reason for this is that the age of the healthy subjects here was roughly 30 years higher than that of the subjects studied before (Chapter 2-4). This suggests that the hypothalamic response to glucose is delayed in older subjects.

The long lasting fMRI signal decrease after glucose ingestion suggests a prolonged decrease in hypothalamic neuronal activity. It was recently found that intravenous administration of glucose resulted in a shorter and attenuated hypothalamic response as compared to response observed after oral administration (Chapter 5). Intravenous glucose (~35 g) resulted in a rapid, pronounced rise in serum glucose and insulin concentrations, while oral administration of glucose resulted in a slow rise in glucose concentration up to ~7 mmol/L, along with a less rapid but pronounced rise in insulin concentration. The shorter duration of the fMRI response after intravenous glucose administration suggests that the incretin effect might account for the prolonged signal decrease observed by oral glucose ingestion.

The hypothalamus, which is known to play a role in the glucose regulation (Benzo, 1983), sends signals to the peripheral organs, including the liver, by stimulating the autonomic nerves (Uyama et al., 2004) and by releasing hormones from the pituitary. Stimulation of the ventromedial hypothalamic nucleus (VMH), which is considered to be involved in the sympathetic outflow (Inoue and Bray, 1977; Niijima et al., 1984; Saito et al., 1989; Yoshimatsu et al., 1984), causes an increase in the hepatic glucose production due to an increase in glycogenolysis (Shimazu, 1981) and glyconeogenesis and suppression of glycolysis (Shimazu and Ogasawara, 1975). Interestingly, activation of the sympathetic nervous system by hypoglycemia (Frizzell et al., 1993; Frohman and Nagai, 1976) has been shown to be prevented by local perfusion of the VMH by glucose. As proposed by Matsuda et al. (Matsuda et al., 1999), this suggests that an

increase in plasma glucose concentration, or in the local glucose concentration within the VMH, inhibits the sympathetic nervous system through direct inhibition of neuronal activity in the VMH. This suggests that the apparent lack of a hypothalamic response to glucose in patients with type 2 diabetes might be associated with a lack of inhibition of the sympathetic nervous system, which would prevent a decrease in hepatic glucose production.

In patients with type 2 diabetes, a significant correlation between the fMRI AUC in the UAH and the UPH and the fasting insulin concentration was found, whereas no such correlation was observed in the control group. Fasting insulin constitutes a long term negative feedback signal of energy intake and body adiposity (Havel, 2001; Schwartz et al., 2000) that acts via the hypothalamus and other brain regions (Baskin et al., 1999; Schwartz et al., 1999). In Chapter 5, we found that the fasting insulin concentration correlated strongly with the hypothalamic fMRI response to intravenous but not to oral glucose in healthy men: higher fasting insulin concentrations were associated with a greater negative fMRI response. In the current study, on the contrary, higher fasting insulin values (at screening) were associated with a greater positive fMRI response in diabetics. Also, in patients with type 2 diabetes, a significant correlation was found between the fMRI AUC in the UPH and the fasting glucose concentration (FPG) at screening. The hypothalamus is involved in the regulation of the plasma glucose concentration, which is impaired in diabetics. A higher FPG might affect the autonomic nervous system and counteract the inhibition of the hypothalamus after glucose ingestion. More studies will be required to determine the mechanisms underlying the effects of the fasting insulin and glucose concentration on the hypothalamic response to glucose.

Since our study was conducted in patients with type 2 diabetes, these results can not be extrapolated to patients with other forms of diabetes that are characterized by an insulin secretion defect rather than by insulin resistance.

In conclusion, our results show that the hypothalamic fMRI signal in response to glucose ingestion is impaired in patients with type 2 diabetes.

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Chapter 6

Effects of satiety on brain activation during chocolate tasting in men and women

Summary

The brain plays a crucial role in the decision to eat, integrating multiple hormonal and neural signals. A key factor controlling food intake is selective satiety, *i.e.*, the phenomenon that the motivation to eat more of a food decreases more than does the motivation to eat foods not eaten. We investigated the effect of satiation with chocolate on the brain activation associated with chocolate taste in men and women. Twelve men and 12 women participated. Subjects fasted overnight and were scanned by use of functional magnetic resonance imaging while tasting chocolate milk, before and after eating chocolate until they were satiated. In men, chocolate satiation was associated with increased taste activation in the ventral striatum, insula, and orbitofrontal and medial orbitofrontal cortex and with decreased taste activation in somatosensory areas. Women showed increased taste activation in the precentral gyrus, superior temporal gyrus, and putamen and decreased taste activation in the hypothalamus and amygdala. Sex differences in the effect of chocolate satiation were found in the hypothalamus, ventral striatum, and medial prefrontal cortex (all $P < 0.005$). Our results indicate that men and women differ in their response to satiation and suggest that the regulation of food intake by the brain may vary between the sexes. Therefore, sex differences are a covariate of interest in studies of the brain's responses to food.

Introduction

The brain plays a crucial role in the decision to eat, integrating multiple hormonal and neural signals that convey information about the body's nutritional status (Schwartz et al., 2000). Among the many factors that influence the decision to start or stop eating, such as social context, the amount of food left and dietary restraint, the volume and energy content of the food consumed are most important. As more food is ingested, the feeling of fullness becomes stronger and the motivation to eat decreases (Rolls et al., 2000; Rolls and Roe, 2002). However, when a certain food is eaten, its pleasantness and the motivation to eat more of it decrease gradually, even though the stomach is not yet full (Rolls et al., 1983). In this case, one is still motivated to consume other foods, particularly those with different sensory characteristics; *i.e.*, one is not satiated per se, but is satiated to the specific food that was consumed. This phenomenon has been termed selective satiety (Kringelbach, 2005). Another widely used and closely related term, introduced by Rolls et al (1981), is sensory-specific satiety. This term is also used to refer to what we defined above as selective satiety, but is usually more specific, referring to selective satiety of one of the senses, *e.g.*, olfactory sensory-specific satiety. Following the definitions of Blundell, we use the term satiation when referring to the process of becoming satiated, which ends with meal termination, and the term satiety when referring to the subsequent state of being satiated (Blundell, 1991). Sensory-specific satiation for odor and taste have been shown to have neural correlates in the orbitofrontal cortex (Kringelbach et al., 2003; O'Doherty et al., 2000).

Although sex differences in eating behavior have often been described (Bates et al., 1999; Beer-Borst et al., 2000; Provencher et al., 2003; Rolls et al., 1991; Woods et al., 2003), sex differences in the brain activation associated with food stimuli are undocumented. Most neuroimaging studies investigating the effects of food stimuli use either men or both men and women. For a better understanding of the regulation of food intake, it might be important to differentiate between men and women. In the present study, we examine the effect of satiation with chocolate on the brain activation associated with chocolate taste in both men and women.

Materials and methods

Subjects

Twenty-four healthy normal-weight volunteers participated [12 men, mean age 21.3 ± 2.8 y, mean body mass index (BMI; in kg/m^2) 21.5 ± 1.6, and 12 women, mean age 20.5 ± 1.4 y, mean BMI 22.0 ± 1.4). Subjects were recruited through an advertisement put up at various locations in the University Medical Center Utrecht. We used a Health and Lifestyle Questionnaire to assess general health and aspects of lifestyle relevant to the study and the Dutch Eating Behavior Questionnaire (Van Strien et al., 1986) to assess eating behavior (restrained eating in particular). The main purpose of these questionnaires was to screen for subjects who would in any way be unwilling to consume a large amount of chocolate, *e.g.*, because they were consuming a medically prescribed diet or were concerned with their weight. Exclusion criteria included a BMI <19 or >25; being under 18 or over 28 y of age on the study day; smoking; a history of alcohol consumption or current alcohol consumption of >28 units/wk; a history of medical or surgical events that may significantly affect the study outcome, such as metabolic or endocrine disease or any gastrointestinal disorder; irregular eating habits; following a weight-reduction diet or a medically prescribed diet; restrained eating [for men, a score >2.50, and for women, a score >3.50 on the Dutch Restrained Eating Questionnaire (Van Strien et al., 1986)]; use of medication (except aspirin, paracetamol, or contraceptive pills); claustrophobia; diabetes; and metal implants or metal objects on the body that cannot be removed (*e.g.*, piercing, hearing aid, or brace). Written informed consent was obtained from all subjects according to the Declaration of Helsinki, and the study protocol was approved by the Medical Ethical Committee of the University Medical Center Utrecht, Utrecht, The Netherlands.

Experimental procedures

The study procedures are summarized in **Figure 6.1**. The subjects were instructed to fast overnight from 10:00 pm (no food or beverages, except water). In the morning, the subjects were scanned twice: before eating bittersweet chocolate until satiety (*i.e.*, fasted) and after (*i.e.*, satiated). During both scans, chocolate milk was administered (0.1 mL/s) followed by water (0.2 mL/s, to wash away the chocolate taste) and a rest period: three blocks of 30 s each, repeated nine times. Stimuli were administered at room temperature (23 °C). To achieve chocolate satiety, the subjects were satiated with bittersweet chocolate because this is a food with a strong taste that
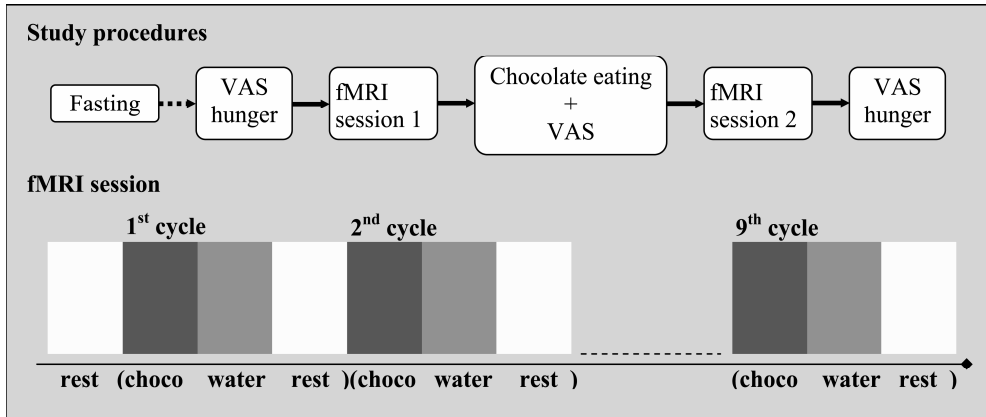
Study procedures

Fasting ┈▶ VAS hunger ▶ fMRI session 1 ▶ Chocolate eating + VAS ▶ fMRI session 2 ▶ VAS hunger

fMRI session

1st cycle   2nd cycle   9th cycle

rest (choco water rest)(choco water rest)   (choco water rest)

Figure 6.1. Summary of study procedures and visual representation of the functional magnetic resonance imaging (fMRI) block design. Choco, administration of chocolate milk; VAS, visual analogue scale; water, administration of water.

requires chewing, which leads to prolonged and enhanced sensory stimulation. Hence it was expected that a relatively small amount would induce chocolate satiety, circumventing overall satiety due to filling of the stomach (volume effect). After eating the chocolate, the subjects rinsed their mouths with tap water to remove chocolate residues, which might otherwise have affected their subsequent taste experience in the scanner. Chocolate milk was used as a stimulus during scanning because it has a chocolate flavor and is relatively easy to administer.

Scans were performed on a 1.5-T Philips Gyroscan ACS-NT system (Philips Medical Systems, Best, The Netherlands) by using a multislice 2D single-shot EPI sequence [repetition time/echo time (TR/TE) = 2500/45 ms, flip angle = 90°, 24 interleaved slices, $4 \times 4 \times 4$ mm^3 voxels). In addition, an anatomical T_1-weighted volume was acquired (TR/TE = 30/4.6 ms, flip angle = 30°, 48 axial slices, $1 \times 1 \times 2$ mm^3 voxels). So that we could assess their general motivation to eat, the subjects filled out a set of four visual analogue scales (VASs, range: 0 to 100 mm) before the first and after the second scan session on which they reported their feelings of general hunger, fullness, general desire to eat, and prospective food consumption (Flint et al., 2000). For every subject, the scores of these four scales were averaged to obtain a single general hunger score. To keep the subjects alert in monitoring the chocolate's taste and their motivation to eat during chocolate eating, palatability and desire to eat more chocolate were assessed after every two pieces of chocolate eaten (≈13 g) with two other VASs.

Data processing and analysis

Functional magnetic resonance imaging (fMRI) data were preprocessed and analyzed with the SPM2 software package (Wellcome Department of Imaging Neuroscience, London, United Kingdom) implemented in MATLAB 6.5 (The Mathworks Inc, Natick, MA). The functional volumes of every subject were realigned to the first volume of the first run, globally normalized (resampling to $2 \times 2 \times 2$ mm^3), and spatially smoothed with a gaussian kernel of 10 mm full width at half maximum. A statistical parametric map was generated for every subject by fitting a boxcar function to each time series, convolved with the canonical hemodynamic response function and its temporal derivative. Data were high-pass filtered with a cutoff of 128 s. Both the chocolate milk and the water delivery blocks were modeled. The response to water, however, was neglected in further analyses, because water delivery only served to wash away the preceding chocolate taste. To regress out motion-related activations, the motion-correction parameters from the realignment procedure were added to the model as covariates.

Within-subject analyses

For every subject, parameters were estimated for six comparisons (usually referred to as contrasts), yielding six contrast images: two for tasting chocolate milk in the hungry state versus rest (activation termed taste hungry and deactivation termed taste hungry neg), two for tasting chocolate milk in the satiated state versus rest (activation termed taste satiated and deactivation termed taste satiated neg), and two contrast images assessing the effects of satiation on taste activation. For the last two contrast images, one assessed the positive effects of satiation, *i.e.*, areas where the taste activation in the satiated state was greater than that in the hungry state (termed taste satiated minus taste hungry), and one assessed the negative effects of satiation, *i.e.*, areas where the taste activation in the hungry state was greater than that in the satiated state (termed taste hungry minus taste satiated). In the last analysis, taste-specific activations unaffected by satiation, *i.e.*, activations common to both states and that were not of interest here, are expected to subtract out (*e.g.*, motor activations related to tongue movement and swallowing). This is because taste activations in the two states are compared within one subject. By activation we refer to fMRI signal increases, and by deactivation to fMRI signal decreases.

Between-subject analyses

Group analyses were performed by using the contrast images. Our main analysis involved subtraction of activation maps in the fasted and the

satiated state. Because the main aim of this study was to study differences in taste activation due to satiation with chocolate, areas of taste deactivation were disregarded during the analyses by means of masking. Mask images were created by performing one-sample t-tests on the taste hungry neg and taste satiated neg contrast images of men, women, and both men and women. The resulting t-maps were thresholded at P = 0.05 (uncorrected for multiple comparisons) and converted to exclusive binary mask images. The following analyses were performed:

1) Effects of satiation on taste activation in men and women. Voxels where satiation resulted in fMRI signal increases were tested for by putting the taste satiated minus taste hungry contrast images of men and women into a one-sample t-test, masked for deactivation during hunger. Similarly, voxels where satiation resulted in fMRI signal decreases were tested for by putting the taste hungry minus taste satiated contrast images of men and women into a one-sample t-test, masked for deactivation during satiety. T-Maps were thresholded at P = 0.005 (uncorrected for multiple comparisons).

2) Sex differences in the effect of satiation on taste activation. Voxels with a differential effect of satiation on taste activation in men and women were tested for by comparing the taste satiated minus taste hungry and taste hungry minus taste satiated contrast images of men and women by using two-sample t-tests. In this analysis, a positive effect of satiation that is greater in men than in women is equivalent to a negative effect of satiation that is greater in women than in men.

3) Region of interest (ROI) analysis. In addition to the whole-brain analyses outlined above, we performed the same tests confined to four a priori ROIs: the hypothalamus, the amygdala, the insula, and the orbitofrontal cortex. All of these regions have been implicated in taste processing, and the hypothalamus and amygdala are areas showing sex differences in response to emotional stimuli (Hamann et al., 2004; Schwartz et al., 2000; Sewards and Sewards, 2003; Small et al., 2003; Wrase et al., 2003). ROIs were analyzed by using the WFU Pickatlas tool (Maldjian et al., 2003), which confines the analysis to a volume of interest and uses SPM's small volume correction. The hypothalamic ROI was defined as an 8-mm sphere centered on Montreal Neurological Institute coordinates (0, -4, -8) and the amygdala ROI as two 8-mm spheres centered on Montreal Neurological Institute coordinates (-20, -4, -20) and (20, -4, -20). For the insula ROI, we used the mask image supplied with the WFU Pickatlas tool. The orbitofrontal cortex ROI consisted of a mask containing all orbital gyri and was generated by use of the software package MARINA (Bender Institute of Neuroimaging, University of Giessen, Giessen, Germany),

which uses the anatomical parcellation of the brain published by Tzourio-Mazoyer et al (2002).

4) Subjective ratings. Mean (general) hunger scores were calculated for every subject by averaging the VAS scores of general hunger, 100 minus fullness, general desire to eat, and prospective food consumption. The effect of chocolate satiation on the mean hunger score and the general desire to eat was calculated by subtracting the score in the fasted state from that in the satiated state. Similarly, the decrease in chocolate palatability and the desire to eat more chocolate were calculated by subtracting the score in the fasted state (after the first two pieces of chocolate) from that in the satiated state (after the last piece of chocolate eaten).

Sex differences in these VAS scores and in the effect of satiation on them were tested for with a two-sample t-test. Also, Pearson correlation coefficients were calculated between these VAS scores (mean general hunger score, general desire to eat, palatability, and desire to eat chocolate) and the amount of chocolate eaten and between the effect of satiation on these scores and the amount of chocolate eaten. These analyses were done with SPSS statistical software (version 12.0.1; SPSS Inc, Chicago, IL). P-values < 0.05 (two-sided) were considered significant.

Results

Our ability to measure significant taste activations in single subjects in the insula and orbitofrontal cortex is shown in **Figure 6.2** (P < 0.05 corrected for multiple comparisons). The latter is an area that can be difficult to image with fMRI because of signal loss caused by the air in the nasal cavity and sinuses.
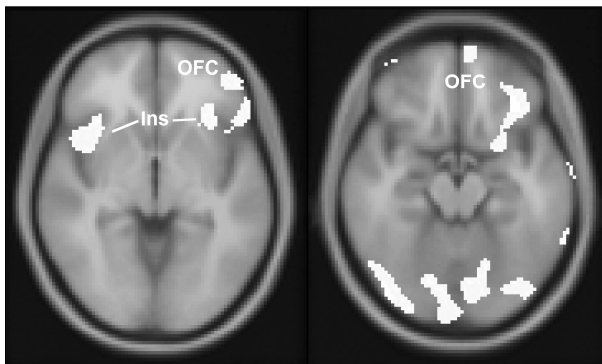
Figure 6.2. T-value maps of two subjects tasting chocolate milk, overlaid on overlaid on axial sections of an average anatomical image in neurologic orientation (*i.e.*, left is left). Images were obtained with the use of magnetic resonance imaging. The T-maps are thresholded at T = 4.79 (P = 0.05, family-wise error-corrected for multiple comparisons). Taste activations were assessed by statistical parametric mapping. Ins, insula; OFC, orbitofrontal cortex.

Effect of satiation on taste activation in men and women

Brain regions in men and women in which chocolate satiation affected taste activation are tabulated in **Table 6.1** and **Table 6.2** respectively. In men, chocolate satiation was associated with increased taste activation in the posterior part of the left ventral striatum (globus pallidus and putamen), left precentral gyrus, dorsolateral prefrontal cortex, left dorsal striatum (putamen), anterior insula, and the orbitofrontal and medial orbitofrontal cortex (anterior cingulate). Decreased taste activation was observed in somatosensory areas (inferior and superior parietal lobules) and the medial prefrontal cortex (medial part of the superior frontal gyrus, just anterior of the supplementary motor area). Selected activations are shown in **Figure 6.3**. Men showed no effect of satiation in the amygdala or hypothalamus.

In women, chocolate satiation was associated with increased taste activation in the precentral gyrus (bilaterally, but predominantly on the right), right superior temporal gyrus, and ventral striatum (putamen). Decreased taste activation was observed in the hypothalamus and amygdala. Selected activations are shown in **Figure 6.4**. Women showed no effect of satiation in the insula or orbitofrontal cortex.

Figure 6.3. Brain regions in men (*n* = 12) where the taste activation in response to chocolate milk was affected by satiation with chocolate. Shown are T-value maps thresholded at T = 3.11 (P = 0.005 uncorrected for multiple comparisons) overlaid on axial sections of an average anatomical image in neurologic orientation (*i.e.*, left is left). Images were obtained with the use of magnetic resonance imaging. Regions with increased taste activation after satiation are shown in white; regions with decreased taste activation after satiation are shown in black. Differences in taste activation induced by satiation with chocolate were assessed by statistical parametric mapping. The figure is intended for visual inspection of some regions of the brain, including the Insula (Ins), medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), putamen (Pu), and ventral striatum (VS). Details of all affected brain regions are shown in Table 6.1.
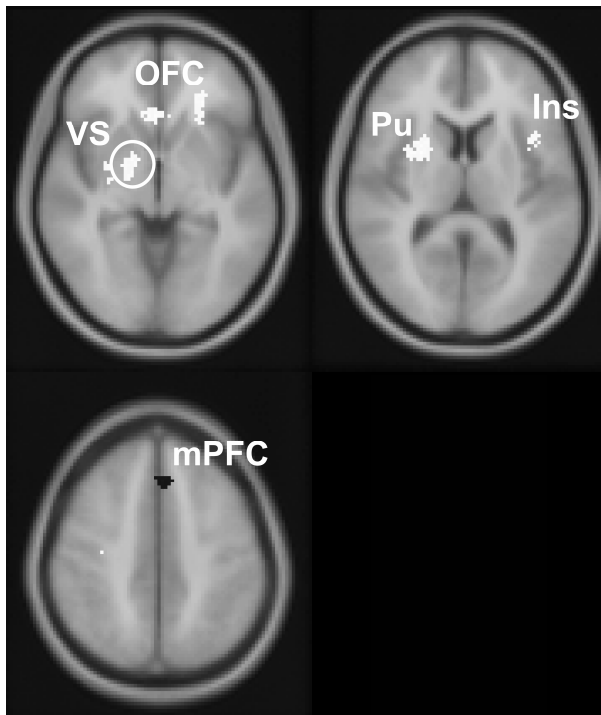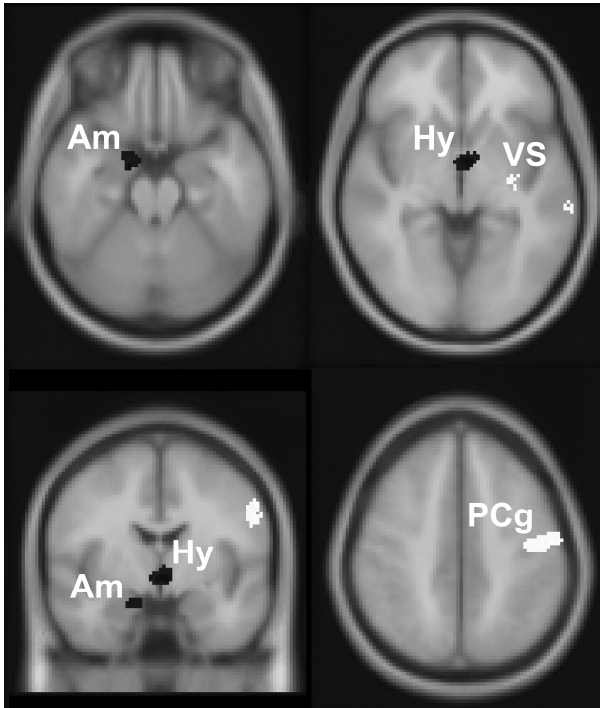
Figure 6.4. Brain regions in women ($n =$ 12) where the taste activation in response to chocolate milk was affected by satiation with chocolate. Shown are T-value maps thresholded at T = 3.11 (P = 0.005 uncorrected for multiple comparisons) overlaid on sections of an average anatomical image in neurologic orientation (*i.e.*, left is left). Images were obtained with the use of magnetic resonance imaging. Regions with increased taste activation after satiation are shown in white; regions with decreased taste activation after satiation are shown in black. Differences in taste activation induced by satiation with chocolate were assessed by statistical parametric mapping. The figure is intended for visual inspection of some regions of the brain, including the amygdala (Am), hypothalamus (Hy), precentral gyms (PCg), and ventral striatum (VS). Details of all affected brain regions are shown in Table 6.2.

Sex differences in the effect of chocolate satiation on taste activation

Brain regions that are differentially affected by chocolate satiation in men and women are tabulated in **Table 6.3**. Sex differences in the effect of chocolate satiation were found in the hypothalamus (a region showing a negative effect of satiation on taste activation in women, see Table 6.2 and Figure 6.4), ventral striatum (a region showing a positive effect of satiation on taste activation in men, see Table 6.1 and Figure 6.3), and medial prefrontal cortex (medial frontal gyrus, a region with a negative effect of satiation on taste activation in men, see Table 6.1 and Figure 6.3). No significant differences between the sexes were found in the insula, amygdala, or orbitofrontal cortex.

Subjective ratings

VAS scores and the amount of chocolate eaten are summarized in **Table 6.4**. On average, the men ate more chocolate than did the women (P < 0.05) and reported to be hungrier than the women were at the start of the experiment (P < 0.05). However, the decrease in the general hunger score due to chocolate eating did not differ between the sexes, nor did the scores

Table 6.1 Brain regions affected by satiation with chocolate in men[1]

Region	Effect of satiation[1]	Cluster size[2]	Peak voxel location[3]			z-score	SD[4]
			x	y	z		
Whole brain							
L ventral striatum / globus pallidus	pos	197[5]	-20	-8	-4	3.83	0.15
L ventral striatum / globus pallidus	pos	197[5]	-16	-2	0	3.39	0.26
L putamen	pos	197[5]	-35	-6	2	3.35	0.22
L precentral gyrus	pos	136	-34	-16	38	3.69	0.37
L dlPFC / superior frontal gyrus	pos	34	-14	12	66	3.55	0.28
R orbitofrontal cortex	pos	81	22	32	-4	3.47	0.34
L medial OFC / anterior cingulate	pos	63	-6	26	-4	3.33	0.28
L anterior insula	pos	112[5]	34	4	10	3.23	0.37
L dorsal striatum / putamen	pos	112[5]	-24	6	8	3.19	0.28
L cuneus	pos	48	-8	-80	20	3.21	0.40
L precentral gyrus	pos	72	-40	-8	36	3.17	0.53
L superior occipital gyrus	pos	92	-20	-90	16	3.09	0.44
L inferior parietal lobule	neg	29	-54	-34	54	3.24	0.57
L superior parietal lobule	neg	47	-42	-46	62	3.18	0.58
mPFC / superior frontal gyrus	neg	40	0	26	46	2.95	0.47
Insula ROI							
L anterior insula	pos	16	-34	4	10	3.23	0.37
R anterior insula / frontal operculum	pos	26	38	12	10	2.96	0.41
Orbitofrontal cortex ROI							
L medial OFC / anterior cingulate	pos	16	-6	26	-4	3.33	0.28

[1] Effects of satiation were tested for by performing t-tests on the difference between taste activation in the fasted state and that in the satiated state for all brain voxels by using statistical parametric mapping. dlPFC, dorsolateral prefrontal cortex; L, left; mPFC, medial prefrontal cortex; neg, decreased taste activation after satiation; OFC, orbitofrontal cortex; pos, increased taste activation after satiation; R, right; ROI, region of interest.

[2] Reported clusters were thresholded at P < 0.005 with a cluster threshold of K > 20 voxels for the whole brain and K > 10 voxels for ROIs.

[3] Voxel coordinates are in the Montreal Neurological Institute (MNI) space (Evans et al., 1993).

[4] The SD was calculated by taking the square root of the residual variance, i.e., the variance in the data that is not explained by the model that was fitted to the data.

[5] A cluster of significant voxels that contains more than one peak voxel or encompasses more than one anatomic location.

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Table 6.2 Brain regions affected by satiation with chocolate in women[1]

Region	Effect of satiation[1]	Cluster size[2]	Peak voxel location[3]			z-score	SD[4]
			x	y	z		
Whole brain							
R precentral gyrus	pos	475[5]	44	-14	48	3.47	0.42
R precentral gyrus	pos	475[5]	56	-2	32	2.98	0.62
R superior temporal gyrus	pos	63	66	-30	12	3.29	0.31
R ventral striatum / putamen	pos	38	30	-16	-6	3.21	0.34
R superior temporal gyrus	pos	41	54	-10	8	2.90	0.79
L precentral gyrus	pos	18	-42	-16	48	2.81	0.50
Hypothalamus	neg	114	2	-4	-4	4.49	0.35
L amygdala	neg	92	-18	-6	-22	4.12	0.33
Amygdala ROI							
L amygdala	neg	65	-18	-6	-22	4.12	0.33
Hypothalamus ROI	neg	93	2	-4	-4	4.49	0.35

[1] Effects of satiation were tested for by performing t-tests on the difference between taste activation in the fasted state and that in the satiated state for all brain voxels by using statistical parametric mapping. L, left; neg, decreased taste activation after satiation; pos, increased taste activation after satiation; R, right; ROI, region of interest.
[2] Reported clusters were thresholded at P < 0.005 with a cluster threshold of K > 20 voxels for the whole brain and K > 10 voxels for ROIs.
[3] Voxel coordinates are in the Montreal Neurological Institute (MNI) space (Evans et al., 1993).
[4] The SD was calculated by taking the square root of the residual variance, *i.e.*, the variance in the data that is not explained by the model that was fitted to the data.
[5] A cluster of significant voxels that contains more than one peak voxel.

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Table 6.3 Brain regions that were differentially affected by satiation with chocolate in 12 men and 12 women[1]

Region	Cluster size[2]	Peak voxel location[3]			z-score	SD[4]
		x	y	z		
Whole brain						
Hypothalamus[5]	122[6]	-2	-4	-6	3.66	0.80
L ventral striatum / globus pallidus[7]	122[6]	-14	-2	-2	3.26	0.43
R medial frontal gyrus[8]	33	6	26	46	3.25	0.55
Hypothalamus ROI	98	-2	-4	-6	3.66	0.80

[1] L, left; R, right; ROI, region of interest. Differential effects of satiation in men and women were tested for by comparing the effect of satiation with chocolate on taste activity during tasting chocolate milk for all brain voxels with a t-test and statistical parametric mapping.

[2] Reported clusters were thresholded at $P < 0.005$ with a cluster threshold of $K > 20$ voxels for the whole brain and $K > 10$ voxels for ROIs.

[3] Voxel coordinates are in the Montreal Neurological Institute (MNI) space (Evans et al., 1993).

[4] The SD was calculated by taking the square root of the residual variance, *i.e.*, the variance in the data that is not explained by the model that was fitted to the data.

[5] Region with a negative effect of satiation on taste activation in women.

[6] A cluster of significant voxels that encompasses more than one anatomical location.

[7] Region with a positive effect of satiation on taste activation in men.

[8] Region with a negative effect of satiation on taste activation in men.

relating to general desire to eat (Table 6.4). The scores of chocolate palatability and desire to eat chocolate and the decreases therein also did not differ significantly between men and women. In both sexes, the decrease in the general desire to eat was smaller than the decrease in the desire to eat chocolate ($P < 0.05$). Correlations between the amount of chocolate eaten and (changes in) subjective ratings in men and women are shown in **Table 6.5**. In both men and women, the decrease in the desire to eat chocolate correlated positively with the amount of chocolate eaten (men, $r = 0.60$; women, $r = 0.62$; both $P < 0.05$), whereas the decrease in chocolate palatability did not. In women, there was a strong negative correlation between the general desire to eat in the fasted state and the amount of chocolate eaten ($r = -0.82$, $P < 0.01$). Thus, women who reported a higher general desire to eat at the start of the experiment subsequently ate a smaller amount of chocolate.

Table 6.4 Subjective ratings in and the amount of chocolate eaten by men and women[1]

Measure	Men (n = 12)	Women (n = 12)
VAS general hunger, fasted	78 ± 10	70 ± 8[2]
VAS general hunger, satiated	32 ± 20	25 ± 16
Decrease VAS hunger (fasted – satiated)	46 ± 22	44 ± 18
VAS general desire to eat, fasted	76 ± 18	70 ± 14
VAS general desire to eat, satiated	27 ± 21	25 ± 27
Decrease VAS general desire to eat (fasted – satiated)[3]	49 ± 27	45 ± 28
VAS palatability of chocolate, fasted	85 ± 13	84 ± 11
VAS palatability of chocolate, satiated	39 ± 27	22 ± 21
Decrease VAS palatability of chocolate (fasted – satiated)	46 ± 33	61 ± 21
VAS desire to eat chocolate, fasted	84 ± 14	79 ± 13
VAS desire to eat chocolate, satiated	8 ± 11	2 ± 3
Decrease VAS desire to eat chocolate (fasted – satiated)[3]	76 ± 20	77 ± 13
Amount of chocolate eaten (g)	157 ± 59	106 ± 48[2]

[1] All values are mean ± SD. Visual analogue scale (VAS) scores were taken before (fasted) and after (satiated) subjects ate bittersweet chocolate until they were satiated.
[2] Significantly different from men, P < 0.05 (two-sample t-test).
[3] The decrease in the general desire to eat was significantly smaller than the decrease in the desire to eat chocolate in men (paired-sample t-test, P < 0.01) and in women (paired-sample t-test, P < 0.05).

Table 6.5 Pearson's correlation coefficients for the correlation between the amount of chocolate eaten and (changes in) subjective ratings in men and women[1]

	Correlation with the amount of chocolate eaten (g)		
Measure	Men (n = 12)	Women (n = 12)	Men and women (n = 24)
VAS general hunger, fasted	0.36	-0.69[2]	0.13
Decrease VAS general hunger (fasted - satiated)	0.34	-0.37	0.08
VAS general desire to eat, fasted	-0.03	-0.82[3]	-0.21
Decrease VAS general desire to eat (fasted - satiated)	-0.01	-0.33	-0.11
VAS palatability of chocolate, fasted	0.17	0.09	0.15
Decrease VAS palatability of chocolate (fasted - satiated)	0.12	0.19	0.00
VAS desire to eat chocolate, fasted	0.56	0.55	0.57[3]
Decrease VAS desire to eat chocolate (fasted - satiated)	0.60[2]	0.62[2]	0.53[3]

[1] Visual analogue scale (VAS) scores were taken before (fasted) and after (satiated) subjects ate bittersweet chocolate until they were satiated.
[2] Significant correlation, P < 0.05.
[3] Significant correlation, P < 0.01.

Discussion

We investigated the effects of satiation with chocolate on the brain activation associated with chocolate taste and found that these are mostly different in men and women. Effects of satiation on brain activation have

been shown for visual, olfactory, and gustatory food stimuli (Hinton et al., 2004; Holsen et al., 2005; Kringelbach et al., 2003; LaBar et al., 2001; O'Doherty et al., 2000; Small et al., 2001). In particular, it has been reported that brain activation in parts of the orbitofrontal cortex decreases after selective satiation (Kringelbach et al., 2003; O'Doherty et al., 2000; Small et al., 2001). These studies used men or men and women. In our study, we examined men and women separately. In men, we found increased taste activation in parts of the orbitofrontal cortex in response to chocolate satiation, whereas in women we found no effects in the orbitofrontal cortex. O'Doherty et al (2000) found decreased olfactory activation in part of the orbitofrontal cortex after satiation with bananas (the sex of the subjects was not reported). Kringelbach et al (2003) found that similar parts of the orbitofrontal cortex showed decreased brain activation in response to tomato juice and chocolate milk after these were drunk to satiety. It should be noted that the method of analysis in both these studies was aimed at detecting activation decreases. In a positron-emission study in which subjects of both sexes were gradually satiated with chocolate, the decreasing reward value correlated with decreased activation in the caudomedial orbitofrontal cortex and increased activation in the caudolateral orbitofrontal cortex (Small et al., 2001). This suggests that changes in orbitofrontal cortex activation after selective satiation relate to the decreased motivation to eat. Taken together, these and our findings warrant further investigation of the roles of the orbitofrontal cortex in the processing of food stimuli, looking at increases as well as decreases in activation and bearing sex in mind.

We found positive effects of satiation on taste activation in men in the ventral and dorsal striatum and in women in the ventral striatum. In men, effects of sensory-specific satiation with chocolate milk on taste activation in the ventral striatum (putamen) were found that were absent when the subjects were satiated with tomato juice (Kringelbach et al., 2003). Moreover, Small et al (2001) reported effects of satiation with chocolate in striatal regions (dorsal striatum, putamen. and caudate). This suggests that these striatal effects of satiation are specific to chocolate.

We found positive effects of chocolate satiation in the precentral gyrus (motor cortex) in both sexes. Other studies, with different methods, also showed effects of satiation on precentral gyrus activation (Holsen et al., 2005; Small et al., 2001). In men, we observed decreased taste activation in response to satiation in somatosensory areas. This agrees with positron-emission data showing increased glucose metabolism in the somatosensory cortex of hungry subjects in response to the multimodal presentation of an attractive food (Wang et al., 2004). Another positron-emission study, which

found enhanced resting state metabolism in the oral somatosensory cortex of obese subjects, suggested that this could indicate increased sensitivity to the rewarding properties of food (Wang et al., 2002). Similarly, in our study, satiation could have caused desensitization in somatosensory areas.

Amygdala

The amygdala is known to respond to both aversive and pleasant taste stimuli (O'Doherty et al., 2001; Zald, 2003). We found that amygdala activation in women decreased after chocolate satiation. This agrees with previous studies reporting decreased activation in the amygdala after odor-specific satiation (O'Doherty et al., 2000) and smaller amygdala responses to food-related visual stimuli in response to satiation (LaBar et al., 2001). Amygdala activation has been linked to emotional intensity (Royet et al., 2003; Small et al., 2003) and, more generally, to the significance of the stimulus being evaluated (LaBar et al., 2001; Pelchat et al., 2004). Our findings in women agree with this: the taste of a liked food is more significant during hunger than during satiety and this is reflected in amygdala activation. In men, we found no effect of satiation on amygdala activation. Sex differences in amygdala response have been reported in the context of visual emotional stimuli (Klein et al., 2003; Wrase et al., 2003; Zald, 2003), but not in the context of satiation.

Hypothalamus

The hypothalamus is important in the regulation of food intake (Schwartz et al., 2000; Sewards and Sewards, 2003). In women, we found decreased taste activation in the hypothalamus in response to satiation. This could reflect the decrease in hunger, *i.e.*, the decreased motivation to eat chocolate. This hypothesis agrees with previous work suggesting that neuronal activity in the lateral hypothalamic area represents reward value (Rolls, 1989) and that activity in the dorsomedial hypothalamus represents hunger (Sewards and Sewards, 2003).

Insula

The insula contains the primary taste cortex. In our study, taste activation in the anterior insula increased after satiation in men. Small et al (2001) reported relative cerebral blood flow decreases with decreasing reward value of chocolate in the dorsal insula/operculum. In contrast, it was shown in macaques that the responsiveness of neurons in the insular gustatory cortex is independent of hunger (Yaxley et al., 1988). Also, the anterior insula responds in a similar way to the oral delivery of water in the thirsty

and the satiated state (De Araujo et al., 2003). This suggests that more studies using different taste stimuli and motivational states are needed to further elucidate the functional neuroanatomy of the insula.

Sex differences

We found sex differences in the effect of satiation in the hypothalamus, ventral striatum, and medial prefrontal cortex. This adds to the growing number of studies reporting sex differences in stimulus processing in the brain, including responses to visual emotional stimuli (Canli et al., 2002; Klein et al., 2003; Lee et al., 2002; Wrase et al., 2003), sadness (Schneider et al., 2000), odors (Royet et al., 2003), and extreme hunger and satiety (Del Parigi et al., 2002). The sex differences we found suggest that satiation might work differently in men and women. There is supportive evidence for this from other fields that suggests that women are more affected than men by the hedonic value of food (Beatty, 1982; Zylan, 1996).

Study design

We maximized chocolate satiety and minimized the amount of chocolate ingested by satiating subjects with small pieces of chocolate with a high cocoa content (52%). As intended, the decrease in the general desire to eat was smaller than the decrease in the desire to eat chocolate in both sexes. As in normal eating behavior, satiation with chocolate in our experiment involved not only satiation for a particular taste (chocolate) but also an inevitable concomitant increase in overall satiety because of the volume and energy content of the food ingested (Bell et al., 2003; Rolls and Roe, 2002). Thus, the observed effects of chocolate satiation on taste activation primarily reflect the decreased motivation to consume more chocolate, but also incorporate a decrease in the general motivation to eat.

We used solid chocolate for satiation and chocolate milk for tasting in the scanner. This approach presumes that satiation for solid chocolate extends to other chocolate substances. With the use of a similar approach with bananas and banana odor, effects of olfactory sensory-specific satiation were shown in the brain for the first time (O'Doherty et al., 2000). Ideally, the same substance is used for satiation and testing. However, in fMRI paradigms, this precludes solid food stimuli.

Another source of variability that is part of every study of this kind lies in the differences in the taste experience of subjects. Some might find the chocolate taste to be stronger than others do. Also, the effect of this strong taste on the subsequent tasting of chocolate milk likely varies between subjects. This could be assessed by obtaining taste intensity ratings.

However, how differences in taste intensity experienced by subjects relate to their patterns of brain activity remains to be investigated.

In summary, we showed different effects of chocolate satiation on taste activation in men and women. Our results suggest that the sexes differ in their response to satiation. Therefore, sex differences are a covariate of interest in studies of the brain's responses to tasting food and the regulation of food intake.

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Chapter 7

General discussion

This thesis describes the changes in brain activity in response to food stimuli as measured by functional MRI, with a focus on the hypothalamus. The results described in the preceding chapters are discussed in relation to the ultimate goal of this research, which was to find biomarkers of satiety in the brain. The sense and non-sense of such biomarkers is discussed and ideas for future research are given.

• Main findings

We found a prolonged and dose-dependent decrease in the hypothalamic fMRI signal that started within few minutes after the ingestion of a glucose solution (Chapter 2). This signal decrease proved to be specific to glucose ingestion in so far that no signal decreases were observed in the hypothalamus after ingestion of a non-sweet carbohydrate (maltodextrin solution) and a sweet-tasting solution (aspartame) (Chapter 3). Compared to ingestion of glucose, the hypothalamic response after infusion of glucose into the bloodstream was smaller and less prolonged. The blood insulin response to ingestion of glucose was also more prolonged than that to infusion of glucose, and was associated with better glucoregulation (Chapter 4). In patients with type 2 diabetes mellitus, ingestion of glucose was not associated with a decrease in the hypothalamic fMRI signal as it was in healthy control subjects (Chapter 5).

In Chapter 6, we found sex differences in the effect of satiation with chocolate on the brain activity associated with tasting chocolate milk in the hypothalamus, ventral striatum and medial prefrontal cortex.

• The meaning of fMRI signal changes

Acute: in response to a sensory stimulus

   Although the physiological mechanisms that underlie increases in the Blood Oxygen Level Dependent (BOLD) fMRI signal in response to a stimulus are not completely understood there is an empirically robust relationship between increased neuronal activity and increased blood flow. Because the local tissue oxygen consumption does not increase to the same extent, the increase in blood flow results in a lower concentration of deoxyhemoglobin. This results in an increase in the BOLD fMRI signal (Berns, 1999; Raichle, 1998). Recently, it has been shown that increases in the fMRI signal are indeed synonymous with increases in local neuronal activity (Logothetis et al., 2001; Mukamel et al., 2005; Smith et al., 2002). BOLD fMRI signal decreases, on the contrary, have been the subject of much more dispute. Apart from signifying neuronal deactivation (Allison et al., 2000; Gusnard et al., 2001; Raichle et al., 2001; Shmuel et al., 2002; Smith et al., 2004), fMRI signal decreases have been viewed as a side-phenomenon to neuronal activation in the so-called vascular blood-steal hypothesis. This states that increased blood flow in areas of increased neuronal activation causes decreased blood flow in adjacent areas (Harel et al., 2002; Kannurpatti and Biswal, 2004). Recently, however, it has been shown that decreases in neuronal activity are a significant part of decreases in the fMRI signal in the visual cortex (Shmuel et al., 2006). In summary, stimulus-induced changes in the BOLD fMRI signal provide an indirect measure of associated changes in neuronal activity. It is important to note that BOLD fMRI provides a relative rather than a quantitative measure. Usually, fMRI signal changes are assessed relative to a 'no stimulus' control condition. Often this is 'resting with eyes closed' but it can also be a more specific control condition, such as tasting artificial saliva (Francis et al., 1999). In Chapter 6, we used a different approach and compared the response to tasting chocolate milk before and after satiation with chocolate, which obviated the need for a control taste stimulus.

Long term: in response to a food stimulus

   In Chapters 2-5 a decrease in the BOLD fMRI signal in the hypothalamus has been measured for up to 30 min after glucose ingestion. The interpretation of such long term fMRI signal changes has not received attention in the literature. Basically, the BOLD signal relates to the degree of oxygenation of the blood, which depends on the rate of oxygen extraction and on the local cerebral blood flow. Changes in the blood flow, as

measured by PET, are considered a marker for neuronal activity such that brain regions with more neuronal activity show a higher blood flow. This relies on the idea that brain regions that are more active need more oxygen and nutrients and therefore have a higher blood flow. Tataranni et al. (1999) reported decreased relative cerebral blood flow in the hypothalamus of normal-weight men in response to satiation with a liquid meal after a 36-h fast. Also, it has been argued that an increased plasma glucose concentration causes inhibition of the sympathetic nervous system by inhibition of neural activity in the hypothalamus (Matsuda et al., 1999). The above suggests that the decreases in the hypothalamic fMRI signal after glucose ingestion are associated with decreased blood flow and decreased neuronal activity.

• The hypothalamic fMRI signal as a biomarker?

The central role of the hypothalamus in the regulation of food intake makes it a brain region of particular interest when looking for a central biomarker. In Chapters 2-4 we investigated the possible use of the hypothalamic fMRI signal as a measure of metabolic satiety, *i.e.*, satiety related to the nutritional value of a food. In favor of this, we reported a dose-dependent decrease in the hypothalamic fMRI signal after glucose ingestion in Chapter 2. However, another carbohydrate (maltodextrin) did not elicit a fMRI decrease (Chapter 3). This suggests that this hypothalamic response is specific to glucose, although other, more common, carbohydrates such as sucrose or fructose remain to be tested. In Chapter 4, the comparison of the effects of intravenous and oral administration of glucose on the hypothalamic fMRI signal showed that preabsorptive signals contribute substantially to the hypothalamic response to glucose. Moreover, a higher hunger score at 30 min after glucose ingestion was associated with a greater fMRI response (signal decrease) in the hypothalamus, whereas it was also associated with a smaller blood glucose response. This suggests a link between the blood glucose response after ingestion of glucose, decreased neuronal activity in the hypothalamus and decreased hunger. In addition, the findings in Chapter 4 emphasized that the central role of the hypothalamus in energy homeostasis as well as other homeostatic processes entails a number of possible confounding factors that also affect the hypothalamic fMRI signal such as blood pressure changes, fasting insulin concentration and thirst. This could explain the high between-subject variability we observed in the hypothalamic fMRI response to glucose. Another factor that could affect hypothalamic responses to satiety is gender. The sex differences in the effect of satiation with chocolate on the brain

activity associated with tasting chocolate milk that we found in Chapter 6 might relate to the effects of metabolic satiety on the hypothalamus rather than to taste-specific satiety effects.

Apart from the investigation of healthy subjects, fMRI measurements of the hypothalamic response to glucose might be useful for characterizing or investigating pathologies that relate to glucose metabolism such as obesity and diabetes mellitus (Matsuda et al. (1999), Chapter 5).

Taken together, the hypothalamic fMRI response to glucose has proven to be a reproducible dose-dependent measure that is affected by preabsorptive signals, the fasting blood insulin concentration and pathology (type 2 diabetes mellitus). Moreover, it correlated with subjective ratings of hunger after glucose ingestion.

• The sense and non-sense of biomarkers of satiety

The physiological and psychological processes that govern the feeling of hunger and the decision to eat are very complex. Although some useful conceptual distinctions can be made such as those between metabolic and sensory satiety, or between the motivation to eat (wanting) and the expected pleasure of eating (liking), the decision to eat ultimately depends on multiple factors, internal as well as external. In my opinion, the feeling of hunger is an example of the organistic nature of biological systems. Up to some point, biological processes can be explained mechanistically, *e.g.*, in terms of the interactions between molecules or cells. However, interactions between such lower-level elements give rise to meta-phenomena on higher levels of organization that cannot be explained in terms of those interactions alone; the whole is more than the sum of its parts. Therefore it is unlikely that there is a single peripheral measure, such as the concentration of a hormone in the blood, that adequately predicts the chance that somebody will start eating or the amount of food that will be eaten. In terms of the theory just sketched, a lower-level element can not account for a meta-phenomenon on a higher level of organization such as the feeling of satiety. If this is indeed a meta-phenomenon the only place where this could be measured is in the brain, because that is where information is integrated and the feeling of satiety resides. The question that emerges then is whether the neuronal activity in a part of the brain, such as the hypothalamus, provides a useful measure of the meta-phenomenon 'satiety'. This question is synonymous with the ultimate goal of this thesis, which was to find central biomarkers of satiety, *i.e.*, physiological measures in the brain that relate to

subjectively rated appetite, actual food intake, or both (De Graaf et al., 2004).

The practical use of fMRI measurements as biomarkers, *e.g.*, to support a claim for the satiety-enhancing capability of a functional food, is unlikely because they are expensive and not easily carried out (De Graaf et al., 2004). Ideally, a biomarker of satiety correlates with subjective ratings of (aspects of) satiety. Now suppose we find a biomarker in the brain that correlates extremely well with the subjective feeling of hunger or even with the amount of calories eaten. For testing novel foods this biomarker would not be particularly useful since measuring subjective hunger or actual food intake would be much easier. Thus, the use of this biomarker would be in the deepened insight that it entails and not in its practical application as a biomarker. Naturally, this newly acquired knowledge could result in an application. For example, it could lead to the development of interventions, such as drug delivery, that affect this biomarker and the feeling of satiety. This could be part of the prevention or treatment of obesity. This has the interest of companies, because health products and anti-obesity drugs sell more and more, as well as of national economies, because obesity and comorbid diseases are costly in terms of health care and the loss of working power. With respect to this I want to emphasize that the basic prevention of affluent diseases in general and obesity in particular lies in maintaining a lifestyle with enough physical exercise and a varied diet. The choice for that is up to the individual consumer (and parent). Basically, high-tech foods are not required, although these are inevitably marketed and could contribute to weight control.

In summary, from the work presented in this thesis and other studies it can be concluded that fMRI is a valuable tool with which more insight into the effects of food on the brain can be gained. The search for biomarkers of satiety, whether successful or not, provides a better understanding of the physiological mechanisms behind the regulation of food intake and energy balance. Such knowledge can help in preventing and curing obesity.

• Other neuroimaging techniques that could provide central biomarkers of satiety

Perfusion fMRI

Perfusion functional MRI relies on a technique called arterial spin labeling (ASL), in which magnetically labeled arterial blood water is used as a diffusible tracer to estimate cerebral blood flow (CBF), in a manner

analogous to that used for [15]O PET scanning. In short, a series of non-labeled (control) images and labeled images are acquired in an interleaved manner. Then, perfusion images are obtained by subtracting the control images from the labeled images pair-wise. Perfusion fMRI has several advantages compared to BOLD fMRI (Detre and Wang, 2002) (Aguirre et al., 2002; Wang et al., 2003), among which are: reduced baseline signal drift and motion artefacts, improved sensitivity for slow changes in neuronal activity, reduced inter-subject variability, and reduced sensitivity to susceptibility artefacts. The regions that are prone to susceptibility artefacts are the limbic system (hypothalamus, amygdala), orbitofrontal and inferior temporal regions. These are all regions of interest when studying the effects of taste or satiation on the brain. An additional advantage over BOLD fMRI is that perfusion fMRI offers the possibility of absolute quantification: under a number of assumptions one can estimate cerebral blood flow from the perfusion measurements (Buxton et al., 1998; Luh et al., 1999; Wang et al., 2003). Current drawbacks, that will likely be resolved in the near future, are that ASL techniques are more difficult to implement and have less imaging coverage than BOLD techniques. A major drawback that has hindered application of ASL techniques in functional imaging is that signal changes are smaller than for BOLD contrast, typically less than 1 % on a 1.5 T scanner. However, on MRI scanners with a higher static field this problem diminishes. In conclusion, perfusion fMRI is a promising technique for studying the relatively slow effects of food satiation in the brain especially at higher field strengths (3 Tesla and higher).

Electroencephalography (EEG) and Event-related potentials (ERPs)

An EEG reflects the electric potential resulting primarily from synaptic trans-membrane currents in neuronal dendrites; its signals that are directly coupled to neuronal electrical activity. An EEG is recorded with electrodes placed on the scalp (currently usually 64 or 128 electrodes). ERPs are a series of positive and negative voltage deflections in the EEG that are time-locked to specific sensory, motor or cognitive events. Compared to PET and fMRI, EEG measurements are cheap and relatively easy to perform, although they have their practical limitations as well. Studies into the effects of satiety on the EEG and ERPs have found that, although there are effects of satiation on the EEG, these are aspecific effects that can be attributed to increased arousal (Hoffman et al., 1999; Hoffman and Polich, 1998). This disqualifies EEG as a technique for measuring effects of satiety in the brain. However, the strength of EEG lies in its high temporal resolution, which is in the order of milliseconds. This makes the combination with fMRI, which

has a high spatial resolution, worthwhile for studying the sequence of neuronal events (Dale and Halgren, 2001), *e.g.*, during tasting.

• Ideas for future research

Other stimuli or designs

It is as yet unclear in how far the hypothalamic response to glucose is specific to glucose. Therefore, it would be of interest to investigate the effects of the ingestion of other sugars, in particular those used in food products and beverages, such as sucrose and fructose. Also, the combination of sweet taste and caloric content might be crucial for a hypothalamic response (Chapter 3). This could be examined by studying the effects of different combinations of sweeteners and carbohydrates, *e.g.*, using solutions of different carbohydrates with different levels of (added) sweetness. In addition to the effects of such relatively simple food stimuli, the effects of more complex food stimuli, such as milk shakes or liquid meals, on the hypothalamic fMRI signal could be assessed.

In this thesis, stimuli (mainly glucose solutions) were mostly administered orally (by ingestion). This corresponds best with normal food intake but entails artefacts in the fMRI data due to the movements that accompany drinking. In Chapter 4, glucose was administered intravenously, which excludes signalling pathways that originate between the mouth and the stomach and which does not suffer from motion artefacts during administration. An intermediate mode of administration would be to infuse food stimuli into the stomach. This would preclude effects that originate in the mouth and esophagus.

Yet another approach would be to infuse hormones involved in the regulation of food intake into the bloodstream while imaging the hypothalamus, *e.g.*, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), Peptide $YY_{(3-36)}$ (PYY) or ghrelin.

Other imaging techniques

In this thesis, the hypothalamus was imaged by scanning a $10 - 12$ mm thick sagittal slice. An important practical reason for this approach was that this enables good movement correction, as most movement associated with swallowing and drinking occurs in this plane. An axial cross-section, however, might enable segmentation of the lateral hypothalamus ('hunger center'). Also, a well choosen axial plane could include the brain's reward center (nucleus accumbens). A prerequisite would be good fixation of the

head and, obviously, scanning of an axial cross-section would combine best with infusion of stimuli into the bloodstream or the stomach (*i.e.*, not with ingestion).

As put forward above, perfusion fMRI has certain advantages over BOLD fMRI, especially when trying to image the effects of satiety. A relatively simple first experiment would be to image the brain before and after consumption of a meal. Perfusion fMRI focussed on the hypothalamus would also be of interest because it is less prone to susceptibility artefacts than BOLD fMRI. This, however, will require a high-field scanner in order to obtain sufficiently high spatial resolution and signal to noise ratio.

Other types of subjects

In this thesis, we mostly investigated young lean men (Chapters 2-4). As Chapter 6 and studies of others suggest, it would be of interest to compare the brain responses to food stimuli of men and women. In studies looking at the effects of food, men are often preferred over women as subjects because women's hormone levels are more variable. Ideally, women are measured in the same phase of their menstrual cycle, but this can entail practical difficulties, *e.g.*, in study planning.

Apart from healthy individuals, people with certain pathologies could be imaged. This can provide insight into hypothalamic dysfunction and characterization of pathologies. In Chapter 5, we found a diminished hypothalamic response in patients with type 2 diabetes mellitus. Other pathologies of interest are obesity (see *e.g.* Matsuda et al. (1999)), type 1 diabetes mellitus, and eating disorders, such as bulimia or anorexia nervosa.

• **Conclusions**

The hypothalamic fMRI response to glucose is a reproducible dose-dependent measure. So far, it has proven to be specific to glucose. Its correlation with subjective ratings of hunger after glucose ingestion makes it an interesting candidate-biomarker of metabolic satiety. It is also affected by preabsorptive signals, the fasting blood insulin concentration and pathology (type 2 diabetes mellitus). Thus, the research in this thesis provides the first steps to much more functional neuroimaging research into the effects of food stimuli on the hypothalamus and the rest of the brain. In general, functional MRI provides valuable and promising tools for investigating the regulation of food intake in the brain.

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Samenvatting

Het uiteindelijke doel van het in dit proefschrift beschreven onderzoek was het vinden van centrale biomerkers van verzadiging: objectieve maten in de hersenen die samenhangen met het subjectieve gevoel van verzadiging. In dit proefschrift zijn veranderingen in hersenactiviteit beschreven in respons op voedingsstimuli, zoals gemeten met functionele Magnetische Resonantie (fMRI), met een focus op de hypothalamus. De hypothalamus is een interessant hersengebied wat verzadiging betreft omdat zij een centrale rol speelt in de regulatie van voedsel-inname. Functionele MRI is een techniek waarmee op niet-invasieve wijze indirect neurale activiteit gemeten kan worden aan de hand van veranderingen in de zuurstofconcentratie in het bloed in de hersenen. In dit proefschrift is onderzocht of fMRI-metingen aan de hypothalamus gebruikt kunnen worden als een biomerker van metabole verzadiging. Eerdere studies hebben laten zien dat het mogelijk is om met één lange fMRI-scan veranderingen in het fMRI-signaal in de hypothalamus te meten na inname van een glucose-oplossing. In **Hoofdstuk 2** is een vergelijkbare experimentele opzet gebruikt om de respons van de hypothalamus op glucose te onderzoeken. Een belangrijke vraag was of deze respons dosis-afhankelijk is. Proefpersonen dronken in de MRI-scanner een oplossing met 25 of 75 gram glucose, of water. Er werd een langdurige en dosis-afhankelijke afname in het hypothalame fMRI-signaal gevonden. Deze afname begon binnen enkele minuten na de stimulus-inname; nog voor de absorptie van glucose in de bloedbaan. Dit riep de vraag op wat de respectievelijke bijdragen van de zoete smaak en van de energie-inhoud van de glucose-oplossing aan deze respons waren. Daarom werd nog een experiment gedaan, wat beschreven is in **Hoofdstuk 3**. In dit experiment kregen proefpersonen niet alleen een glucose-oplossing en water te drinken, maar ook oplossingen van aspartaam (zoete smaak) en van maltodextrine (een niet-zoet koolhydraat). Ook werden de glucose- en insuline-concentraties in het bloed gedurende het experiment bepaald. Er werd gevonden dat alleen glucose een afname in het fMRI signaal in de hypothalamus teweeg bracht. Deze afname begon vóór de stijging van de glucose-spiegel in het bloed. Zoete smaak (aspartaam) en energie-inhoud (maltodextrine) daarentegen veroorzaakten geen veranderingen in het fMRI-signaal. Deze bevindingen suggereren dat er glucose-specifieke preabsorptieve mechanismen zijn die de hypothalame

respons veroorzaken en dat deze niet gestuurd worden door de zoete smaak van glucose. Om de bijdrage van dergelijke mechanismen aan de hypothalame respons op glucose te onderzoeken werd het effect van orale inname van glucose vergeleken met dat van intraveneuze toediening van glucose. Ook werden weer glucose- en insuline-concentraties in het bloed bepaald. Dit experiment is beschreven in **Hoofdstuk 4**. Er werd een verminderde respons op glucose gevonden na infusie; het fMRI-signaal daalde minder en de daling duurde minder lang vergeleken met die na orale glucose inname. De insuline respons na orale glucose inname was ook langer dan die na glucose infusie en was geassocieerd met een betere regulatie van de bloedsuikerspiegel. Ook werden correlaties gevonden tussen de totale fMRI respons na glucose infusie en de nuchtere insuline concentratie en tussen de totale fMRI respons na orale glucose inname en subjectieve honger scores. In **Hoofdstuk 5** is de hypothalame respons op glucose van gezonde controles vergeleken met die van patienten met diabetes type 2. Diabeten vertoonden geen daling in het hypothalame fMRI signaal na glucose inname. In **Hoofdstuk 6** is gekeken naar de effecten van sensorische verzadiging in plaats van die van metabole verzadiging. Zowel mannen als vrouwen proefden chocolademelk in de MRI-scanner, voor en na verzadiging met pure chocolade. Er werden sexe-verschillen gevonden in het effect van de verzadiging met chocolade op de hersenactiviteit geassocieerd met het proeven van chocolademelk in de hypothalamus, het ventrale striatum en de mediale prefrontale cortex.

Geconcludeerd kan worden dat de respons van de hypothalamus op glucose inname reproduceerbaar en dosis-afhankelijk is. Voor zover nu bekend is deze respons specifiek voor glucose. De hypothalame respons op glucose is een kandidaat-biomerker voor metabole verzadiging, temeer daar zij correleert met subjectieve honger-scores na glucose inname. Zij wordt ook beïnvloed door preabsorptieve signalen, de nuchtere insuline concentratie en pathologie (diabetes type 2). Derhalve is het onderzoek beschreven in dit proefschrift de eerste stap op weg naar meer niet-invasief hersenfunctie-onderzoek naar de effecten van voedingsstimuli op de hypothalamus en de rest van het brein. In het algemeen kan gezegd worden dat functionele MRI-technieken waardevol en veelbelovend zijn voor het onderzoeken van de regulatie van voedsel-inname in de hersenen. De zoektocht naar biomerkers voor verzadiging, of zij nu succesvol is of niet, resulteert in een beter begrip van de fysiologische mechanismen die ten grondslag liggen aan de regulatie van voedsel-inname en energiebalans. Dergelijke kennis kan van nut zijn bij de preventie en behandeling van overgewicht.

List of Publications

International Journals

Smeets P.A.M., Van Osch M.J.P., De Graaf C., Stafleu A., Van der Grond J., 2005. Functional MRI of human hypothalamic responses following glucose ingestion. *NeuroImage* 24(2): 363-368.

Smeets P.A.M., De Graaf C., Stafleu A., Van Osch M.J.P., Van der Grond J., 2005. Functional magnetic resonance imaging of human hypothalamic responses to sweet taste and calories. *American Journal of Clinical Nutrition* 82(5): 1011-1016.

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Smeets P.A.M., Van Osch M.J.P., Stafleu A., De Graaf C., Van der Grond J., 2004. Human hypothalamic responses following glucose ingestion. *Proceedings of the International Society for Magnetic Resonance in Medicine* 11: 595.

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Other publications

Smeets P.A.M., Duchateau M., 2001. Feeding behaviour in the bumble bee *Bombus terrestris*. *Belg. J. Zool.* 131 suppl. 2: 11-18.

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Dat waren de min of meer direct betrokkenen, maar er zijn nog veel meer mensen te noemen. De eerste paar jaar werkte ik iedere donderdag bij TNO, later minder. Dat was een aangename verandering van omgeving; andere sfeer, andere collega's. Qua bureaucratisch gehalte overtreft TNO het UMCU, wat er toch ook wel wat van kan. Ik diende, naast een handtekening, zelfs een paraaf te ontwikkelen. Wendy Blom, deeltijd-kamergenoot samen met Elleny, en projectgenoot, je bent inmiddels een ongetwijfeld glansrijke carriere begonnen bij Unilever, veel succes daarmee! Henk Hendriks, ook jij hebt een inspirerend enthousiasme voor onderzoek en ik was verheugd dat je in de beoordelings-commissie wilde plaatsnemen. Wat me van TNO naast het ochtendlijke koffie-kletsen in een kringetje het meeste bijstaat zijn de jaarlijkse afdelingsuitjes. Nog steeds als ik een ongemeen smerige sloot zie denk ik terug aan de overwinning van mijn team in de poldersport-competitie en de bijbehorende kaasschaaftrofee. Toen TNO Voeding onderdeel werd van TNO Kwaliteit van Leven, een naamvervaging die ik betreur, en ik door een gemaakt-enthousiaste virtuele organisator benaderd werd om mee te doen heb ik bedankt.
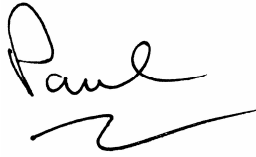
Dan het ISI, zoals gezegd een heterogene en gezellige groep. Allereerst Hans Peeters. Niet alleen het prototype van een ideale schoonzoon volgens sommigen, maar ook van een ideale kamergenoot volgens mij. Ik vond het machtig mooi dat wij over alles konden praten. We houden allebei van reizen en zijn niet vies van een beetje lichaamsbeweging. Ik denk dat niet velen trouwen en promoveren in hetzelfde jaar. Hans, ik hoop dat jullie huwelijksdag net zo onvergetelijk en overweldigend wordt als de onze en wens jullie nu alvast heel veel geluk! Dan Rashindra Manniesing, inmiddels meer vriend dan collega en mijns inziens een rasechte wetenschapper. Ik hoop dat we nog vaak een saunaatje pakken en dat we nog veel interessante gesprekken hebben. Dan wil ik noemen, in willekeurige volgorde, anders moet ik er zo over nadenken: Gerard van Hoorn, à la minute probleem-oplosser en 1 van de stabiele factoren van het ISI, niet alleen als systeembeheerder maar ook als deelnemer aan sportieve en gezellige activiteiten. Roei ze! Sandra Boeijink, financieel baken en vraagbaak. Andere stabiele factoren: het creatieve duo Koen zuip-je-lens-voor-een-

medemens Vincken en Wilbert MRI Bartels, die blijven bijdragen aan een niet aflatende stroom aan promotie-stukjes danwel Ardennen-weekenden, Xmas-parties, Fietsenrallies etc.. Daarnaast heeft Koen mij, zeker in de eerste jaren, vaak van een scriptje of een unix-tipje voorzien. Verder: Theo van Walsum, aimabele iX-guru. Everine enthousiast! van der Kraats, veel geluk! Marloes gelukkig-getrouwd Letteboer. Nelly Anbeek, die meer humor heeft dan je wellicht op het eerste gezicht denkt. De oprechte en vriendelijke Jan-Henry Seppenwoolde. Josien registratie-vraagbaak Pluim. Bas fijnproever Gobets. Eleftheria spitting image Astreinidou, good luck, too bad our joint experiments did not really work out. Casper van Oers, altijd goed voor een gezellige danwel diepzinnige babbel. Stefan Elastix Klein en Marius Elastix Staring. Andriy temperature mapping Shmatukha, good luck with finishing your thesis! Van de CAD-groep: de onverbeterlijke Bram van Ginneken en Meindert easy on Niemeijer. De 'nieuwe lichting': Peter, Niels, Martijn, Sandra, Sara, Patrick, Adriënne en Jaco: veel succes! Het secretariaat: Marjan, Jacqueline en Renée, altijd behulpzaam en in voor een praatje. De bedachtzame fysicus Chris Bakker die nooit naliet een praatje te maken, ook als Hans er even niet was. Nog zeer bedankt dat ik op het MR-Fysica Pride-station mijn data mocht verwerken; dat heeft me veel kostbare tijd gescheeld. Mijn klinische van der Grond-genoten Jeroen turbo-TILT klini-clown Hendrikse en Peter-Jan van Laar.

Dan het pre-promotie tijdperk. Allereerst mijn ouders, die Ben en mij met veel zorg en liefde hebben grootgebracht en ons een zorgeloze jeugd hebben bezorgd. Jullie hebben altijd benadrukt dat ik leuk moest vinden wat ik deed en waren altijd onvoorwaardelijk behulpzaam bij het realiseren van mijn keuzes. Mijn drang tot doorgronden van de wereld en mezelf kon ik mede uitleven door de lessen van Ben van de Baar, een vrijdenker met humor, die ons jongelingen inleidde in de geschiedenis van de filosofie en aanzette tot kritische reflectie. Dat was eind middelbare school. Wat ik daar geleerd had werd weer aangewakkerd door de erudiete en vol vuur gebrachte colleges van inmiddels emeritus hoogleraar Jan van Hooff. Het was mij een voorrecht om daarbij werkcolleges te maken teneinde eerstejaars biologen tot kritische reflectie te brengen, wat overigens niet gelukt zou zijn zonder de steun van de bedachtzame doch enthousiaste didacticus Joop Buddingh'. Later in mijn studie was het Marie-José Duchateau die mij met haar enthousiasme inspireerde om onderzoek te doen, en met haar schreef ik mijn eerste twee artikelen die handelden over de aardhommel. Ondertussen bood aikido een goed tegengewicht aan al dat hoofdwerk. Na dik 10 jaar heb ik er nog altijd niet genoeg van, sterker nog, de groeiende diepgang maakt het alleen maar boeiender. Ik ben zeer verguld met mijn mede-aikidoka van

dojo Heiwakan in Den Haag. Een ander belangrijk onderdeel van mijn bestaan, waar ik ook na dik 10 jaar nog steeds geen genoeg van heb is het Centrum voor Leven en Intuïtie, een unieke down-to-earth plek ter zelf-ontplooïng, die laat zien dat intuïtie niet iets zweverigs is maar een volwaardige menselijke kwaliteit, net als de veel geprezen ratio. Verder dank ik voor hun voortdurende vriendschap Yink spitsvondig liefje Goossens, Wendy Hee You Veling en Paula mmm Mommersteeg.

Dan ten laatste mijn lieve spontane vrouw Marion en mijn zoon Timo. Ik hou van jullie en heb een onwankelbaar vertrouwen in onze toekomst!

Curriculum vitae

Paulus Anna Mathijs Smeets (Paul) was born on 14 February 1975 in the hospital of Roermond, The Netherlands. He spent his childhood in the countryside of the Province of Limburg near the Belgium border, and went to primary school in the small village of Neeritter. After that, he attended the Gymnasium in Horn (1986-1993). After half a year of studying Mechanical Engineering at the Technical University Eindhoven, he acknowledged that the practice of engineering was much more fun that its theory and decided to study living things instead of dead ones. As a result, he studied Biology at Utrecht University (1994 – 2000), with an eventual focus on Behavioral Biology. He carried out two research projects. One at the Dept. of Child Psychiatry of the University Medical Center Utrecht, involving children with ADHD/Conduct Disorder, and one at the Department of Socio-ethology of the Faculty of Biology focused on the feeding behavior of the bumble bee. Other highlights included a year of being the Secretary of the board of the Utrechtse Biologen Vereniging (the student society of the Faculty of Biology) and a half-year stay at Tohoku University in Sendai, Japan. After his graduation, he shortly worked as a garbage man and then as a laboratory analyst in a genome fingerprinting project at the Hubrecht Laboratory in Utrecht. Eventually, in 2002, he started his PhD project at the Image Sciences Institute (ISI) of the University Medical Center Utrecht. The main aim of his project, which was a collaboration between TNO Nutrition and Food Research (now part of TNO Quality of Life) and the ISI, was to find biomarkers of satiety in the human brain with the use of functional Magnetic Resonance Imaging (fMRI). The results of this research are presented in this thesis.