“Well," said Pooh, "what I like best," and then he had to stop and think. Because although Eating Honey was a very good thing to do, there was a moment just before you began to eat it which was better than when you were, but he didn't know what it was called.”

— A.A. Milne, Winnie-the-Pooh

Functional neuroimaging of satiation and satiety

Maartje Sara Spetter

The studies described in this thesis were performed at the Images Sciences Institute, University Medical Center Utrecht, Utrecht, the Netherlands and at the J.B. Pierce Laboratory, Department of Psychiatry, Yale University, New Haven, United States of America. In co-operation with Wageningen University, the Netherlands and the Technical University of Dresden, Germany.

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door

Maartje Sara Spetter

geboren op 25 mei 1984 te ‘s-Gravenhage

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	Prof. dr. ir. C. de Graaf
Co-promotor:	Dr. P.A.M. Smeets

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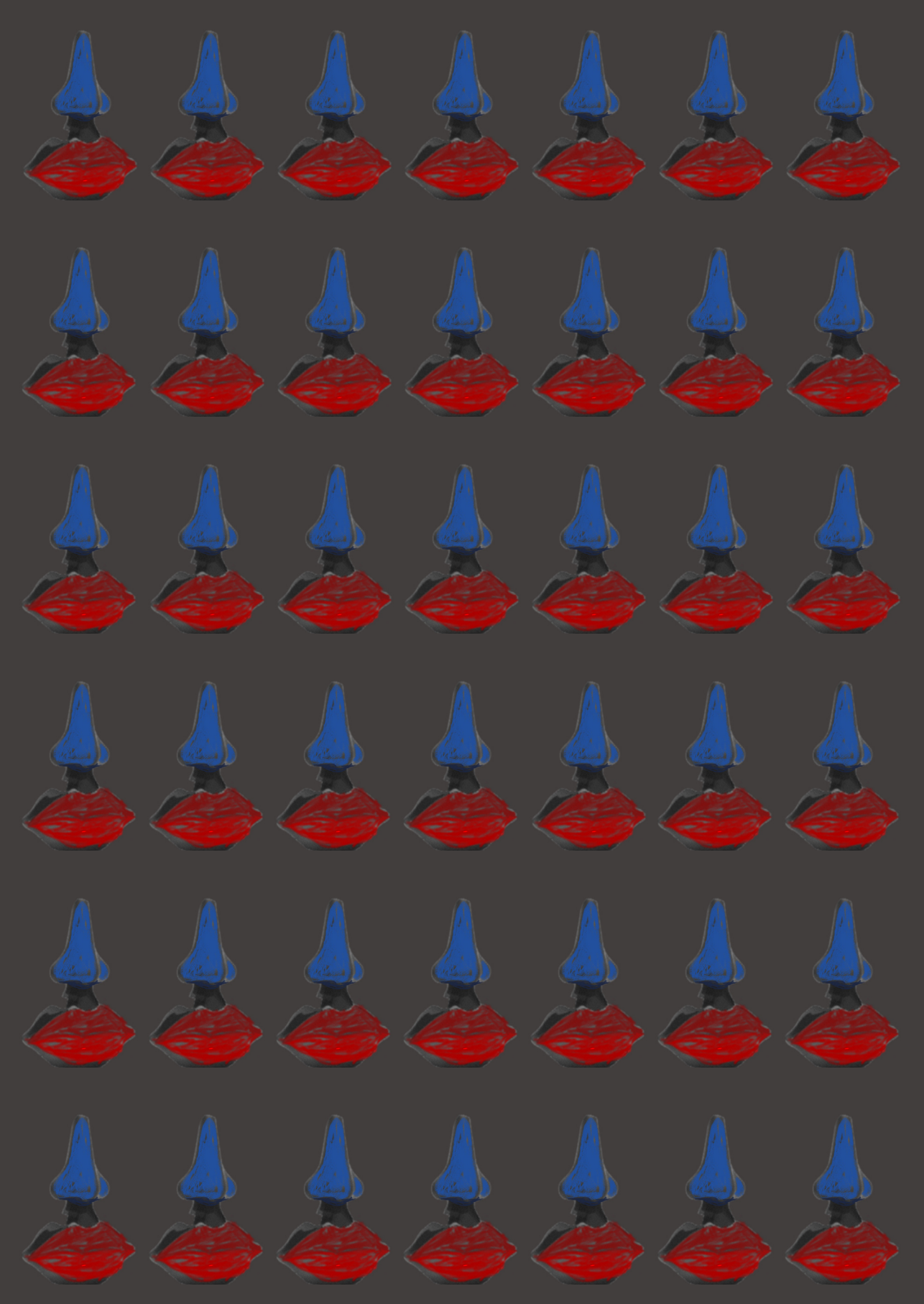


He who possesses art and science has religion;
he who does not possess them, needs religion

Chapter 1

General Introduction

Why do we eat certain things and avoid others, what makes us like or dislike a meal? There are a numerous of factors that influence human feeding behaviour. Internal state or hormone levels (internal cues) play an important role, but also external cues, e.g. food properties: the flavour, taste, smell, intensity, viscosity, complexity of the food, or the social occasion. All these cues are processed in the brain to make a decision to eat or not to eat. The main aim of the research described in this thesis was to understand the effect of internal state on brain activity associated with different taste and odour properties. To introduce this research some information about flavour perception, regulation of food intake, and related brain areas and pathways is provided.

FLAVOUR

When eating a food the flavour is a key point to experience pleasure or aversion (1-3), it is the sensory impression of a food (or drink). The total sensation of flavour is the result of three different inputs: chemical stimulation by the taste buds (known as gustation or taste), stimulation of olfactory receptors (known as olfaction or smell) and stimulation of chemical-sensitive and somasensory free nerve endings of trigeminal and other nerves (e.g. temperature, texture or spiciness) (4-6). The taste on its own is often assumed to be the total sensation of flavour when we eat or drink, sometimes people think of smell. T the common chemical senses are considered even less, but are more important than we realize; e.g. whether a food or drink is hot or cold can influence the quality of the flavour. All these senses have both rewarding and warning functions, e.g. the enjoyment of a chocolate cake, or to alert for spoilt food (7).

The different terms, flavour, odour, smell, olfaction, gustation and taste commonly causes confusion, people often mix up these phrases when expressing the experience of a food, and refer to taste when they mean flavour or smell. This happens especially in countries as the Netherlands; according to the Dutch dictionary, flavour and taste are the same word: smaak. There is no distinction between taste and flavour in Dutch, so becoming aware of this difference is not something the Dutch are used to. However, the sensory experience of a food or drink due to tastes and odours are distinguishable in the neuronal network, which in the end makes a person like or dislike a food or drink.

Gustation - Taste

Taste, also called gustation, refers to the ability to detect substances in the mouth on the tongue. At this moment the world recognizes that there are five basic tastes (8); sweet (associated with sucrose and permits the identification of energy-rich nutrients), salty (associated with NaCl and ensures the electro balance) (9), bitter (associated with quinine, which warns against the intake of potential harmful chemicals) (10), sour (associated with citric acids, which also warns against the intake of potential harmful chemicals) and umami (associated with monosodium glutamate (MSG), which functions as a signal for amino acids containing food) (8). Accumulating evidence suggests that there perhaps is a sixed basic taste, fat (11). Umami (Japanese for good flavour/taste) is just recently generally accepted as a basic taste in the Western world (12) and is associated with the taste of proteins. In the Western culture umami often is referred to as savoury. Although umami alone does not represents the savoury taste, savouriness is associated with a combination of umami, fatty and salty tastes (often protein- and fat-containing foods) (13, 14). Therefore savoury is often confused to be a basic taste, but is not, it is

a food category. According to the Oxford English Dictionary it refers to morally wholesome or acceptable (pleasant or agreeable taste or smell), or it refers to a category which is salty or spicy rather than sweet (15). In the main Western diet 90% of the food is categorized as either sweet or savoury (16).

The five basic tastes are received through taste buds in the human tongue, every taste has an own taste receptor cell (8). Even newborns can already distinguish the differences between the basic tastes; Rosenstein showed in 1988 (17) that infants can already discriminate the four basic tastes; sour, sweet, salty and bitter.

Olfaction - Smell

Olfaction, the sense of smell, detects odorants in the nasal cavity during breathing or eating. Humans can distinguish over one thousand different odours. The smell of a food can draw our attention to the food, or can withdraw our attention. Smelling a food already starts before ingestion occurs and can reach the olfactory epithelium through two pathways; food odours that are in air are smelled orthonasally, but when eating a food, the odour molecules in the food are smelled through the mouth, retronasally (18, 19). Often retronasal olfaction is confused with taste (20), but it is the retronasal olfactory system which senses the complex flavours, the dominant role that olfaction plays in flavour perception when we eat is largely unrecognized (21).

Somasensory senses

Texture, temperature and spiciness also play an important role when eating a food or drinking a fluid. The viscosity, hardness and chewiness influence the sensory experience of a food. The same accounts for the temperature, e.g. hot versus cold chocolate milk, and spiciness, e.g. the heat of hot chilli pepper, or the coolness that is experienced when eating menthol. All these sensory properties have an effect on the pleasure or aversion of the ingested food.

NEURONAL PATHWAYS OF GUSTATION AND OLFACTION

To identify or evaluate a food, multiple sensory properties play an important role. Already before the food is ingested in the mouth, sight (visual) and smell (orthonasal olfactory) of a food are major factors in identifying and even evaluating the food (22). When putting a food or drink in the mouth, the function of tasting is mainly to identify if a substance in the food is to promote or disturb the homeostasis, by identifying the five basic tastes and their associated nutrients. When a food is in the mouth, retronasal olfaction is experienced. Retronasal olfaction is often confused with taste (20, 23), when people lose the sense of smell they refer to loss of taste functions, but it is the retronasal olfactory system which senses the complex flavours. The representation of flavour, gustatory and olfaction information, starts in the mouth and nose and travels through several nervous tracts to be represented in the human brain. The paragraphs below will describe the relevant pathways to provide a better understanding of ‘tasting’ a food or drink.

Gustation pathways

Linking brain responses to gustation and olfaction started in the 80’s. First primates were studied, but in the 90’s it became possible to study humans, when fMRI and PET became more widely used meth-

ods. In the first primate studies, it was proposed that the gustatory pathways travel from the central relay in the brainstem, the nucleus of the solitary tract (see Figure 1), via the thalamus to the primary taste cortex in the frontal operculum and insula to reach a secondary cortical taste area in the caudolateral orbitofrontal cortex (24). Schoenfeld et al. (25) observed the taste activation of all five basic taste in the brain with the use of fMRI. They found a high inter-individual variability in the exact part of the insula which was activated by the five basic tastes, but considerable overlap between the insular regions activated by the different tastes. Taste sensation is frequently observed to activate the orbitofrontal cortex (OFC) (26-33), insula and overlying operculum (25-28, 34-36). Also amygdala, thalamus and anterior cingulate cortex (ACC) activation was linked to taste stimulation (27, 28, 30, 36). Gustation probably activates a wide network of cerebral areas (37).

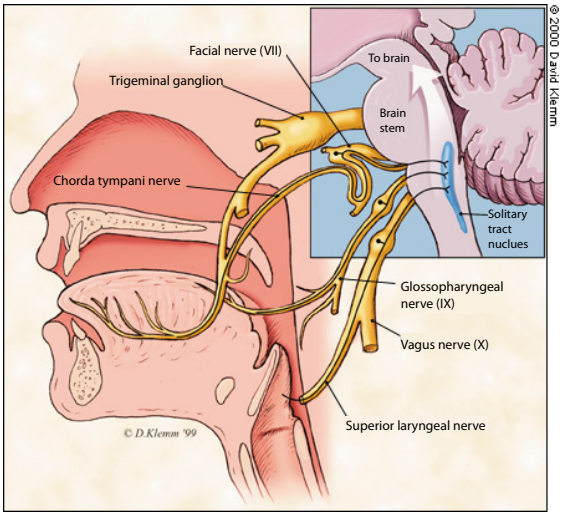


Figure 1 Anatomy of peripheral taste pathways. Multiple nerves, including cranial nerves VII, IX and X, transmit taste information from the mouth and pharynx to the brain via the brain stem. Copyright 1999-2000 David Klemm.

Olfaction pathways

The olfactory pathway starts in the olfactory bulb (see Figure 2), followed by a projection via the lateral olfactory tract to several areas, e.g. the olfactory cortex (piriform) and amygdala (38). It also travels through the agranular part of the insula. This area is just at the caudal border of the OFC and from here reaches the orbitofrontal cortex (39). Other higher order projections converge on the mediodorsal thalamus and hypothalamus (40) (see Figure 3).

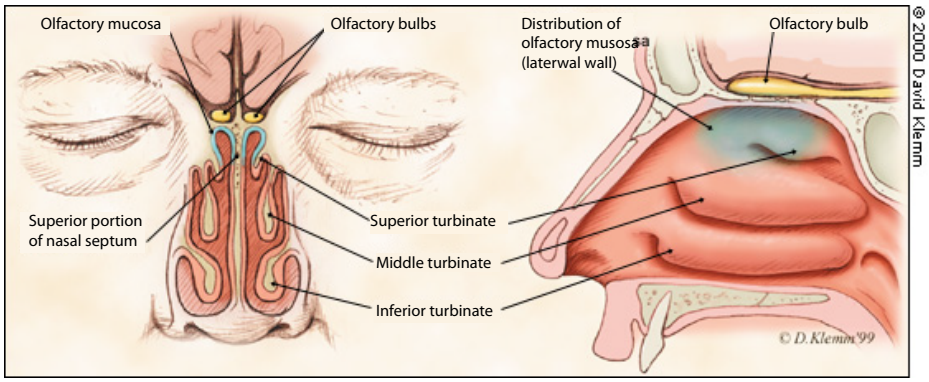


Figure 2 Anatomy of olfactory neural pathways, showing the distribution of olfactory receptors in the roof of the nasal cavity. Copyright 1999-2000 David Klemm.

Since Rozin (19) proposed that smell has two pathways, retro- and orthonasal, and that people may evaluate an identical olfactory stimulus differently depending on how it is perceived, various research has been done to discriminate these two (41-46). Both pathways activate the piriform cortex, insula, hippocampus and OFC when olfactory stimulated (47-49). Small et al (44) found that a food odour is perceived differently when comparing orthonasal olfaction brain responses to retronasal olfaction, whereas for non-food odours this was not found. They found that when a chocolate odour was perceived orthonasally compared to retronasally an increased response in the insula/operculum, thalamus, hippocampus, amygdala and caudolateral OFC was observed. But when comparing retronasal with orthonasal, an increased response in the perigenual cingulate and medial OFC was found for a food odour. This suggests that odorant category might be important for how an odour is processed, with dissociable regions of the olfactory system devoted to the processing of food and non-food odours.

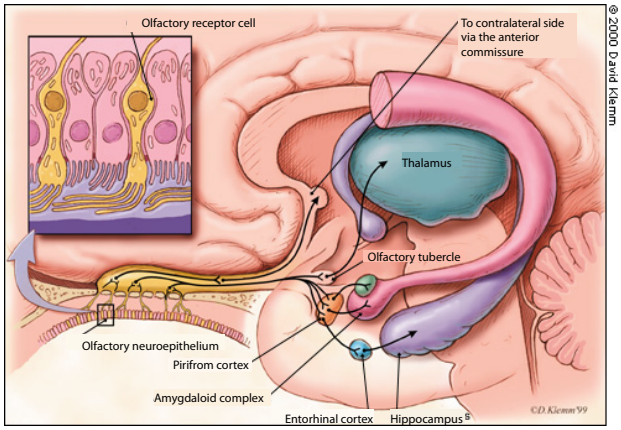


Figure 3 Simplified diagram of cortical regions thought to be involved in the processing of olfactory information as it passes from the olfactory epithelium to the brain. Copyright 1999-2000 David Klemm

APPETITE, SATIATION AND SATIETY

The amount of food that a person consumes is influenced by external and internal factors (50, 51). The internal factors, also referred to as the physiological status of a person, are influenced by satiety, satiation, sensory-specific satiety (SSS), hunger and appetite, and are determined by an interaction of the brain and the gut. The terminology of these statuses may not be universally agreed, for this thesis the following definitions will be used: appetite refers to qualitative aspects of eating, sensory aspects or responsiveness to environmental stimulation (52). Satiation is a process that leads to the termination of eating; therefore controls meal size and is also known as intra-meal satiety. Satiety is the state that leads to inhibition of further eating, decline in hunger, increase in fullness after a meal has finished and is also known as post-ingestive satiety or inter-meal satiety. Hunger is the sensation reflecting a mental urge to eat. It can be traced to changes in physical sensations in parts of the body (stomach, limbs or head). In its strong form it may include feelings of light-headedness, weakness or emptiness in stomach (52). The interaction between satiation and satiety is also referred to as the satiety cascade (53). During an eating episode satiation develops, the volume, weight or caloric content of the food determines satiation. While consuming, hunger and appetite decline and fullness increases, reaching its low or top at the end of the meal. The process of satiation stops food intake. After food consumption a person is in the process of satiety, and further eating is inhibited. The intensity of satiety is determined by the time before the next eating moment, or the amount that is consumed during the next meal (53).

Sensory-Specific Satiation

In the regulation of food intake, satiation plays an important role because it determines meal size. Two types of satiation can be distinguished: metabolic satiation and sensory-specific satiation. Metabolic satiation refers to the body's need for food and is strongly affected by the fullness of the stomach and the release of hormones. Sensory-specific satiation (SSS) has been defined as the decrease in pleasantness of an eaten food relative to an uneaten food (54). It relates to how much a particular food is liked (hedonic value) and is the result of a neurobiological process. Other foods not eaten to satiety remain relatively pleasant (55). When SSS occurs for a sweet food, a savoury food is still relatively more pleasant and vice versa. A decrease in liking due to sensory-specific satiation stops consumption, or in case there are more foods available, leads to consumption of other foods (56). Thus, sensory-specific satiation influences food intake. All sensory properties of a food can contribute to sensory-specific satiety. Major factors are the duration of oral exposure, taste intensity and complexity of the food. They play an underlying role in understanding sensory-specific satiation. These properties and their related pleasantness therefore can influence food intake. SSS occurs within 2 minutes after consumption, when there has been little opportunity for digestion and absorption and it is specific for the sensory aspects of products (57). Texture, flavour, and colour have been described as important factors affecting the degree of sensory-specific satiety, but also the influence of macronutrients.

Satiety Signalling

When controlling eating behaviour, specific regions in the brain, neurotransmitters, hormones and the gut all play an important role. The hypothalamus and brainstem are two brain areas that play a role in appetite control and when to start eating (58, 59). Activation in these two brain areas are influenced by periphery hormone levels, which themselves depend on what and how much is in the stomach and intestines. When a meal is eaten the food is broken down in macro- and micronutrients, the stomach produces acidic digestive juices to initiate the process of digestion. The time it takes to fully break down a food is called gastric emptying, which is controlled by a complex interaction between the brainstem and gut hormones (60). This food signalling appears pre- and post absorptive. Before consumption, the body reacts to the smell and sight of a food, this pre-absorptive phase is called the cephalic phase response, where hormones are already released to prepare the body for ingestion (61). During and after ingestion receptors throughout the gastrointestinal tract detect the nutrients and send signals. Many of the nutritional feedback are mediated by peptide hormones (62). The hormones that interact are insulin, ghrelin, cholecystokinin (CCK), peptide YY (PYY), leptin and glucagon-like peptide 1 (GLP-1), which influence the process of feeling full and to stop eating (63-66). After the gut, the duodenum and intestines are the next step in the digestion process and also secret different hormones which influence eating behaviour control. The role of all these hormones in the interaction with brain activation when eating is still not totally understood.

Satiation/Satiety and the Brain

The role of the brain during food consumption has been studied in the last decades. In primates the caudolateral orbitofrontal cortex (24) and hypothalamus (67) neuronal signalling decreased to zero after being satiated. In the human brain, effects of hunger and satiety on activation to chemosensory stimuli were found in the insular cortex (68, 69), OFC (68, 70-72), hippocampus, anterior cingulate cortex (35) and striatum (68). The interplay between the sensory stimulation and gastric filling in the

brain is difficult to separate. It has been proposed that the oral stimulation of food and the gastric effect of the nutrients and gastric distension all play a role in the process of satiety. In neuroimaging studies, in which the stomach was distended with a gastric balloon, activation was observed in the insula, amygdala, posterior insula, left inferior frontal gyrus and anterior cingulate cortex (73, 74). No study so far has studied the acute effect of food infusion.

In the past decades, researchers have come to understand the pathways in the brain of gustation and olfaction, although the interaction of these pathways when eating or the influence of internal state is still not totally clear. To understand the regions which are involved in flavour perception, and to be able to discriminate between gustatory and olfactory processes during a hunger and satiety state further research is necessary. These findings will provide inside to understand eating behaviour.

HEDONICS AND THE BRAIN

Eating behaviour is strongly influenced by the reward value of the food. Food reward refers to the preference or intake of a food in humans. According to Berridge (75) bitterness activated brain systems of aversion and disgust, whereas sweet taste activates the reward system of liking and wanting. But in foods and drinks such as beer, coffee or olives, bitterness can become pleasant and will activate the hedonic brains systems. Understanding pleasure and aversion in the brain may open the way to understand the impact of food rewards on eating behaviour. The reward value of food contributes significantly to why people eat or even overeat.

The hedonic value that changes during consumption is defined as 'liking', referring to the palatability of the food, whereas the motivation to eat is associated with 'wanting', but both terms refer to food reward (76). Separating and measuring wanting and liking in behavioural eating studies is of interest but has proven to be very difficult (77-79), although there is some evidence that there is a different neural response (1, 80, 81). The impact of food reward on eating behaviour can perhaps be better understood by understanding the underlying brain pathways of motivation to eat and aversion of a food or taste (81). Reward in the brain is often associated with the striatum, an area which consists of the caudate, pallidum, and putamen (75, 76). When looking at the effects of SSS in the brain, the changes of pleasantness for an eaten taste, the orbitofrontal cortex has been associated with satiation-induced changes in pleasantness (liking) (68, 82). Pleasure itself, food and non-food related, is often associated with subcortical regions such as the nucleus accumbens, striatum, amygdala, and cortical regions such as the orbitofrontal cortex, cingulate cortex, and anterior insula (28, 68, 83-87).

One of the main questions in the research area of food behaviour is to understand why people eat what they eat, and how the brain plays a role in this. According to Berthoud (88) one can understand the neural mechanisms behind food intake by observing pleasure and reward in the brain, and by studying satiety and satiation in the brain and its neural and hormonal afferent pathways.

THIS THESIS

The mechanisms and networks of gustatory and olfactory processes and the effect of internal state will provide an insight on normal eating behaviour. Although most of these networks are known, the effect of internal state on stimuli induced brain activity should be expand further. How does the internal state influence brain activity associated with different taste and odour properties?

In this thesis we try to further expand the knowledge about the effect of internal state on brain activity associated with different taste and odour properties, by first addressing the intensity properties of the food, second and third we studied the effect of consumption on brain activation associated with savoury and sweet tastes or food and non food odours. Last we measured the acute effect of satiation on the brain.

Contents of this Thesis

The aim of this thesis is to better understand how the brain plays a role in gustatory and olfactory processes and in the process of satiation and satiety. Sensory properties play an important role in the process of sensory specific satiation; e.g. the intensity of taste. Taste intensity and the related responses in the brain can help us to understand the gustatory pathways. (Chapter 2)

In previous studies the effects of consumption on brain responses was observed in the OFC, but were mostly related to hedonic, liking changes. But the motivation to eat, wanting-related changes, also affects brain activation. The usefulness of a specific food decreases after it has been ingested, whereas another food is still relatively useful for the human body, especially when distinguish between sweet and savoury, the two main food categories in daily life. The changes in brain responses that are wanting-related will help understanding the effect of consumption on reward areas of the brain.(Chapter 3)

Is it possible to have a look in the brain and see how much a person will eat later on? Brain response to a specific taste perhaps can predict subsequent intake. (Chapter 3)

Odours can lead to attraction or aversion. Food odours can work appetizing when hungry, whereas non food odours have no such effect. But when full, the experience of odours, especially food odours evokes different brain responses. Even the route of delivery, are odours perceived ortho- or retronasally, can influence the response differently when full or hungry. (Chapter 4)

The satiation process starts in the mouth, gustatory response, and is followed by an interaction of the gut and brain responses, through hormones of the periphery. The interplay between the sensory stimulation and gastric distension and the related brain responses is yet to be observed. Also the acute effect of a nutrient load in the stomach and the related brain responses has never been studied. When a nutrient load is directly infused into the stomach, does this cause a difference in hormonal responses compared to normal oral ingestion. (Chapter 5)

Overall, the aim of this thesis is to create a better understanding of the effect and process of satiation and satiety in the brain, for taste and odour.

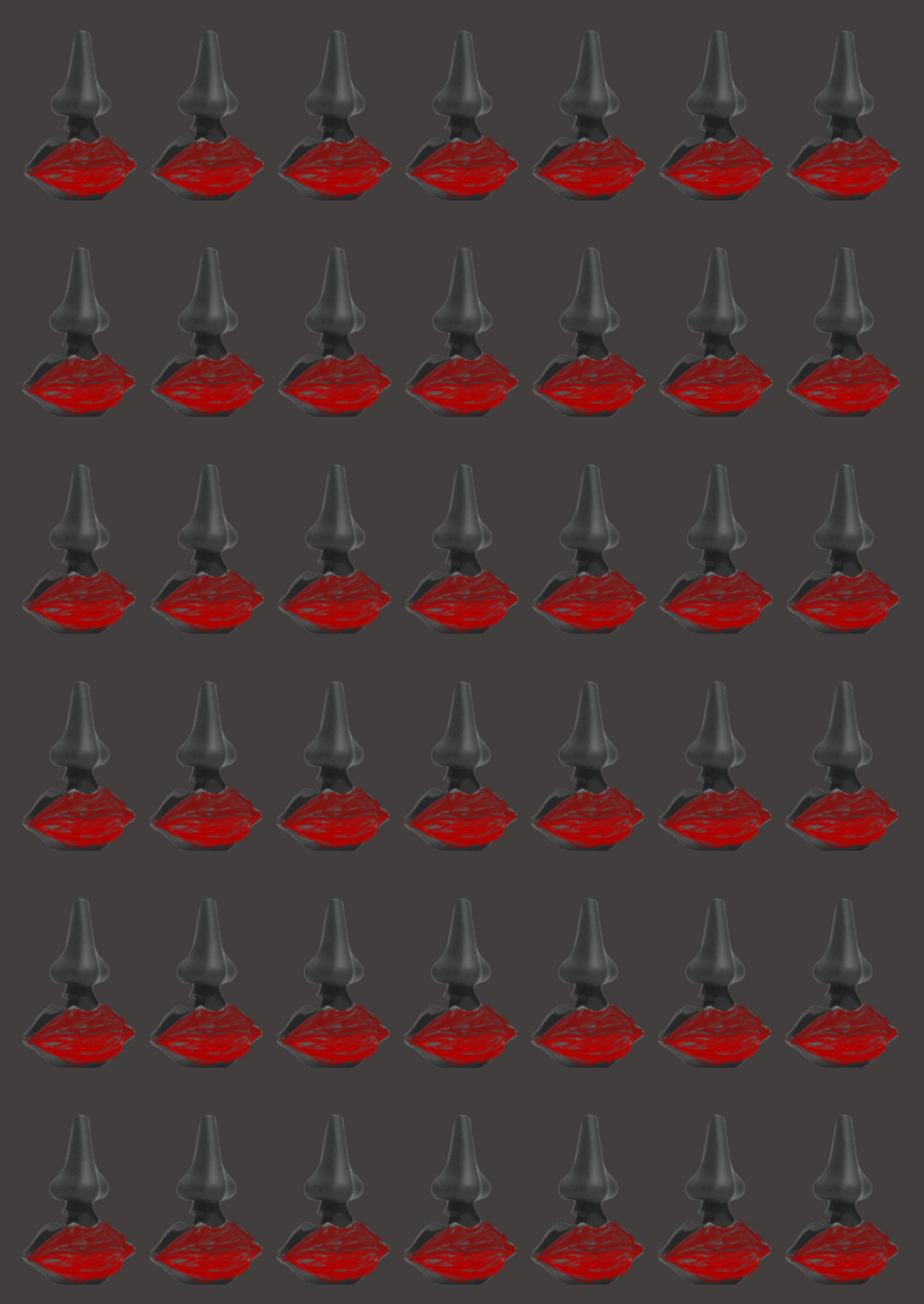
REFERENCES

1. Berridge, K. C. & Kringelbach, M. L. (2008) Affective neuroscience of pleasure: reward in humans and animals. Psychopharmacology (Berl) 199: 457-480.
2. Cabanac, M. (1971) Physiological role of pleasure. Science 173: 1103-1107.
3. Berridge, K. C., Ho, C. Y., Richard, J. M. & DiFeliceantonio, A. G. (2010) The tempted brain eats: pleasure and desire circuits in obesity and eating disorders. Brain Res. 1350: 43-64.
4. Travers, J. B. (1988) Efferent projections from the anterior nucleus of the solitary tract of the hamster. Brain Res. 457: 1-11.
5. Small, D. M. & Green, B. (2011) A Proposed Model of a Flavor Modality. In: The Neural Bases of Multisensory Processes , pp. 705-726.
6. Lundstrom, J. N., Boesveldt, S. & Albrecht, J. (2011) Central Processing of the Chemical Senses: an Overview. ACS Chem. Neurosci. 2: 5-16.
7. Scott, T. R. (2011) Taste as a basis for body wisdom. Physiol Behav. 104: 57-63.
8. Chandrashekar, J., Hoon, M. A., Ryba, N. J. & Zuker, C. S. (2006) The receptors and cells for mammalian taste. Nature 444: 288-294.
9. Galindo-Cuspinera, V. & Breslin, P. A. (2006) The liaison of sweet and savoury. Chem. Senses 31: 221-225.
10. Scott, T. R. & Plata-Salaman, C. R. (1991) Coding of taste quality. Smell and taste in health and disease 345-368.
11. Chale-Rush, A., Burgess, J. R. & Mattes, R. D. (2007) Evidence for human orosensory (taste?) sensitivity to free fatty acids. Chem. Senses 32: 423-431.
12. Kurihara, K. (2009) Glutamate: from discovery as a food flavor to role as a basic taste (umami). Am. J. Clin. Nutr. 90: 719S-722S.
13. Small, D. M. (2006) Central gustatory processing in humans. Adv. Otorhinolaryngol. 63: 191-220.
14. Pritchard, T. C., Hamilton, R. B., Morse, J. R. & Norgren, R. (1986) Projections of thalamic gustatory and lingual areas in the monkey, Macaca fascicularis. J. Comp Neurol. 244: 213-228.
15. J.Simpson & E.Weiner (1989) Oxford English Dictionaries.
16. Mattes, R. D., Pierce, C. B. & Friedman, M. I. (1988) Daily caloric intake of normal-weight adults: response to changes in dietary energy density of a luncheon meal. Am. J Clin. Nutr. 48: 214-219.
17. Rosenstein, D. & Oster, H. (1988) Differential facial responses to four basic tastes in newborns. Child Dev. 59: 1555-1568.
18. Negoias, S., Visschers, R., Boelrijk, A. & Hummel, T. (2008) New ways to understand aroma perception. Food Chemistry 108: 1247-1254.
19. Rozin, P. (1982) "Taste-smell confusions" and the duality of the olfactory sense. Percept. Psychophys. 31: 397-401.
20. Murphy, C., Cain, W. S. & Bartoshuk, L. M. (1977) Mutual action of taste and olfaction. Sens. Processes 1: 204-211.
21. Shepherd, G. M. (2006) Smell images and the flavour system in the human brain. Nature 444: 316-321.
22. Small, D. M. (2006) Central gustatory processing in humans. Adv. Otorhinolaryngol. 63: 191-220.
23. Murphy, C. & Cain, W. S. (1980) Taste and olfaction: independence vs interaction. Physiol Behav. 24: 601-605.
24. Rolls, E. T. (1989) Information processing in the taste system of primates. J. Exp. Biol. 146: 141-164.
25. Schoenfeld, M. A., Neuer, G., Tempelmann, C., Schussler, K., Noesselt, T., Hopf, J. M. & Heinze, H. J. (2004) Functional magnetic resonance tomography correlates of taste perception in the human primary taste cortex. Neuroscience 127: 347-353.
26. de Araujo, I., Rolls, E. T., Kringelbach, M. L., McGlone, F. & Phillips, N. (2003) Taste-olfactory convergence, and the representation of the pleasantness of flavour, in the human brain. Eur. J. Neurosci. 18: 2059-2068.
27. Haase, L., Cerf-Ducastel, B., Buracas, G. & Murphy, C. (2007) On-line psychophysical data acquisition and event-related fMRI protocol optimized for the investigation of brain activation in response to gustatory stimuli. J. Neurosci. Methods 159: 98-107.
28. O'Doherty, J., Rolls, E. T., Francis, S., Bowtell, R. & McGlone, F. (2001) Representation of pleasant and aversive taste in the human brain. J. Neurophysiol. 85: 1315-1321.

29. Veldhuizen, M. G., Bender, G., Constable, R. T. & Small, D. M. (2007) Trying to detect taste in a tasteless solution: modulation of early gustatory cortex by attention to taste. Chem. Senses 32: 569-581.
30. Zald, D. H., Lee, J. T., Fluegel, K. W. & Pardo, J. V. (1998) Aversive gustatory stimulation activates limbic circuits in humans. Brain 121 (Pt 6): 1143-1154.
31. Zald, D. H., Hagen, M. C. & Pardo, J. V. (2002) Neural correlates of tasting concentrated quinine and sugar solutions. J. Neurophysiol. 87: 1068-1075.
32. Small, D. M., Jones-Gotman, M., Zatorre, R. J., Petrides, M. & Evans, A. C. (1997) Flavor processing: more than the sum of its parts. Neuroreport 8: 3913-3917.
33. Small, D. M., Jones-Gotman, M., Zatorre, R. J., Petrides, M. & Evans, A. C. (1997) A role for the right anterior temporal lobe in taste quality recognition. J. Neurosci. 17: 5136-5142.
34. Bender, G., Veldhuizen, M. G., Meltzer, J. A., Gitelman, D. R. & Small, D. M. (2009) Neural correlates of evaluative compared with passive tasting. Eur. J. Neurosci. 30: 327-338.
35. Haase, L., Cerf-Ducastel, B. & Murphy, C. (2009) Cortical activation in response to pure taste stimuli during the physiological states of hunger and satiety. Neuroimage. 44: 1008-1021.
36. Grabenhorst, F. & Rolls, E. T. (2008) Selective attention to affective value alters how the brain processes taste stimuli. Eur. J. Neurosci. 27: 723-729.
37. Veldhuizen, M. G., Albrecht, J., Zelano, C., Boesveldt, S., Breslin, P. & Lundstrom, J. N. (2011) Identification of human gustatory cortex by activation likelihood estimation. Hum. Brain Mapp. 32: 2256-2266.
38. Gottfried, J. A. (2010) Central mechanisms of odour object perception. Nat. Rev. Neurosci. 11: 628-641.
39. Carmichael, S. T., Clugnet, M. C. & Price, J. L. (1994) Central olfactory connections in the macaque monkey. J. Comp Neurol. 346: 403-434.
40. Haberly, L. B. (1998) Olfactory cortex. In: The synaptic organization of the brain (4th ed.) (Shepherd, G. M. ed.), pp. 377-416. New York, NY, US: Oxford University Press.
41. Sun, B. C. & Halpern, B. P. (2005) Identification of air phase retronasal and orthonasal odorant pairs. Chem. Senses 30: 693-706.
42. Heilmann, S. & Hummel, T. (2004) A new method for comparing orthonasal and retronasal olfaction. Behav. Neurosci. 118: 412-419.
43. Heilmann, S., Strehle, G., Rosenheim, K., Damm, M. & Hummel, T. (2002) Clinical assessment of retronasal olfactory function. Arch. Otolaryngol. Head Neck Surg. 128: 414-418.
44. Small, D. M., Gerber, J. C., Mak, Y. E. & Hummel, T. (2005) Differential neural responses evoked by orthonasal versus retronasal odorant perception in humans. Neuron 47: 593-605.
45. Bender, G., Hummel, T., Negoias, S. & Small, D. M. (2009) Separate signals for orthonasal vs. retronasal perception of food but not nonfood odors. Behav. Neurosci. 123: 481-489.
46. Small, D. M. & Prescott, J. (2005) Odor/taste integration and the perception of flavor. Exp. Brain Res. 166: 345-357.
47. Cerf-Ducastel, B. & Murphy, C. (2001) fMRI activation in response to odorants orally delivered in aqueous solutions. Chem. Senses 26: 625-637.
48. Gottfried, J. A., Deichmann, R., Winston, J. S. & Dolan, R. J. (2002) Functional heterogeneity in human olfactory cortex: an event-related functional magnetic resonance imaging study. J. Neurosci. 22: 10819-10828.
49. Zatorre, R. J., Jones-Gotman, M., Evans, A. C. & Meyer, E. (1992) Functional localization and lateralization of human olfactory cortex. Nature 360: 339-340.
50. Wansink, B. (2004) Environmental factors that increase the food intake and consumption volume of unknowing consumers. Annu. Rev. Nutr. 24: 455-479.
51. Woods, S. C. (2005) Signals that influence food intake and body weight. Physiol Behav. 86: 709-716.
52. Blundell, J., de, G. C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., Mela, D., Salah, S., Schuring, E. et al. (2010) Appetite control: methodological aspects of the evaluation of foods. Obes. Rev. 11: 251-270.
53. Blundell, J. E., Lawton, C. L., Cotton, J. R. & Macdiarmid, J. I. (1996) Control of human appetite: implications for the intake of dietary fat. Annu. Rev. Nutr. 16: 285-319.
54. Rolls, B. J., Rowe, E. A. & Rolls, E. T. (1982) How sensory properties of foods affect human feeding behavior. Physiol Behav. 29: 409-417.
55. Rolls, B. J., Rolls, E. T., Rowe, E. A. & Sweeney, K. (1981) Sensory specific satiety in man. Physiol Behav. 27: 137-142.
56. Rolls, B. J., van Duijvenvoorde, P. M. & Rowe, E. A. (1983) Variety in the diet enhances intake in a meal and contributes to the development of obesity in the rat. Physiol Behav. 31: 21-27.

57. Hetherington, M. M. (1996) Sensory-specific satiety and its importance in meal termination. Neurosci. Biobehav. Rev. 20: 113-117.
58. Hillebrand, J. J., de, W. D. & Adan, R. A. (2002) Neuropeptides, food intake and body weight regulation: a hypothalamic focus. Peptides 23: 2283-2306.
59. Williams, G., Bing, C., Cai, X. J., Harrold, J. A., King, P. J. & Liu, X. H. (2001) The hypothalamus and the control of energy homeostasis: different circuits, different purposes. Physiol Behav. 74: 683-701.
60. Naslund, E. & Hellstrom, P. M. (2007) Appetite signaling: from gut peptides and enteric nerves to brain. Physiol Behav. 92: 256-262.
61. Smeets, P. A., Erkner, A. & de Graaf C. (2010) Cephalic phase responses and appetite. Nutr. Rev. 68: 643-655.
62. Schloegl, H., Percik, R., Horstmann, A., Villringer, A. & Stumvoll, M. (2011) Peptide hormones regulating appetite--focus on neuroimaging studies in humans. Diabetes Metab Res. Rev. 27: 104-112.
63. Batterham, R. L., Cohen, M. A., Ellis, S. M., Le Roux, C. W., Withers, D. J., Frost, G. S., Ghatei, M. A. & Bloom, S. R. (2003) Inhibition of food intake in obese subjects by peptide YY3-36. N. Engl. J. Med. 349: 941-948.
64. Batterham, R. L., Cowley, M. A., Small, C. J., Herzog, H., Cohen, M. A., Dakin, C. L., Wren, A. M., Brynes, A. E., Low, M. J. et al. (2002) Gut hormone PYY(3-36) physiologically inhibits food intake. Nature 418: 650-654.
65. Muurahainen, N., Kissileff, H. R., Derogatis, A. J. & Pi-Sunyer, F. X. (1988) Effects of cholecystokinin-octapeptide (CCK-8) on food intake and gastric emptying in man. Physiol Behav. 44: 645-649.
66. Beglinger, C. & Degen, L. (2006) Gastrointestinal satiety signals in humans--physiologic roles for GLP-1 and PYY? Physiol Behav. 89: 460-464.
67. Burton, M. J., Rolls, E. T. & Mora, F. (1976) Effects of hunger on the responses of neurons in the lateral hypothalamus to the sight and taste of food. Exp. Neurol. 51: 668-677.
68. Kringelbach, M. L., O'Doherty, J., Rolls, E. T. & Andrews, C. (2003) Activation of the human orbitofrontal cortex to a liquid food stimulus is correlated with its subjective pleasantness. Cereb. Cortex 13: 1064-1071.
69. Del, P. A., Gautier, J. F., Chen, K., Salbe, A. D., Ravussin, E., Reiman, E. & Tataranni, P. A. (2002) Neuroimaging and obesity: mapping the brain responses to hunger and satiation in humans using positron emission tomography. Ann. N. Y. Acad. Sci. 967: 389-397.
70. Small, D. M., Zatorre, R. J., Dagher, A., Evans, A. C. & Jones-Gotman, M. (2001) Changes in brain activity related to eating chocolate: from pleasure to aversion. Brain 124: 1720-1733.
71. Gottfried, J. A., O'Doherty, J. & Dolan, R. J. (2003) Encoding predictive reward value in human amygdala and orbitofrontal cortex. Science 301: 1104-1107.
72. Del, P. A., Chen, K., Gautier, J. F., Salbe, A. D., Pratley, R. E., Ravussin, E., Reiman, E. M. & Tataranni, P. A. (2002) Sex differences in the human brain's response to hunger and satiation. Am. J. Clin. Nutr. 75: 1017-1022.
73. Wang, G. J., Tomasi, D., Backus, W., Wang, R., Telang, F., Geliebter, A., Korner, J., Bauman, A., Fowler, J. S. et al. (2008) Gastric distention activates satiety circuitry in the human brain. Neuroimage. 39: 1824-1831.
74. Stephan, E., Pardo, J. V., Faris, P. L., Hartman, B. K., Kim, S. W., Ivanov, E. H., Daughters, R. S., Costello, P. A. & Goodale, R. L. (2003) Functional neuroimaging of gastric distention. J. Gastrointest. Surg. 7: 740-749.
75. Berridge, K. C. (2009) Wanting and Liking: Observations from the Neuroscience and Psychology Laboratory. Inquiry (Oslo.) 52: 378.
76. Berridge, K. C. (1996) Food reward: brain substrates of wanting and liking. Neurosci. Biobehav. Rev. 20: 1-25.
77. Finlayson, G., King, N. & Blundell, J. E. (2007) Liking vs. wanting food: importance for human appetite control and weight regulation. Neurosci. Biobehav. Rev. 31: 987-1002.
78. Lemmens, S. G., Schoffelen, P. F., Wouters, L., Born, J. M., Martens, M. J., Rutters, F. & Westerterp-Plantenga, M. S. (2009) Eating what you like induces a stronger decrease of 'wanting' to eat. Physiol Behav. 98: 318-325.
79. Havermans, R. C. (2011) "You Say it's Liking, I Say it's Wanting ...". On the difficulty of disentangling food reward in man. Appetite 57: 286-294.
80. Pecina, S., Smith, K. S. & Berridge, K. C. (2006) Hedonic hot spots in the brain. Neuroscientist. 12: 500-511.
81. Berridge, K. C. (2009) 'Liking' and 'wanting' food rewards: brain substrates and roles in eating disorders. Physiol Behav. 97: 537-550.

82. O'Doherty, J., Rolls, E. T., Francis, S., Bowtell, R., McGlone, F., Kobal, G., Renner, B. & Ahne, G. (2000) Sensory-specific satiety-related olfactory activation of the human orbitofrontal cortex. Neuroreport 11: 893-897.
83. Grabenhorst, F., Rolls, E. T., Margot, C., da Silva, M. A. & Velazco, M. I. (2007) How pleasant and unpleasant stimuli combine in different brain regions: odor mixtures. J. Neurosci. 27: 13532-13540.
84. Small, D. M., Bender, G., Veldhuizen, M. G., Rudenga, K., Nachtigal, D. & Felsted, J. (2007) The role of the human orbitofrontal cortex in taste and flavor processing. Ann. N. Y. Acad. Sci. 1121: 136-151.
85. Kringelbach, M. L. & Berridge, K. C. (2009) Towards a functional neuroanatomy of pleasure and happiness. Trends Cogn Sci. 13: 479-487.
86. Kringelbach, M. L. & Rolls, E. T. (2004) The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. Prog. Neurobiol. 72: 341-372.
87. Everitt, B. J. & Robbins, T. W. (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat. Neurosci. 8: 1481-1489.
88. Berthoud, H. R. (2002) Multiple neural systems controlling food intake and body weight. Neurosci. Biobehav. Rev. 26: 393-428.



Wenn du nicht irrst, kommst du nicht zu Verstand

Chapter 2

Representation of sweet and salty taste intensity in the brain

ABSTRACT

The intensity of the taste of a food is affected mostly by the amount of sugars (mono- and disaccharides) or salt it contains. To season savoury-tasting foods mainly table salt (NaCl) is used, and to sweeten foods, sugars like sucrose are used. Foods with highly intense tastes are consumed in smaller amounts. The optimal taste intensity of a food is the intensity at which it is perceived as most pleasant. When taste intensity decreases or increases from optimal, the pleasantness of a food decreases. Here, we investigated the brain representation of sweet and salty taste intensity using functional MRI. 15 subjects visited twice and tasted a range of four watery solutions (0 to 1M) of either sucrose or NaCl in water. Middle insula activation increased with increasing concentration for both NaCl and sucrose. Despite similar subjective intensity ratings, anterior insula activation by NaCl increased more with concentration than that by sucrose. Amygdala activation increased with increasing NaCl concentration but not sucrose concentration. In conclusion, sweet and salty taste intensity are represented in the middle insula. Amygdala activation is only modulated by saltiness. Further research will need to extrapolate these results from simple solutions to real foods.

INTRODUCTION

The interplay between taste function and food intake is an important research area, of particular interest recently (1), due to the increase of obesity in the western world. Sensory properties of eaten foods influence the tasting process and thereby food intake (2). Major factors which affect satiation and thereby meal size are related to the degree of orosensory stimulation and include oral exposure time (3-5), sensory complexity of the food (6, 7) and taste intensity (8). Taste intensity of a food depends strongly on sugar (mono- and disaccharides) and salt (NaCl) content: these are the two main types of seasoning agents. Savoury foods are mainly seasoned with table salt (NaCl) and sweet foods contain sugars (often sucrose). Foods with highly intense tastes are consumed in smaller amounts (8), possibly due to a high degree of sensory-specific satiation. Taste intensity, i.e., sweetness or saltiness, is tightly coupled to pleasantness (9). When intensity deviates from the (subjective) optimum, pleasantness declines.

The neurological processes underlying the perception of different food properties are still relatively unclear. The five basic tastes, the intensity and the affective value are a part of the gustatory pathway in the brain (10). So far, only one study has investigated the representation of taste intensity in the brain, using a low and a high concentration of a sweet (pleasant) and a bitter (unpleasant) taste in a 2×2 design (11). In this study, it was found that the cerebellum, pons, putamen, middle insula and amygdala respond to differences in taste intensity (11). Here, we aim to reproduce these results for sweetness and extend them to saltiness. In addition, we aim to refine previous findings by employing a range of intensities rather than two extremes. Thus, our objective was to determine the brain regions where taste activation covaries with sweet and salty taste intensity.

MATERIALS AND METHODS

Subjects

Subjects were recruited with flyers posted at the University Medical Center Utrecht. After applying, subjects were invited for a screening session. During the screening, they rated all taste stimuli used in the experiment. This was done to verify that subjects could discriminate concentration differences. Subjects also completed a medical questionnaire and the restrained eating part of the Dutch Eating Behaviour Questionnaire (12). Exclusion criteria included smoking, dieting for weight loss or having a medically prescribed diet, restrained eating (13), use of medication and having an eating disorder, a history of or current alcohol consumption > 28 units per week, or any medical diseases (including taste and smell disorders). 15 normal-weight right-handed men (mean age 23.3 ± 1.7 y, mean body mass index 22.0 ± 1.5 kg/m2) participated. All experimental procedures were approved by the Medical Ethical Committee of the University Medical Center Utrecht and written informed consent was obtained from all subjects before the experiment. Data from one subject was not included in the analyses because of motion artefacts.

fMRI paradigm

This study is a randomized cross-over design study with two taste conditions; sweetness (sucrose) and

Figure 1 fMRI paradigm. Timeline of one cycle of tasting during a fmri run. Black squares represent screen with cues that subjects saw during scan.* Trials that included ratings took 54s (three out of seven trails), trails without ratings lasted for 38s.

saltiness (NaCl). There were two scan sessions, one per taste condition, on separate days at least one week apart. The order of the two scans was randomized across subjects. Subjects fasted for at least two hours before the scan sessions (no food or beverage except water). During the functional run, subjects tasted four concentrations of sucrose or NaCl dissolved in water, with concentrations of 0 M, 0.13 M, 0.50 M and 1.0 M and resulting intensities from 'zero' to 'high'. The four stimuli were pseudo-randomly presented, seven times each. After tasting 1 mL for 11s, subjects either rated the intensity and pleasantness of a stimulus on Visual Analog Scales (VAS duration 8s, after three out of seven trials) or were directly cued on a screen to swallow (4s) and 'rest' (fixation on a crosshair for 4s). Subsequently, subjects received a rinse with water (11s), followed again by a cue to swallow (4s) and rest (4s). This is depicted in Figure 1. Inside the scanner, subjects held the tips of five bound flexible tubes in their mouth (diameter of 3 mm per tube). The tips where positioned comfortably between the lips, such that the tubes delivered the stimuli on the front of the tongue. All stimuli and water (for rinsing) were administered at room temperature (23°C) by use of five programmable syringe pumps (NE-500, New Era Pump Systems, Wantagh, NY, USA). The pumps were programmed to administer 1 mL of solution in 2.5 s. Also, VAS ratings of intensity and pleasantness were made during the scan by use of an custom built button box. Instructions were displayed on a screen through a computer interface, run by the computer program PRESENTATION (Neurobehavioral Systems Inc, Albany, USA).

Stimuli

In a pilot study, ten solutions of sucrose in water and ten solutions of NaCl in water ranging from 0 to 1.25 M were rated by 30 normal-weight subjects (male, mean age 29.6 ± 2.7 y) on perceived intensity, pleasantness, sweetness and saltiness on 10 cm VAS's, labelled "not at all" and "extremely". Using the average intensity ratings from this pilot study, the concentrations for low, middle and high intensity were determined such that low intensity corresponded with an average VAS score of 3, middle intensity corresponded with 5.5 and high intensity corresponded with 8.0 cm. The four concentrations chosen for both sucrose and NaCl were 0 M, 0.13 M, 0.50 M and 1.0 M. These concentrations are referred to as 'zero', 'low', 'middle' and 'high' intensity.

fMRI data acquisition

The scans were performed on a 3 Tesla Philips Achieva MRI scanner at the University Medical Center Utrecht. A 2D single-shot EPI sequence was used (TR/TE= 1600/23 ms, flip angle = 90°, FOV = 256 × 208 mm, 30 interleaved axial slices, voxel size = 4 × 4 × 4 mm). The total duration of each functional run was 21 min, during which 799 scans were obtained. After the functional run, a T1-weighted anatomical scan was made (TR/TE = 61/8.4 ms, flip angle = 30°, FOV = 288 × 175mm, 175 axial slices, voxel size = 1 × 1 × 2 mm).

Data analyses

FMRI data were preprocessed and analyzed using SPM5 (Wellcome Department of Imaging Neuroscience, London, UK,) run with MATLAB 7.5 (The Mathworks Inc, Natick, MA) and the WFU Pickatlas tool (14). First the functional volumes of every subject were realigned to the first volume of the first run. Next, the anatomical image was co-registered with the mean functional image. Then the images were normalized (retaining 4 × 4 × 4 mm voxels) to Montreal Neurological Institute space (MNI space) (15), and spatially smoothed with a Gaussian kernel of 8mm 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. Data were high-pass filtered with a cut off of 128s.

For every subject, two types of analyses were performed: parametric modulation analyses (1) and analyses of taste activation (2). (1) For both sessions, two taste conditions were modelled with two parameters: an intensity parameter (first parameter) and pleasantness ratings (second parameter), once using the subjective intensity ratings (NaCl/sucrose intensity ratings) and once using the concentrations (NaCl/sucrose concentration; objective measure) as the intensity parameter. Since pleasantness and intensity are closely related (9), pleasantness needs to be taken into account when examining intensity effects. The responses to swallowing, rinsing and rating were modelled, but were not included in further analyses. The contrast images for linear parametric modulation of taste activation by subjective intensity ratings and by concentration were calculated for both sessions (sucrose and NaCl). (2) For both sessions eight conditions were modelled: tasting of zero, low, middle and high concentration solutions, swallowing, rinsing and giving VAS ratings. The responses to swallowing, rinsing and rating were not included in further analyses.

In summary, these analyses yielded two modulation contrast images (for modulation by concentration and by subjective intensity) and four taste activation contrast images (zero, low, middle and high intensity) per subject per session. The motion correction parameters from the realignment procedure were added to all models as regressors to regress out motion-related variance.

For the group analyses, the modulation contrast images of both sessions of all subjects were entered into a paired t-test. Two paired t-tests were done using the parametric modulation contrast images: one with the contrast images of modulation by intensity ratings and one with the contrast images of modulation by concentration. Lastly, the contrast images of taste activation were entered into a 2 × 4 ANOVA with taste (sweet and salt) and concentrations (0 M, 0.13 M, 0.50 M and 1 M) as factors. Parameter estimates of taste activation by the different solutions were obtained from this ANOVA with the use of Marsbar (MARSeille Boite A Regions d'interet, Marseille France). Per subject eight mean parameter estimates were calculated (one for every concentration in the two taste sessions). Parameter estimates were normalized per subject by using the parameter estimate of the zero concentration as a baseline measurement. A priori regions of interest (ROIs) were the insula, amygdala, striatum (putamen + caudate), pons and cerebellum. These regions have been shown to respond to differences in taste (11) and/or odour intensity (16, 17). ROI masks were made using the WFU Pickatlastool (14).

The subjective ratings obtained during the scans were analyzed as follows: mean intensity ratings were calculated per condition (sucrose and NaCl) and per concentration (0 M, 0.13 M, 0.50 M and 1.0 M).

Subsequently, these average intensity ratings were compared between the sweet and the salty session with paired t-tests, for every concentration. The same was done for the mean pleasantness ratings. The correlation (r) between the subjective intensity ratings and the given concentration were also calculated. Statistical analyses of the subjective ratings were done with SPSS 16.0 (SPSS Inc, Chicago, USA).

RESULTS

Subjective ratings

Intensity and pleasantness ratings are shown in Figure 2. Mean intensity ratings did not differ between the sucrose and NaCl solutions for any concentration (paired t-tests $p>0.05$). Higher concentrations of both tastes were rated as more intense. Mean ±SD VAS ratings of NaCl intensities were 1.5 ± 0.9 (zero), 3.0 ±1.5 (low), 6.2 ±1.8 (middle) and 8.5 ±0.9 cm (high). Mean sweet intensities ratings were at 1.7 ± 0.6 (zero), 2.6 ±1.2 (low), 6.3 ±1.4 (middle) and 8.0 ± 1.0 cm (high). Subjective intensity ratings were highly correlated with concentration for both tastes (sucrose, $r = 0.92$ $p < 0.01$ and NaCl, $r = 0.90$, $p < 0.01$) (Figure 3). Mean pleasantness ratings dropped with increasing concentration, for both tastes as shown in Figure 2 right panel (Mean saltiness: 5.7 ± 1.6, 4.9 ±1.0, 3.5 ± 1.2 and 1.9 ± 1.1 cm. Mean sweetness ratings were 5.1 ± 1.3, 5.1 ± 1.2, 5.0 ±1.7 and 4.4 ± 1.9 cm) However, only the pleasantness of the highest concentration of the NaCl solutions decreased significantly compared with the zero concentration (paired t-test, $p < 0.05$).

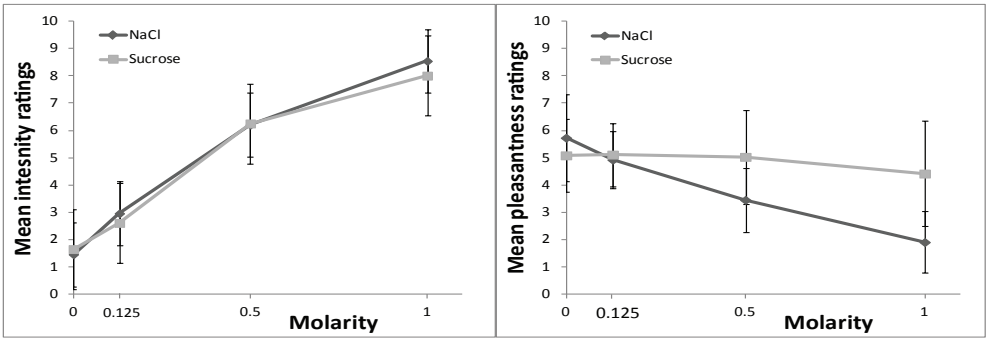


Figure 2 Mean (±SD) VAS ratings of intensity (two left graphs) and pleasantness (two right graphs) of the four salt and sucrose solutions obtained during the fMRI task (N = 14). The mean intensity ratings per concentration did not differ between the two conditions (paired t-tests, $p > 0.05$). The mean pleasantness ratings per concentration only differ when tasting the highest concentration (paired t-tests, $p < 0.05$).

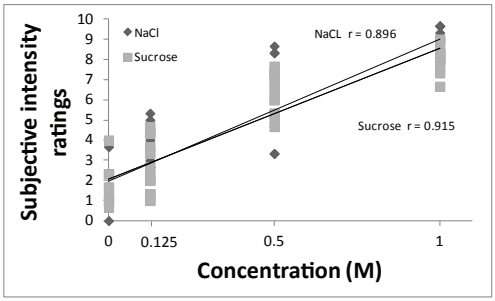


Figure 3 Correlation between VAS ratings of stimulus intensity and concentration of tastes solutions. The correlation for the sucrose is represented by the black (r = 0.915). NaCl correlation is represented with the gray line (r = 0.896) (Pearson's correlation, $p < 0.01$).when tasting the highest concentration (paired t-tests, $p < 0.05$).

Neuroimaging data

Intensity in the brain: NaCl and sucrose

When combining the responses to sweet and salty taste, taste activation in the middle insula was bilaterally modulated by concentration, as well by intensity ratings (L: MNI (-40, -12, 16), z = 4.26 and R: MNI (32, 12, -4), z = 5.07). Taste activation in the right amygdala (MNI (32, 12, 4), z = 5.07) and right putamen (MNI (28, 0, -12), z = 3.62) covaried with concentration but not with perceived intensity.

NaCl

Brain regions whose response covaried with NaCl intensity ratings and NaCl concentration are shown in Table 1. In the NaCl condition, taste activation was modulated by intensity ratings in the middle insula (bilaterally), right amygdala, left hippocampus, right putamen and in the caudate (bilaterally). Activation in the middle insula (bilaterally), right amygdala, and the right putamen was modulated by NaCl concentration. Positive modulation of taste activation in the middle insula by NaCl concentration and intensity ratings is shown in detail in Figure 4. Modulation of amygdala taste activation by NaCl concentration and intensity is shown in Figure 6.

Sucrose

Brain regions whose response covaried with sweet intensity ratings and sucrose concentration are shown in Table 2. Modulation of taste activation by sweet intensity ratings was found in the right middle insula (Figure 5). Modulation by sweetness intensity in the left insula was not statistically significant (MNI -42, -20, 20), Z-score = 3.0 $p < 0.01$). Also, in the left thalamus modulation of taste activation by sweetness was found. Taste activation in the right middle insula and in the right putamen were positively modulated by sucrose concentration (Table 2). Taste activation in the amygdala was not modulated by sucrose concentration or perceived sweetness intensity.

NaCl versus Sucrose

The differences between modulation of taste activation by NaCl and by sucrose are shown in Table 3 (NaCl>Sucrose). Modulation of taste activation in the anterior insula was stronger from saltiness than from sweetness, i.e., anterior insula activation increased more with NaCl concentration then with sucrose concentration. There were no brain areas that were modulated more strongly by sucrose concentration than by NaCl concentration.

Table 1 Brain regions where taste activation is modulated by the degree of saltiness[1]

Region	Cluster size[2]	Peakvoxel coordinates[3]			Z score	Region	Cluster size[2]	Peakvoxel coordinates[3]			Z score
		x	y	z				x	y	z	
Intensity ratings						**Concentration**					
Whole brain											
Insula L	98	-40	-12	16	4.87	Insula R	149	32	16	0	4.05
Hippocampus L		-36	-28	-8	4.17	Inferior frontal gyrus R	42	44	36	8	3.82
Insula R	311	32	20	16	4.78	Precentral gyrus L	47	-36	-20	64	3.69
Inferior frontal gyrus R		56	16	12	4.38			56	16	12	4.38
Insula ROI											
Insula L	18	-40	-12	16	4.68	Insula R	41	32	16	4	4.60
		16	20	-8	3.99		78	36	12	4	4.30
Insula R	78	36	12	4	4.30	Insula L	13	-40	-12	4	3.27
	17	40	-12	8	3.51						
Striatum ROI											
Caudate R	14	20	-20	24	5.00	Putamen R	24	32	12	0	4.56
Caudate L	11	-16	-8	24	4.15						
Putamen R	24	24	8	0	3.39						
Amygdala ROI											
Amygdala R	11	32	4	-20	4.04	Amygdala R	11	24	0	-12	3.60

[1]Saltiness modulation was tested by performing a t-test on the contrast images for modulation of taste activation by intensity ratings and concentration for all brain voxels by using statistical parametric mapping. L, left: R, right; ROI, region of interest. [2]Reported clusters were thresholded at $p < 0.005$ (uncorrected for multiple comparisons) with a cluster extent of $K > 20$ voxels for whole brain and $K > 8$ voxels for ROIs.[3]Voxel coordinates are in Montreal Neurological Institute (MNI) space (15).

Table 2 Brain regions where taste activation is modulated by the degree of sweetness [1]

Region	Cluster size[2]	Peakvoxel coordinates[3]			Z score	Region	Cluster size[2]	Peakvoxel coordinates[3]			Z score
		x	y	z				x	y	z	
Intensity ratings						**Concentration**					
Whole brain											
Precental gyrus R	34	64	-4	12	4.05	Thalamus L	146	-16	-20	12	4.41
						Insula R	20	40	-20	20	3.31
Insula ROI											
Insula R	15	40	-20	20	3.83						
Striatum ROI											
Putamen R	11	28	8	8	3.69						

[1]Sweetness modulation was tested by performing a t-test on the contrast images of modulation of taste activation by intensity ratings and concentration for all brain voxels by using statistical parametric mapping. L, left: R, right; ROI, region of interest. [2]Reported clusters were thresholded at $p < 0.005$ (uncorrected for multiple comparisons) with a cluster extent of $K > 20$ voxels for whole brain and $K > 8$ voxels for ROIs.[3]Voxel coordinates are in Montreal Neurological Institute (MNI) space (15).

Table 3 Brain regions where taste activation is differentially modulated by sweetness and saltiness

Region	Cluster size[2]	Peakvoxel coordinates[3]			Z score	Region	Cluster size[2]	Peakvoxel coordinates[3]			Z score
		x	y	z				x	y	z	
Intensity ratings						**Concentration**					
Whole brain											
Frontal middle gyrus R	34	64	-4	12	4.05						
Insula ROI											
Anterior insula R	38	-32	20	-8	4.47	Insula R	12	44	-12	8	4.01
		-40	20	-8	4.21						
Anterior insula L	35	36	16	4	4.22						
		28	24	-12	3.77						

[1]Modulation was tested by performing a t-test on the contrast images of modulation of taste activation by intensity ratings and concentration for all brain voxels by using statistical parametric mapping. L, left: R, right; ROI, region of interest. [2]Reported clusters were thresholded at $p < 0.005$ (uncorrected for multiple comparisons) with a cluster extent of $K > 20$ voxels for whole brain and $K > 10$ voxels for ROIs. [3]Voxel coordinates are in Montreal Neurological Institute (MNI) space (15).

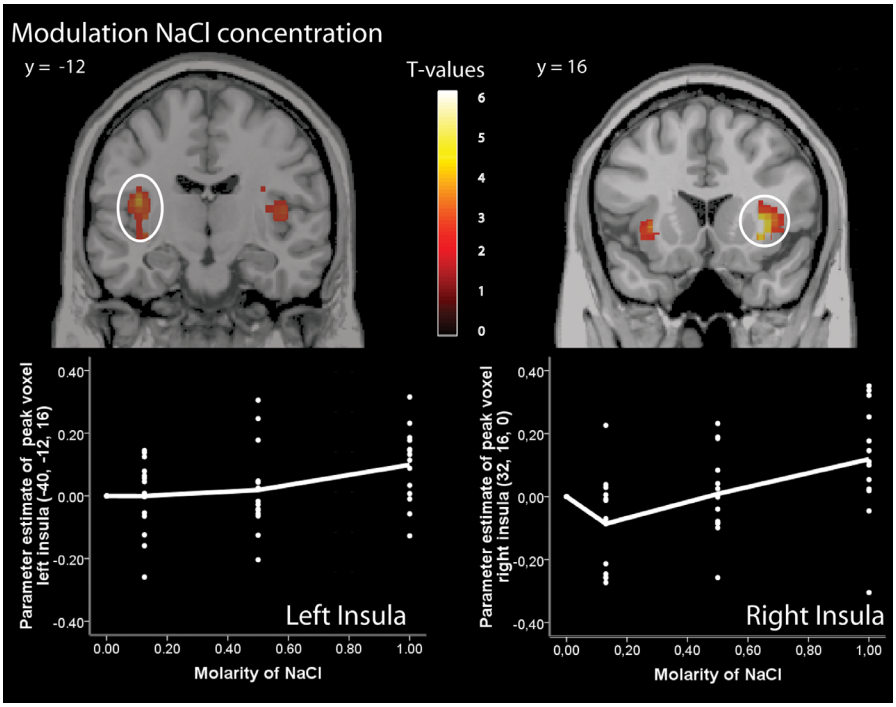


Figure 4 Modulation of insula taste activation by saltiness. Left: Statistical parametric maps of the t-tests, thresholded at $t = 2.8$ ($p < 0.005$ uncorrected) and mean parameter estimates of taste activation for the left insula peak voxels per concentration. Right: Statistical parametric maps of the t-tests, thresholded at $t = 2.8$ ($p < 0.005$ uncorrected) and mean parameter estimates of taste activation for the right insula peak voxels per concentration. Circles indicate the insula clusters.

DISCUSSION

We determined the brain areas where taste activation covaries with stimulus intensity, using a range of NaCl and sucrose solutions. Perceived intensity and concentration were highly correlated and therefore modulation by these two factors yielded similar brain areas.

The first study examining the representation of taste intensity in the brain compared brain responses between two low intensity tastes and two high intensity tastes (sweet and bitter) (11). This classical fMRI approach compares taste activation, i.e., how robustly tasting induces a blood oxygen level-dependent (BOLD) response. In contrast, we used parametric modulation analyses in conjunction with a range of four concentrations of each stimulus type. Parametric modulation is a more recently developed approach (18), which tests for a linear correlation between a parameter and the amplitude of the BOLD response. Using this approach, we examined several ROI's based on the study of Small et al. (2003) and found modulation of taste activation by intensity in the middle insula (bilaterally), amygdala, striatum and hippocampus, but not in the pons and cerebellum.

Insula

We found that middle insula taste activation was modulated by intensity differences, bilaterally. This is in line with the findings of Small et al. (2003), that high intensity tastes activate the middle insula more strongly than low intensity tastes. Schoenfeld et al. (19) found a high inter-individual variability in the exact part of the insula activated by the five basic tastes, but considerable overlap between the insular regions activated by the different tastes. However, they did not account for possible effects of differences in pleasantness and intensity. We found in additional analyses on the group level that patterns of taste activation show great overlap and do not differ significantly between sweet and salty solutions (this study) and between sweet and savoury drinks (unpublished data). Nevertheless, inter-individual differences may have decreased the power of our group analyses in the insula.

We found that taste activation in the anterior insula increased more with NaCl concentration than with sucrose concentration. The anterior insula is the putative primary taste cortex (20) and is known to respond to taste compared with a tasteless solution (21). However, it is also known to play a role in negative valence-specific responses in taste (11). In our study, high saltiness was perceived as less pleasant than high sweetness. This suggests that the stronger modulation of anterior insula by taste activation by saltiness could be due to a valence difference.

Amygdala

Our results show that saltiness (both perceived intensity and concentration) modulates taste activation in the amygdala whereas sweetness did not affect amygdala activation. The amygdala has been associated with emotional processing of positive as well as negative stimuli (22, 23). For food stimuli amygdala activation has been shown to be associated with reward (24) and with the intensity of odours (17). Winston et al. (16) found that amygdala odour activation was only affected by odour intensity when an odour was perceived as pleasant or unpleasant, i.e., the amygdala did not respond to neutral odours. In our study, pleasantness ratings of the sucrose solutions remained approximately neutral, whereas the pleasantness of NaCl solutions decreased with increasing concentration. Thus our results suggest, in line with previous findings for odours (16), that the amygdala codes for intensity

but only for non-neutral stimuli, i.e., not independent of valence. According to Veldhuizen et al (2007), the hedonic tone and subjective intensity of a stimulus are related and therefore difficult to separate. Previous brain imaging studies (11) (16, 17) (25) and our study show there is a functional interplay between intensity and pleasantness.

Striatum

Both sweetness and saltiness modulated taste activation in the striatum (putamen and caudate). This is in line with the findings of Small et al. (11) that striatal (putamen) activation is affected by intensity differences and may reflect (the assessment of) reward value and/or affective value. Rudenga et al. (26) showed that when tasting a potentially nutritive stimulus (sucrose and NaCl) the connectivity between the insula and striatum was enhanced compared to potentially harmful tastes. The dorsal striatum encodes consummatory food reward, while the ventral striatum responds preferentially to food anticipation and is involved in forming predictions of affective value. For sweetness this is not surprising, given the rewarding nature of sucrose solution, but for saltiness this is a novel finding (27).

Hippocampus

We found modulation of hippocampal taste activation by saltiness ipsilateral to the site of amygdala activation. This concurs with the findings of Zald et al. (28) that tasting saline activates the hippocampus (contrasted with water) but has not been reported in other neuroimaging studies that administered basic taste stimuli (29). Zald et al. (28) noted that hippocampus activation by saline primarily occurred among subjects who found the saline extremely aversive. This suggests that, similar to the amygdala, the modulation of taste activation in the hippocampus may be caused by aversive taste of salt rather than by intensity alone.

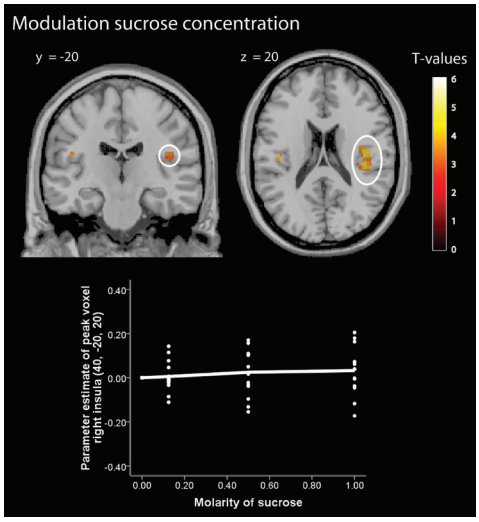


Figure 5 Modulation of insula taste activation by sweetness. Top: Statistical parametric maps of the t-tests, thresholded at t = 2.8 ($p < 0.005$ uncorrected). Circles indicate the insula cluster. Bottom: Mean parameter estimates of taste activation for the insula peak voxel per concentration.

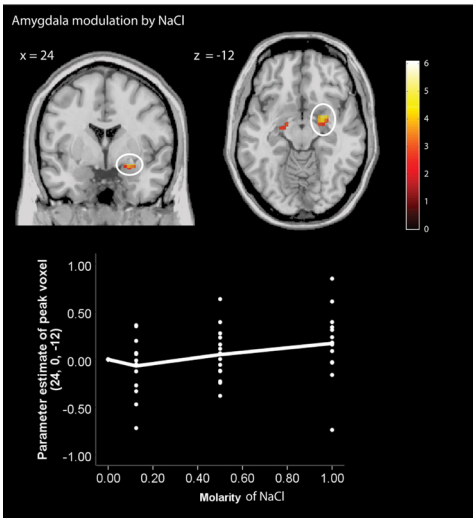


Figure 6 Modulation of amygdala taste activation by saltiness. Top: Statistical parametric maps of the t-tests, thresholded at t = 2.8 ($p < 0.005$ uncorrected). Bottom: Mean parameter estimates of taste activation for the amygdala peak voxels per concentration. Circles indicate the amygdala cluster.

Sweetness versus saltiness

The process of tasting starts on the tongue. All areas of the tongue can respond to the five basic tastes (30). In rodents, the mean firing rate of taste cells is similar for sucrose and NaCl (31). Moreover, the mean responses to both NaCl and sucrose have been found to increase monotonically with concentration (31). Although the mean firing rate of taste cells is similar for sucrose and NaCl, stimulation by sucrose or NaCl subsequently results in different patterns of taste transduction (32), and involves different neurons (33). This difference in taste transduction could explain the differences in modulation of amygdala and anterior insula activation by sweetness and saltiness. However, little is known about the NaCl taste transduction pathway (34). In addition these differences in peripheral taste transduction and the differences in modulation by sucrose and NaCl could be explained by their different physiological function. Both NaCl and sucrose are essential nutrients (34). NaCl plays an essential role in maintaining electrolyte balance, as well as in the regulation of blood pressure and blood volume and in water homeostasis (1). Since a high NaCl intake can disturb the electrolyte balance and other regulation processes NaCl intake is strictly regulated. Sucrose intake, on the other hand, is not so strictly regulated. Sucrose is a source of energy for the body and carbohydrate (as well as other macronutrient) intake is mainly limited by availability and by satiety mechanisms (35). As a result, sucrose intake is tolerable for the body in far greater amounts. This may explain why neuronal activation in the amygdala and anterior insula was higher for NaCl than for sucrose solutions. The higher sensitivity of these brain areas to NaCl concentration may reflect the strict monitoring of NaCl intake.

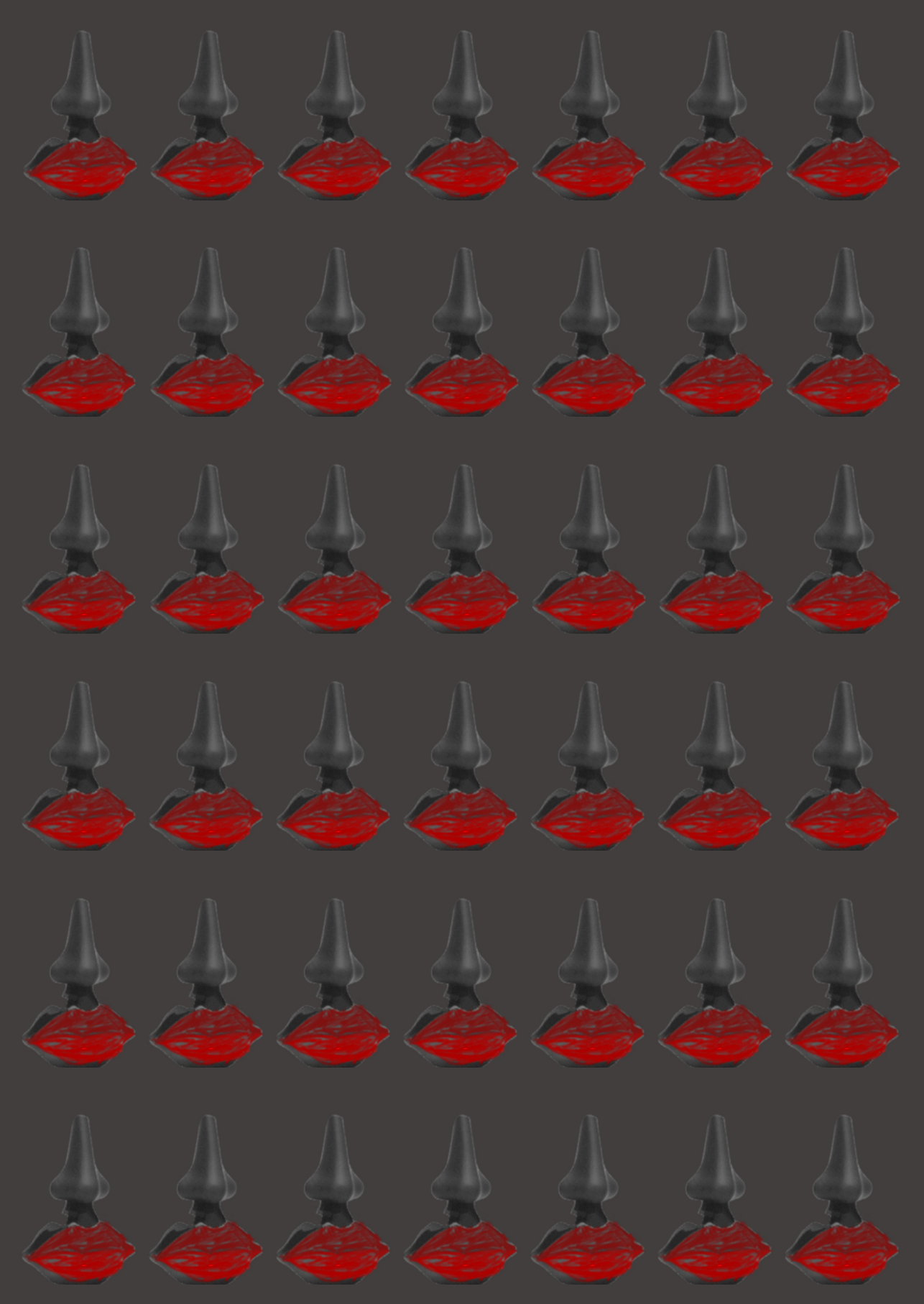
CONCLUSION

In conclusion, our results suggest that taste intensity is represented in the middle insula. Despite similar subjective intensity ratings, modulation of taste activation in the anterior insula by NaCl increased more with concentration than that by sucrose. This greater responsiveness of the anterior insula to saline (compared to sucrose) intensity differences, as well as the modulation of amygdala activation by NaCl taste intensity and concentration can be explained by the fact that intensity and pleasantness are closely related (9), and valence is an important factor when perceived intensity changes. Given the potentially unpleasant and artificial nature of a pure NaCl and sucrose solutions, subsequent studies should use sweet and savoury foods to corroborate and extend our finding. This may further elucidate potential differences in satiation for sweet and savoury foods (36).

REFERENCES

1. Garcia-Bailo, B., Toguri, C., Eny, K. M. & El-Sohemy, A. (2009) Genetic variation in taste and its influence on food selection. OMICS. 13: 69-80.
2. Sorensen, L. B., Moller, P., Flint, A., Martens, M. & Raben, A. (2003) Effect of sensory perception of foods on appetite and food intake: a review of studies on humans. Int. J. Obes. Relat Metab Disord. 27: 1152-1166.
3. Weijzen, P. L., Smeets, P. A. & de Graaf C. (2009) Sip size of orangeade: effects on intake and sensory-specific satiation. Br. J. Nutr. 102: 1091-1097.
4. Zijlstra, N., de Wijk, R. A., Mars, M., Stafleu, A. & de Graaf C. (2009) Effect of bite size and oral processing time of a semisolid food on satiation. Am. J. Clin. Nutr. 90: 269-275.
5. de Wijk, R. A., Zijlstra, N., Mars, M., de Graaf C. & Prinz, J. F. (2008) The effects of food viscosity on bite size, bite effort and food intake. Physiol Behav. 95: 527-532.
6. Weijzen, P. L., Liem, D. G., Zandstra, E. H. & de, G. C. (2008) Sensory specific satiety and intake: the difference between nibble- and bar-size snacks. Appetite 50: 435-442.
7. Ruijschop, R. M., Boelrijk, A. E., Burgering, M. J., de, G. C. & Westerterp-Plantenga, M. S. (2010) Acute effects of complexity in aroma composition on satiation and food intake. Chem. Senses 35: 91-100.
8. Vickers, Z. & Holton, E. W. J. (2001) Effect of ideal–relative sweetness on yogurt consumption. Food Quality and Preference 12: 521-526.
9. Veldhuizen, M. G., van Rooden, A. P. & Kroeze, J. H. (2006) Dissociating pleasantness and intensity with quinine sulfate/sucrose mixtures in taste. Chem. Senses 31: 649-653.
10. Small, D. M. (2006) Central gustatory processing in humans. Adv. Otorhinolaryngol. 63: 191-220.
11. Small, D. M., Gregory, M. D., Mak, Y. E., Gitelman, D., Mesulam, M. M. & Parrish, T. (2003) Dissociation of neural representation of intensity and affective valuation in human gustation. Neuron 39: 701-711.
12. Van Strien, T., Frijters JER, Bergers GPA & Defares PB (1986) The Dutch Eating Behaviour Questionnaire (DEBQ) for assessment of restrained, emotional and external eating behaviour. Int J Eat Disord 295-315.
13. Van Strien, T. (1997) The concurrent validity of a classification of dieters with low versus high susceptibility toward failure of restraint. Addict. Behav. 22: 587-597.
14. Maldjian, J. A., Laurienti, P. J., Kraft, R. A. & Burdette, J. H. (2003) An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage. 19: 1233-1239.
15. Evans, A. D., Collins, D. L., Millst, S. R., Brown, E. D., Kelly, R. L. & Peters, T. M. (1993) 3D statistical neuroanatomical models from 305 MRI volumes. Proc IEEE Nucl Sci Symp Med Imaging 3: 1813-1817.
16. Winston, J. S., Gottfried, J. A., Kilner, J. M. & Dolan, R. J. (2005) Integrated neural representations of odor intensity and affective valence in human amygdala. J. Neurosci. 25: 8903-8907.
17. Anderson, A. K., Christoff, K., Stappen, I., Panitz, D., Ghahremani, D. G., Glover, G., Gabrieli, J. D. & Sobel, N. (2003) Dissociated neural representations of intensity and valence in human olfaction. Nat. Neurosci. 6: 196-202.
18. Buchel, C., Wise, R. J., Mummery, C. J., Poline, J. B. & Friston, K. J. (1996) Nonlinear regression in parametric activation studies. Neuroimage. 4: 60-66.
19. Schoenfeld, M. A., Neuer, G., Tempelmann, C., Schussler, K., Noesselt, T., Hopf, J. M. & Heinze, H. J. (2004) Functional magnetic resonance tomography correlates of taste perception in the human primary taste cortex. Neuroscience 127: 347-353.
20. Pritchard, T. C., Hamilton, R. B., Morse, J. R. & Norgren, R. (1986) Projections of thalamic gustatory and lingual areas in the monkey, Macaca fascicularis. J. Comp Neurol. 244: 213-228.
21. Bender, G., Veldhuizen, M. G., Meltzer, J. A., Gitelman, D. R. & Small, D. M. (2009) Neural correlates of evaluative compared with passive tasting. Eur. J. Neurosci. 30: 327-338.
22. Zald, D. H. (2003) The human amygdala and the emotional evaluation of sensory stimuli. Brain Res. Brain Res. Rev. 41: 88-123.
23. O'Doherty, J., Rolls, E. T., Francis, S., Bowtell, R. & McGlone, F. (2001) Representation of pleasant and aversive taste in the human brain. J. Neurophysiol. 85: 1315-1321.
24. Baxter, M. G. & Murray, E. A. (2002) The amygdala and reward. Nat. Rev. Neurosci. 3: 563-573.

25. Small, D. M., Zatorre, R. J. & Jones-Gotman, M. (2001) Increased intensity perception of aversive taste following right anteromedial temporal lobe removal in humans. Brain 124: 1566-1575.
26. Rudenga, K., Green, B., Nachtigal, D. & Small, D. (2010) Evidence for an Integrated Oral Sensory Module in the Human Anterior Ventral Insula. Chem. Senses. 35: 693-703.
27. de Araujo, I., Oliveira-Maia, A. J., Sotnikova, T. D., Gainetdinov, R. R., Caron, M. G., Nicolelis, M. A. & Simon, S. A. (2008) Food reward in the absence of taste receptor signaling. Neuron 57: 930-941.
28. Zald, D. H., Lee, J. T., Fluegel, K. W. & Pardo, J. V. (1998) Aversive gustatory stimulation activates limbic circuits in humans. Brain 121 (Pt 6): 1143-1154.
29. Small, D. M. (2006) Central gustatory processing in humans. Adv. Otorhinolaryngol. 63: 191-220.
30. Chandrashekar, J., Hoon, M. A., Ryba, N. J. & Zuker, C. S. (2006) The receptors and cells for mammalian taste. Nature 444: 288-294.
31. Nishijo, H. & Norgren, R. (1990) Responses from parabrachial gustatory neurons in behaving rats. J Neurophysiol. 63: 707-724.
32. Lindemann, B. (2001) Receptors and transduction in taste. Nature 413: 219-225.
33. Schiffman, S. S. (2000) Taste quality and neural coding: implications from psychophysics and neurophysiology. Physiol Behav. 69: 147-159.
34. Roper, S. D. (2009) Parallel processing in mammalian taste buds? Physiol Behav. 97: 604-608.
35. Schwartz, M. W., Woods, S. C., Porte, D., Jr., Seeley, R. J. & Baskin, D. G. (2000) Central nervous system control of food intake. Nature 404: 661-671.
36. Weenen, H., Stafleu, A. & de Graaf, C. (2005) Dynamic aspects of liking: post-prandial persistence of sensory specific satiety. Food Quality and Preference.



Thinking is easy, acting is difficult,
and to put one's thoughts into action is the most difficult thing in the world

Chapter 3

Anterior cingulate taste activation predicts ad libitum intake of sweet and savoury drinks in healthy normal-weight men

ABSTRACT

After food consumption the motivation to eat (wanting) decreases and associated brain reward responses change. Wanting-related brain responses and how these are affected by consumption of specific foods are ill-documented. Moreover, the predictive value of food-induced brain responses for subsequent consumption has not been assessed. We aimed to determine the effects of consumption of sweet and savoury foods on taste activation in the brain, and to assess how far taste activation can predict subsequent ad libitum intake. 15 healthy men (age 27 ±2 y, BMI 22.0 ±1.5 kg/m2) participated in a randomized cross-over trial. After a >3h-fast subjects were scanned with the use of functional magnetic resonance imaging before and after consumption of a sweet or savoury preload (0.35 L fruit or tomato juice) on 2 occasions. After the scans the preload juice was consumed ad libitum. During scanning, subjects tasted the juices and rated their pleasantness. Striatal taste activation decreased after juice consumption, independent of pleasantness. Sweet and savoury taste activation were not differentially affected by consumption. Anterior cingulate taste activation predicted subsequent ad libitum intake of sweet ($r=-0.78$, $P < 0.001$unc) as well as savoury juice ($r=-0.70$, $P < 0.001$unc). In conclusion, we showed how taste activation of brain reward areas changes following food consumption. These changes may be associated with the food's physiological relevance. Further, the results suggest that anterior cingulate taste activation reflects food-specific satiety. This extends our understanding of the representation of food specific-appetite in the brain and shows that neuroimaging may provide objective and more accurate measures food motivation than self-report measures.

INTRODUCTION

The process of satiation, which ultimately results in meal termination, consists of a complex interaction between neural, hormonal and gastro-intestinal signals (1, 2). Satiation, and thereby intake, is affected by numerous internal (physiological) and external (environmental) factors (3). All these factors are integrated in the brain and ultimately result in a particular pattern of food intake. 90% of the foods that people consume can be categorized as either sweet or savoury (4). Sweetness is associated with mono- and disaccharides, which are an important source of energy for the body. Savoriness is associated with umami, fatty and salty tastes (often protein- and fat-containing foods) (5, 6), which also signal a source of energy and are essential for maintaining electrolyte balance (7).

It has been suggested that SSS, which is defined as the relative decrease in pleasantness of a food (8), is higher for savoury than for sweet foods (9). While SSS, by definition, refers to (changes in) liking, consumption of a food also decreases the motivation to eat, i.e., wanting, and both contribute to a food's reward value (10). Because wanting reflects a need state, it seems likely that changes in wanting are also food-specific. Several studies have suggested that liking and wanting have different neural substrates (11-13), but are, however, hard to separate in experimental practice (14-16).

Interestingly, a recent study showed no difference in ad libitum intake between sweet and savoury foods matched on palatability, texture, energy density, and macronutrient composition (17). This suggests that differential effects of sweet and savoury foods on satiation and possibly on the brain are due to differences in these food properties rather than to learned satiety effects associated with sweet and savoury taste.

Effects of food consumption on the brain have been examined in several neuroimaging studies, e.g. by comparing taste or odour responses before and after consumption (18-20) and by scanning during satiation (21). Areas affected by consumption include the insula, striatum, anterior cingulate cortex, hippocampus and amygdala (18, 19, 21). However, the predictive value of food-induced brain responses for subsequent intake has not been established. In particular taste cues might elicit brain responses indicative of subsequent intake because tasting provides an assessment of food quality, composition and palatability.

Thus, firstly we aimed to determine which brain areas showed food-specific changes in sweet and savoury taste activation following consumption of a preload. We hypothesized that taste responses to an eaten food would decrease in reward-related brain areas like the striatum and amygdala. Secondly, we aimed to establish the predictive value of taste activation with regard to subsequent food intake for a sweet as well as for a savoury food. We expected that brain areas found to be affected by satiety may predict ad libitum intake: candidate regions were the insula, striatum, anterior cingulate cortex, hippocampus and amygdala (18, 19, 21).

MATERIALS AND METHODS

Subjects

Healthy normal weight righted-handed men were recruited by flyers posted at the University Medical Center Utrecht. Exclusion criteria were restrained eating (22) (Dutch Eating Behaviour Questionnaire (23): score > 2.5), disliking one of the products, smoking, an energy-restricted diet or changes in body weight >5 kg the last 2 months, eating disorder, history of or current alcohol consumption > 28 units per week, or any diseases (including neurological, psychiatric diseases and taste and smell disorders) and use of medication. In total, 15 normal-weight right-handed men (mean age ± SD 27.3 ± 1.9 yr, mean BMI ± SD 22.0 ± 1.5 kg/m2) enrolled in the study. All experimental procedures were approved by the Medical Ethical Committee of the University Medical Center Utrecht (NL22266.041.08). Before the experiment written informed consent was obtained from all subjects. Sample size was based on previous fMRI studies (20, 24).

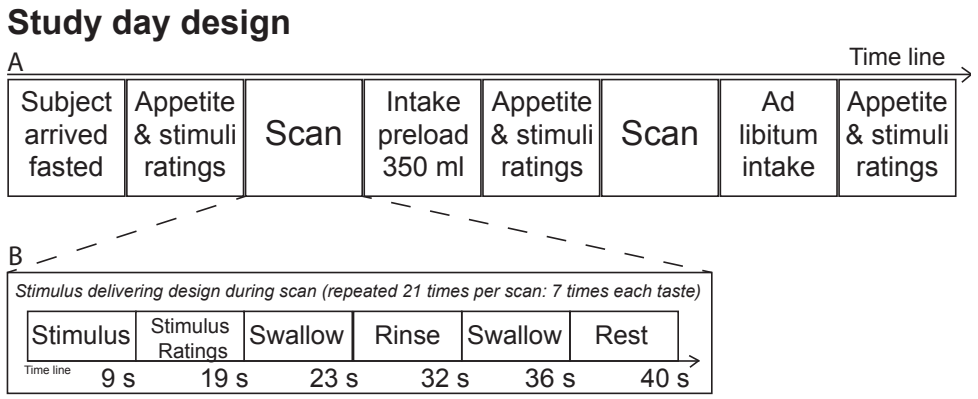


Figure 1 Overall design of a study day (A) and the scan design (B). The savory and sweet condition had the same design. During the 2 fMRI scans all 3 stimuli were tasted 7 times in randomized order, making a total of 21 taste cycles.

Design

This study has a randomized cross-over design with 2 conditions (sweet and savoury). Each condition involved 2 scans sessions with at least 1 week between the 2 study days. The order of the conditions was randomized per subject (Fig. 1).

Test foods

Three stimuli were used. The first was a sweet fruit juice, which was a thick peach and orange juice mix (Appelsientje Dubbeldrank Sinaasappel & Perzik, FrieslandCampina, Ede, the Netherlands) (Nutritional value of 0.1 L: 13.1 g carbohydrates, of which 12.9 g sugars; 0 g fat; 0.28 g fibres; 0.001 g sodium; 8 mg vitamin C and an energy content of 230 kJ). The second was a savoury tomato juice (Appelsientje Zontomaatje, FrieslandCampina, Ede, the Netherlands) (Nutritional value of 0.1 L: 2.9 g carbohydrates,

of which 2.9g sugars; 9 g fat; 0.40 g fibres; 0.25 g sodium; 15 mg vitamin C and an energy content of 69 kJ). The third stimulus was Dutch tap water; this was used as a control stimulus. However, subjects did not know that the control stimulus was water; it was presented to them as a control taste. The fruit juice is referred to as sweet stimulus, tomato juice as the savoury stimulus, and water as the control. The taste drunk as the preload (independent of which taste) is referred to as the target taste. The other taste, i.e. the one not drunk as a preload, is referred to as the non-target taste.

Experimental procedures

Subjects fasted for at least 3h before the scan sessions. All sessions were scheduled in the afternoon and subjects were instructed to eat lunch around noon and to not consume anything afterwards.

When subjects arrived they first filled in the appetite questionnaire, in which hunger, fullness, thirst and desire to eat something sweet and savoury were rated on 100 mm VAS. Secondly, they tasted the 3 stimuli and rated them on pleasantness, intensity, sweetness and saltiness on 100 mm VAS (stimulus questionnaire). After the ratings subjects were placed in the scanner for their first scan.

Inside the scanner subjects had the tip of 4 bounded tubes in their mouth (internal diameter of 3 mm per tube). The tips where positioned comfortably between the lips so that the tubes delivered the stimuli on the front of the tongue. The 3 stimuli and water (for rinsing) were delivered at room temperature (23°C) by use of 4 programmable syringe pumps. The pumps were programmed to administer 1 mL at 0.028 L/min. The 3 stimuli were pseudo-randomly presented, 7 times each. VAS ratings of pleasantness and intensity during the scan were made by use of a button box. Instructions were displayed on a screen through a computer interface, run by the computer program PRESENTATION. After tasting for 9 s subjects gave either VAS ratings (8 s) (after 4 out of 7 trials) or were directly cued to swallow (4 s). Then subjects received a rinse with water (9 s), followed again by a cue to swallow (4 s) and rest (fixation on a crosshair for 4 s).

After the scanner a 0.35 L preload of either tomato juice (savoury taste condition) or fruit juice (sweet taste condition) was consumed. The preload was drunk through a straw from a blinded cup. When the preload was finished the subjects filled in the appetite and stimulus questionnaires again. Subsequently, subjects were scanned again according to the same protocol as the first scan. Finally subjects were given a cup with the preload juice from which they were instructed to drink the juice ad libitum. The subjects could not see the amount they drunk. After consumption the appetite and stimulus questionnaires was filled in again.

fMRI data acquisition

The scans were performed on a 3-Tesla Philips Achieva at the University Medical Center Utrecht. First a T1-weighted anatomical scan was acquired (TR/TE = 61/8.4 ms, flip angle = 30°, FOV = 288 × 175mm, 175 axial slices, voxelsize = 1 × 1 × 1 mm). Second a fMRI scan was made (3D presto EPI sequence, TR/TE= 906/15.6 ms, flip angle =90°, FOV = 224 × 224 × 150mm, 43 interleaved axial slices, voxelsize = 3.5 × 3.5 × 3.5 mm). The total duration of each functional scan was 15 min, during which 990 volumes were obtained. After the functional scan, one additional functional volume was acquired, but with a flip angle of 27° for better anatomical contrast.

fMRI data processing and analysis

fMRI data were pre-processed and analyzed using SPM8 run with MATLAB 7.5 and the WFU Pickatlas-tool (25). First the functional volumes of every subject were realigned to the first volume of the first run. Second, the anatomical image was co-registered with the additional functional volume with a flip angle of 27°, after which this was co-registered with the mean functional image. Third the images were normalized (retaining 3.5 × 3.5 × 3.5 mm voxels) to MNI space (26), and spatially smoothed with a Gaussian kernel of 8mm 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. Data were high-pass filtered with a cut-off of 128 s.

Within subject analyses

For every scan session 7 conditions were modelled: tasting the control, sweet stimuli, savoury stimuli, swallowing, rinsing and giving ratings of pleasantness and intensity. The responses to swallowing, rinsing and rating were neglected in further analyses. Taste activation contrast images were calculated by first subtracting the control stimulus from the other stimuli (sweet and savoury stimuli), resulting in 2 contrast images (sweet vs. control and savoury vs. control) for each subject per scan.

In total, 8 contrast images for taste activation per subject were calculated (2×2×2); sweet before target, sweet after target, savoury before target, savoury after target, sweet before non-target, sweet after non-target, savoury before non-target and savoury after non-target.

Group analyses

To determine the effect of preload on target and non-target taste activation all target and non-target contrast images were entered into a 2 times (before and after preload) ×2 targets (target and non-target) RMANOVA with the mean pleasantness ratings per subject as measured during the scan added as a covariate. To determine the effect of preload per taste category all 8 contrast images were entered into a 2×2×2 (time x target x taste) RMANOVA. The resulting statistical parametric maps were thresholded at $P < 0.001$, $k > 10$, this threshold resembles an overall significance level of $P < 0.05$,corrected for multiple comparisons across the whole brain based on Monte Carlo simulations of random noise distribution using the 3DClustSim module of AFNI (27, 28).

A priori regions of interest (ROIs) were the orbitofrontal cortex, striatum, hippocampus, thalamus, amygdala and insula. These regions have been shown to be involved in effects of consumption and satiation on taste activation in previous neuroimaging studies (20, 29, 30). ROI masks were made using the WFU Pickatlas tool (25).

To determine the brain regions where taste activation covary with ad libitum intake after the scans, the two target contrast images during the second scan were entered into a repeated measure ANOVA. The ad libitum intake of the sweet juice was used a covariate for the sweet target contrast image, and the savoury juice intake was used as a covariate for the savoury target contrast image. The liking and desire to eat something sweet and savoury ratings were also added as covariates, to excluded effect of these factors on the correlations of interest. The same model was run with the non-target taste

activation contrast, to check for taste specificity. Significant correlations ($P < 0.001$, uncorrected for multiple comparisons) between brain activation and ad libitum intake, liking ratings or desire to eat ratings were tested for in SPM8.

For all significant clusters mean parameter estimates of taste activation were obtained with the use of MarsBaR .

Statistical analyzes

All subjective ratings were analyzed using SPPS 16.0 (SPSS Inc, Chicago, USA). Data are presented as means ± SD. Mean pleasantness, intensity, desire to eat something sweet or savoury, sweetness, saltiness, hunger and fullness ratings were compared using a 3 × 2 × 2 and a 2 × 2 × 2 RMANOVA with time point (baseline, after preload and after ad libitum (3) or before and after preload (2)), condition (sweet or savoury) and taste (target, non-target and control) as factors. Bonferroni-corrected paired t-tests were used for post hoc comparison. Pearson correlation coefficients ($P < 0.05$) were calculated to test associations between pleasantness, desire to eat something sweet and savoury, hunger, fullness ratings (during and after the second scan) and ad libitum intake.

With the use of the subjective pleasantness ratings SSS scores were calculated (31). This was done by subtracting the changes in ratings of liking (pleasantness) of the target juice (tomato or fruit) from before the preload to after the preload from the corresponding mean change in ratings of the reference stimuli (control or non-target juice). Thus, the more negative the SSS scores for a food, the higher the degree of SSS.

RESULTS

Subjective data

Hunger and fullness

There were main effects of time on hunger ($P < 0.001$) and fullness ($P < 0.001$). Hunger ratings decreased after preload and after ad libitum intake compared to baseline (both $P < 0.05$).

Fullness ratings increased after the preload and after ad libitum intake compared with baseline (both $P < 0.05$). Hunger and fullness ratings were not differentially affected by the two conditions (Table 1).

Pleasantness

There was a main effect of time (2x3 RMANOVA ($P < 0.001$), 3x3 RMANOVA ($P < 0.005$)) and there was an interaction between taste and time (Outside ($P < 0.001$), Inside ($P < 0.005$)). Pleasantness ratings for the target taste decreased over time ($P < 0.05$ between all time points). For the control and non-target taste no changes were observed ($P > 0.05$). Condition did not affect pleasantness ratings (Table 1 and Supplemental Table 1).

Desire to eat something sweet and savoury

There was a main effect of time for both desire to eat sweet ($P < 0.05$) and savoury ($P < 0.05$). Note that there were 4 missing values at time point 3. Desire to eat something sweet decreased over time

in the sweet ($P < 0.05$), but not in the savoury condition. Desire to eat something savoury decreased after the preload compared to baseline for the savoury ($P < 0.05$), but not for the sweet condition.

Intensity

There was a main effect of taste ($P < 0.005$). Intensity ratings (outside and inside the scanner) were different between the 3 stimuli ($P < 0.05$). The control stimulus was perceived as less intense ($P < 0.05$) (Table 1). Condition and time did not affect the intensity ratings.

Saltiness and sweetness

There was a main effect of taste (saltiness ($P < 0.001$), sweetness ($P < 0.001$)).The tomato juice was perceived as more salty, and the fruit juice as more sweet ($P < 0.05$) (Supplemental Table 2). Condition and time did not affect the saltiness ratings.

Correlations

There was no correlation between changes in pleasantness of the fruit juice and the desire to eat something sweet ($r = 0.51$, $P = 0.11$), or between the changes in pleasantness of the tomato juice and the desire to eat something savoury ($r = 0.19$, $P = 0.51$).

SSS

SSS occurred during both sessions. SSS scores did not differ between the 2 conditions after preload ($P = 0.29$) (Table 1).

Table 1 Pleasantness ratings and SSS scores for the fruit (sweet) and tomato (savoury) stimuli and desire to eat something sweet/savoury, hunger and fullness ratings during the sweet and savoury condition in health, normal weight, young men[1,2]

	Sweet condition			**Savoury condition**		
	Baseline	After preload	After ad libitum	Baseline	After preload	After ad libitum
Hunger	51 ± 23^{a}	37 ± 21^{b}	28 ± 23^{c}	66 ± 17^{a}	44 ± 23^{b}	30 ± 21^{c}
Fullness	29 ± 17^{c}	54 ± 23^{b}	69 ± 22^{a}	36 ± 18^{c}	61 ± 16^{b}	74 ± 14^{a}
Pleasantness						
Fruit	65 ± 19^{a}	54 ± 28^{b}	37 ± 24c	63 ± 20^{a}	62 ± 20^{a}	60 ± 23^{a}
Tomato	50 ± 26^{a}	43 ± 24^{a}	38 ± 27a	57 ± 21^{a}	42 ± 23^{b}	30 ± 21^{c}
Desire to eat						
Sweet	50 ± 20^{a}	41 ± 22^{b}	25 ± 16^{c}	46 ± 18^{a}	52 ± 22^{a}	44 ± 22^{b}
Savoury	59 ± 19^{a}	62 ± 18^{a}	49 ± 18^{b}	54 ± 20^{a}	44 ± 17^{b}	36 ± 20^{b}
SSS scores		-10 ± 23^{a}	-16± 21^{a}		-15 ± 27^{a}	-23 ± 25^{a}

[1]Values are means ± SD, n = 15. a,b,c Entries within a row having different lower case letter superscripts differ significantly (repeated measure ANOVA, post- hoc paired t-test $P < 0.05$). There was no effect of condition and taste stimuli. [2]Rating performed on 100 mm VAS.

Ad libitum intake

Ad libitum intake (mean ± SD) of fruit juice was 0.28 ± 0.19 L and of tomato juice, 0.26 ±0.22 L. There was no difference between the fruit and tomato juice ad libitum intake (P > 0.05). Ad libitum intake was not correlated with pleasantness (r = 0.04, P = 0.82), hunger (r = 0.10, P = 0.60), fullness (r = -0.19, P = 0.32), and desire to eat the associated taste category (r = 0.29, P = 0.12) ratings just before the juice was ad libitum ingested.

Neuroimaging data

Consumption

Overall effects of consumption on overall taste activation were observed in the right amygdala, insula and hippocampus (Supplemental Table 3). For the target taste, taste activation in the striatum (pallidum and putamen) and thalamus decreased with consumption of the preload. After consumption taste activation in the hippocampus was increased (Table 2, Fig. 2 and Fig. 3). Non-target taste activation increased after consumption in the midbrain (MNI (11, -32, -18), z = 4.39), cingulate gyrus (MNI (7, -49, -18), z = 4.25) and the right insula (MNI (39, 11, -14), z = 3.78). The effect of consumption on target taste activation differed between the 2 tastes. When subjects consumed the fruit preload, the target taste showed an increased response in the amygdala, midbrain and ACC after the preload. There was a decreased response in the striatum (pallidum) after consumption, when the target taste was sweet (Table 3). In the savoury condition, the target taste elicited a decreased response in putamen and striatum (caudate) after consumption, whereas activation in the ventral striatum and hippocampus were increased (Fig. 3 and Table 3). Activation did not differ between the sweet and the savoury target tastes.

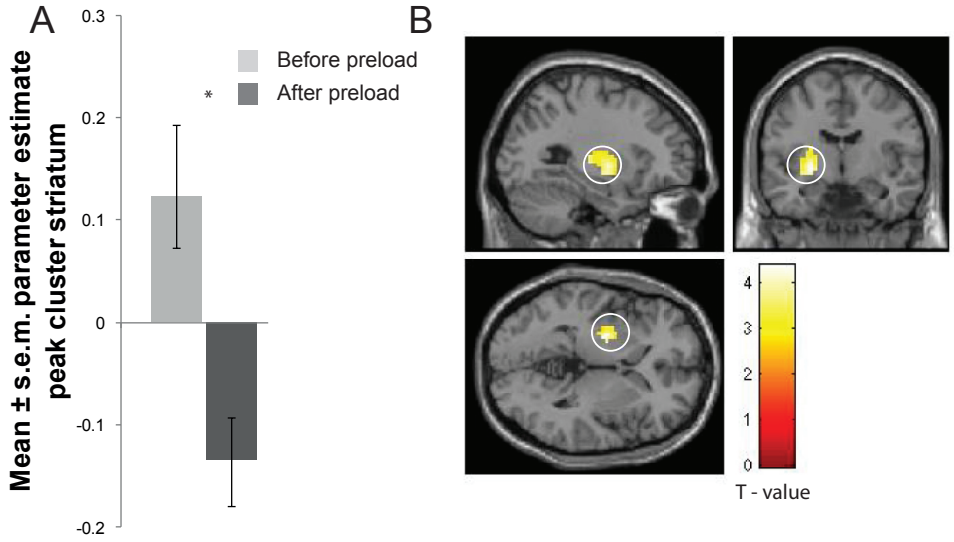


Figure 2 Striatum response for the target taste in healthy, normal weight, young men in both sweet and savoury conditions before and after the preload, n = 15. In A, mean ± S.E.M parameter estimates of the taste activation are presented in bars. In B, a T-map of the taste activation in the striatum (marked with a white circle). Activation is thresholded at T = 2.8, which corresponds to P < 0.005 uncorrected for multiple comparisons for better visualization. * Activation was significantly greater before the preload (ROI analysis, post-ANOVA t-test P < 0.001 uncorrected for multiple comparisons).

Table 2 Effect of consumption on target taste activation for both sweet and savoury condition in healthy, health, normal weight, young men[1]

Regions	Cluster size[2]	Peakvoxel coordinates[3]			Z-score
		x	y	z	
Before > After Preload					
Striatum ROI					
Pallidum L	67	-25	-7	-4	4.20
Putamen L		-25	-11	14	3.63
		-28	-21	7	3.53
Thalamus L	51	-25	-28	7	5.02
Before < After Preload					
Hippocampus L	31	-32	-28	-11	4.75
Hippocampus R	22	42	-35	-11	4.00

[1]Values are clusters of mean brain activation, n = 15. All are ROI analyzes. L: left hemisphere and R: right hemisphere. Clusters are differences in brain activation before and after preload intake. [2]Reported clusters were thresholded at P < 0.001 uncorrected for multiple comparisons, with a cluster extent threshold k > 10 voxels. *Also significant at FWE-corrected P < 0.05. [3]Voxel coordinates are in MNI space (26).

Table 3 Effect of consumption on sweet target taste activation in sweet condition, left table, and savory target taste activation in savory condition, right table in health, normal weight, young men[1]

Region	Cluster size[2]	Peakvoxel coordinates[3]			Z-score	Region	Cluster size[2]	Peakvoxel coordinates[3]			Z-score
		x	y	z				x	y	z	
Sweet						**Savoury**					
Before > After Preload											
Pallidum L	20	-25	-7	-4	4.09	Putamen L	3	-28	-21	4	3.72
							3	-28	-39	4	3.64
							9	-21	-7	14	3.59
Before < After Preload											
Cerebellum	98	28	-56	-28	4.16	Caudate L	7	-14	18	4	3.49
Amygdala R	13	28	4	-14	3.86	Hippocampus L	9	-35	-32	-11	3.48
Midbrain	17	-4	-25	-14	3.78	Hippocampus R	3	42	-35	-11	3.48
ACC	35	0	4	28	3.43						
		0	18	28	3.42						

[1]Values are clusters of mean brain activation, n = 15. Contrasts were calculated using t-tests on the contrast images of before and after preload of the sweet and savory target taste. L: left hemisphere and R: right hemisphere. [2]Reported clusters were thresholded at P < 0.001, uncorrected for multiple comparisons, which corresponds to a Z-score > 3.0. [3]Voxel coordinates are in MNI space (26).

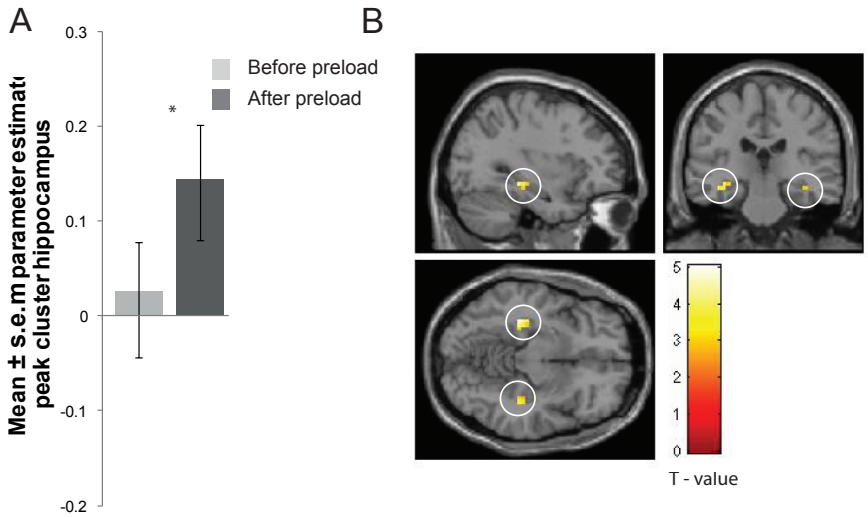


Figure 3 Hippocampus response for target taste in healthy, normal weight, young men in both sweet and savoury conditions before and after the preload, n = 15. In A, mean ± S.E.M parameter estimates of the taste activation are presented in bars. In B, a T-map of the taste activation in the hippocampus (marked with a white circle). Activation is thresholded at T = 2.8, which corresponds to P < 0.005 uncorrected for multiple comparisons for better visualization.* Activation was significantly greater after the preload (ROI analysis, post-ANOVA t-test P < 0.001 uncorrected for multiple comparisons)

Prediction of ad libitum intake

Target taste activation in the ACC during the second scan correlated negatively with subsequent ad libitum intake (Table 4). The same was observed when the sweet and savory condition were analyzed separately (Table 4, Fig. 4, sweet r = -0.78 and savory r = -0.70, both P < 0.001 uncorrected for multiple comparisons in SPM8). Liking ratings were not correlated with any taste activation. The desire to eat a food of the same taste category was correlated with activation in part of the ACC (MNI (0, 49, 7,) z =3.55, P < 0.001 uncorrected for multiple comparisons). Non-target taste activation was not correlated with ad libitum intake.

Table 4 Brain areas during the second scan that covariate negatively with ad libitum intake in the sweet and savory condition in health, normal weight, young men[1]

Region	Cluster size[2]	Peakvoxel coordinates[3]			Z-score
		x	y	z	
Overall					
ACC	28	7	39	4	4.17
Sweet					
ACC	11	4	42	4	3.45
Savoury					
ACC	15	7	32	4	3.23

[1]Values are clusters of mean brain activation that correlated with ad libitum intake, n = 15. Contrasts were calculated using t-tests on the contrast images of after preload of the sweet and savory target taste. L: left hemisphere and R: right hemisphere. ROI analyzes. [2]Reported clusters were thresholded at P < 0.001, uncorrected for multiple comparisons, which corresponds to a Z-score > 3.0, with a cluster extent threshold k > 10. * Also significant at FWE-corrected P < 0.05. [3]Voxel coordinates are in MNI space (26).

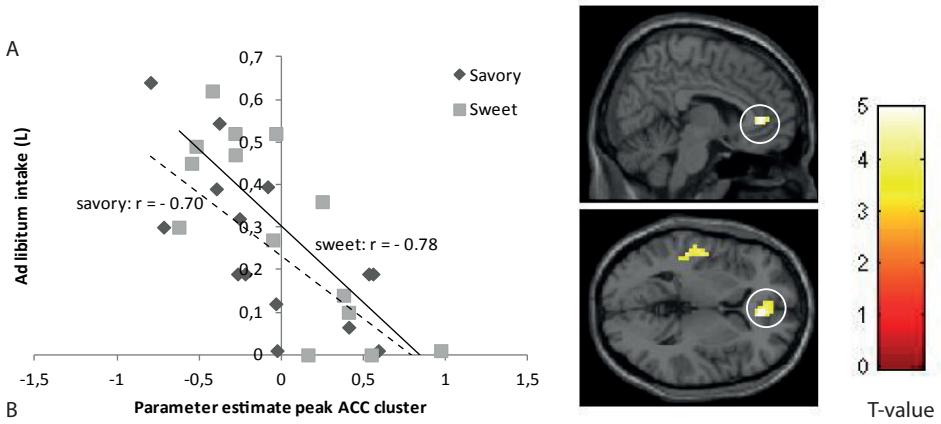


Figure 4 Correlation between taste activation in the ACC and ad libitum intake in healthy, normal weight, young men in both sweet and savoury conditions before and after the preload, n = 15. In A, a scatter plot represents the parameter estimates of the ACC cluster during the second scan and ad libitum intake of all participants in the sweet and savoury condition. The line represents the sweet taste, the dashed line the savoury ($P < 0.001$ uncorrected for multiple comparisons). In B, a T-map of the taste activation in the ACC (marked with a white and black circle). Activation is thresholded at $T = 2.8$, which corresponds to $P < 0.005$ uncorrected for multiple comparisons for better visualization.

DISCUSSION

We found that after sweet and savoury food consumption taste activation decreased in the striatum for the target taste, independent of pleasantness. This was not the case for the non-target taste. Moreover ACC taste activation predicted subsequent ad libitum intake, this was independent of the type of taste.

Effect of consumption

In previous studies, effects of consumption related to changes in pleasantness have been observed in the orbitofrontal cortex (32, 33). The hedonic value that changed during consumption is defined as 'liking', referring to the palatability of the food, whereas the motivation to eat is associated with 'wanting'; both contribute to food reward (10). In behavioural studies wanting and liking are assessed by asking subjects to rate their desire to eat (wanting) and the pleasantness of a taste on that moment (liking) (34). In our study the pleasantness and desire to eat something sweet and savoury ratings changed during both conditions, however the relative change of wanting and liking were not correlated. Because SSS occurred for both taste (during both conditions the pleasantness of the target-taste decreased relative to that of the non-target taste and control following preload consumption (35)), we added pleasantness ratings as a covariate, to be able to examine wanting related changed in taste activation. The brain responses that increased or decreased as an effect of consumption were due to the specific wanting-related changes. Preload consumption induced changes in the target taste activation in the striatum, amygdala, anterior cingulate cortex, hippocampus, midbrain and the cerebellum. These areas are known to be involved in reward and memory (21, 32, 36). In

addition non-target taste activation in the midbrain, mid cingulate gyrus and anterior insula, i.e., knows as primary taste cortex areas (6). Effect of consumption occurred in reward-related areas for the consumed taste and in gustatory areas for the non-consumed taste.

Sweet and savoury target taste

When comparing sweet and savoury target taste activation, no difference was observed. When only looking at sweet or savoury target taste activation after consumption we found food-specific effects in the amygdala, anterior cingulate cortex, ventral striatum and hippocampus, even changes in palatability and desire to eat the associated taste did not differ between conditions. There was one notable exception, for the sweet, as well as the savoury target taste, the response in the left ventral striatum decreased after consumption. This is in line with the role of this reward area in food craving (37), and putamen activation has been shown in the prediction of reward (38). Moreover, note that this decreased striatal response was not due to changes in pleasantness, since pleasantness was added as a covariate, and because it was not observed for the non-target taste. This suggests that the decrease in ventral striatal taste activation reflects a diminished motivation to eat a specific food, i.e., a food-specific decline in wanting. According to Cabanac's theory of alliesthesia (39), taste pleasantness decreases after eating a food because there is a signal of the absence of any need for the associated food's nutrients (e.g. sweet taste is associated with sugar and salty/savoury taste with salt). This theory illustrated how difficult it is to separate liking and wanting (16). Brain responses changes, independent of pleasantness, could provide a better understanding of the neural correlation of the desire to eat (wanting) (40).

Activation in the amygdala, anterior cingulate cortex and midbrain only increased after a sweet preload consumption. It has been shown in previous studies that activation in the anterior cingulate increased with satiation by a sweet food (19, 21). Also the right amygdala response increased. This area involved in both positive and negative reward processes (32, 36, 41), response increased. Smeets et al. (20) observed an increased amygdala response for an energy rich sweet juice after consumption of the same juice, an effect which did not occur when tasting a non-energy rich sweet juice. This is in line with our results: the savoury juice, which contains very little carbohydrate, did not affect amygdala activation (20).

In the savoury condition, taste activation in the ventral striatum and hippocampus was greater after the preload. The ventral striatum receives signals from the hippocampus, a limbic region, which is involved in learning and memory processes, but also in the regulation of feeding (42). DelParigi et al. found an increase in posterior hippocampus activity after satiation in lean subjects (43) and some studies suggsted that the ventral striatum is involved in satiation (18, 44). Jensen et al. (45, 46) showed that the ventral striatum is crucial in the reward system and is activated in the anticipation of an aversive stimulus, this is in accordance with the savoury findings were the wanting ratings decreased.

The different changes in brain response for sweet and savoury consumption might be explained by the macro-nutrient differences and viscosity, in particular the energy content. The fruit juice contains more energy than the tomato juice, and drinking the fruit juice may therefore be more rewarding for the body, although when comparing the two no difference was found

A study limitation is that our findings cannot be extrapolated to other sweet and savoury foods: this remains to be explored in future studies. In general, the use of sweet and savoury as categories is challenging because it is very hard to match on food properties like macronutrient composition. To our knowledge, only one study has succeeded in this (17). What would be more feasible is to show general and macro-nutrient-specific effects of consumption on brain response to food stimuli. In addition, it needs to be established whether the same effects occur for more viscous (solid) foods. Such studies are needed to confirm or refine the general and food-specific effects reported here.

Prediction of ad libitum intake

We found that anterior cingulate activation correlated negatively with subsequent ad libitum intake for both sweet and savoury juice, i.e., stronger anterior cingulate activation was associated with lower ad libitum intake. To our knowledge this is the first time that taste activation has been linked to subsequent ad libitum intake. Previous studies implied that the ACC along with the insula, striatum, hippocampus and amygdala is involved in the process of satiation which ultimately leads to meal termination (18, 19, 21, 47). In particular our results extend previous findings of increased anterior cingulate activation in response to satiety and decreasing reward (21). Other studies suggest that this part of the anterior cingulate reflects satisfaction or even aversion (33, 48). Moreover, Small et al. (21) found that the anterior cingulate activation increased during satiation with chocolate, i.e. , a sweet high-energy food. In our study both sweet and savoury taste activation in the ACC correlated with ad libitum intake, but only when the taste had been consumed as a preload. This suggest that previous findings of sweet taste associated activation (21) are also indicative for responses to a savoury tasting product. Importantly non-target taste activation did not correlate with ad libitum intake, which suggests that anterior cingulate activation represents food-specific satiety. In how far this is related to specific repletion of nutrients remain to be established. This of importance when understanding people's eating motivation and consumption. Thus, our results strongly suggest that taste activation of the anterior cingulate is indicative of the degree of fullness, i.e., it is inversely related to the degree of specific-wanting of that particular taste. Future studies should clarify the importance of the ACC during the process of satiety. The ACC is a extremely active region and is involved in many processes, i.e. desire, addiction and cognition (49) and it activation is observed in a numerous studies were the internal state was altered (19, 21).

In conclusion, when a juice was consumed activation of the striatum, a brain reward area, decreased for the associated taste, but not for the other tastes, which indicated a food specific-wanting related activation change, independent of pleasantness changes. However sweet and savoury differences in macro-nutrients and viscosity could evoke the more food-specific wanting related changes in the amygdala, hippocampus and anterior cortex. This could reflect associated changes in physiological relevance and may underlie food-specific changes in wanting. Furthermore anterior cingulate response predicted subsequent ad libitum juice intake, irrespective of the type of taste. These findings extended our understanding of the representation of food specific appetite in the human brain and suggest that neuroimaging may provide objective and more accurate measure of food motivation than self-report measures.

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REFERENCES

1. Schwartz, M. W., Woods, S. C., Porte, D., Jr., Seeley, R. J. & Baskin, D. G. (2000) Central nervous system control of food intake. Nature 404: 661-671.
2. Woods, S. C. (2005) Signals that influence food intake and body weight. Physiol Behav. 86: 709-716.
3. Wansink, B. (2004) Environmental factors that increase the food intake and consumption volume of unknowing consumers. Annu. Rev. Nutr. 24: 455-479.
4. Mattes, R. D. (1985) Gustation as a determinant of ingestion: methodological issues. Am. J. Clin. Nutr. 41: 672-683.
5. Small, D. M. (2006) Central gustatory processing in humans. Adv. Otorhinolaryngol. 63: 191-220.
6. Pritchard, T. C., Hamilton, R. B., Morse, J. R. & Norgren, R. (1986) Projections of thalamic gustatory and lingual areas in the monkey, Macaca fascicularis. J. Comp Neurol. 244: 213-228.
7. Galindo-Cuspinera, V. & Breslin, P. A. (2006) The liaison of sweet and savory. Chem. Senses 31: 221-225.
8. Rolls, E. T., Rolls, B. J. & Rowe, E. A. (1983) Sensory-specific and motivation-specific satiety for the sight and taste of food and water in man. Physiol Behav. 30: 185-192.
9. Weenen, H., Stafleu, A. & de Graaf, C. (2005) Dynamic aspects of liking: post-prandial persistence of sensory specific satiety. Food Quality and Preference.
10. Berridge, K. C. (1996) Food reward: brain substrates of wanting and liking. Neurosci. Biobehav. Rev. 20: 1-25.
11. Pecina, S., Smith, K. S. & Berridge, K. C. (2006) Hedonic hot spots in the brain. Neuroscientist. 12: 500-511.
12. Berridge, K. C. (2009) 'Liking' and 'wanting' food rewards: brain substrates and roles in eating disorders. Physiol Behav. 97: 537-550.
13. Berridge, K. C. & Kringelbach, M. L. (2008) Affective neuroscience of pleasure: reward in humans and animals. Psychopharmacology (Berl) 199: 457-480.
14. Finlayson, G., King, N. & Blundell, J. E. (2007) Liking vs. wanting food: importance for human appetite control and weight regulation. Neurosci. Biobehav. Rev. 31: 987-1002.
15. Lemmens, S. G., Schoffelen, P. F., Wouters, L., Born, J. M., Martens, M. J., Rutters, F. & Westerterp-Plantenga, M. S. (2009) Eating what you like induces a stronger decrease of 'wanting' to eat. Physiol Behav. 98: 318-325.
16. Havermans, R. C. (2011) "You Say it's Liking, I Say it's Wanting ...". On the difficulty of disentangling food reward in man. Appetite 57: 286-294.
17. Griffioen-Roose, S., Mars, M., Finlayson, G., Blundell, J. E. & de, G. C. (2009) Satiation due to equally palatable sweet and savory meals does not differ in normal weight young adults. J. Nutr. 139: 2093-2098.
18. Smeets, P. A., de Graaf C., Stafleu, A., van Osch, M. J., Nievelstein, R. A. & van der Grond J. (2006) Effect of satiety on brain activation during chocolate tasting in men and women. Am. J. Clin. Nutr. 83: 1297-1305.
19. Haase, L., Cerf-Ducastel, B. & Murphy, C. (2009) Cortical activation in response to pure taste stimuli during the physiological states of hunger and satiety. Neuroimage. 44: 1008-1021.
20. Smeets, P. A., Weijzen, P., de Graaf C. & Viergever, M. A. (2011) Consumption of caloric and non-caloric versions of a soft drink differentially affects brain activation during tasting. Neuroimage. 54: 1367-1374.
21. Small, D. M., Zatorre, R. J., Dagher, A., Evans, A. C. & Jones-Gotman, M. (2001) Changes in brain activity related to eating chocolate: from pleasure to aversion. Brain 124: 1720-1733.
22. Van Strien, T. (1997) The concurrent validity of a classification of dieters with low versus high susceptibility toward failure of restraint. Addict. Behav. 22: 587-597.
23. Van Strien, T., Frijters JER, Bergers GPA & Defares PB (1986) The Dutch Eating Behaviour Questionnaire (DEBQ) for assessment of restrained, emotional and external eating behaviour. Int J Eat Disord 295-315.
24. Desmond, J. E. & Glover, G. H. (2002) Estimating sample size in functional MRI (fMRI) neuroimaging studies: statistical power analyses. J. Neurosci. Methods 118: 115-128.
25. Maldjian, J. A., Laurienti, P. J., Kraft, R. A. & Burdette, J. H. (2003) An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage. 19: 1233-1239.
26. Evans, A. D., Collins, D. L., Millst, S. R., Brown, E. D., Kelly, R. L. & Peters, T. M. (1993) 3D statistical neuroanatomical models from 305 MRI volumes. Proc IEEE Nucl Sci Symp Med Imaging 3: 1813-1817.

27. Cox, R. W. (1996) AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput. Biomed. Res. 29: 162-173.
28. Forman, S. D., Cohen, J. D., Fitzgerald, M., Eddy, W. F., Mintun, M. A. & Noll, D. C. (1995) Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. Magn Reson. Med. 33: 636-647.
29. Kringelbach, M. L., O'Doherty, J., Rolls, E. T. & Andrews, C. (2003) Activation of the human orbitofrontal cortex to a liquid food stimulus is correlated with its subjective pleasantness. Cereb. Cortex 13: 1064-1071.
30. O'Doherty, J., Rolls, E. T., Francis, S., Bowtell, R., McGlone, F., Kobal, G., Renner, B. & Ahne, G. (2000) Sensory-specific satiety-related olfactory activation of the human orbitofrontal cortex. Neuroreport 11: 399-403.
31. Weijzen, P. L., Liem, D. G., Zandstra, E. H. & de, G. C. (2008) Sensory specific satiety and intake: the difference between nibble- and bar-size snacks. Appetite 50: 435-442.
32. O'Doherty, J., Rolls, E. T., Francis, S., Bowtell, R. & McGlone, F. (2001) Representation of pleasant and aversive taste in the human brain. J. Neurophysiol. 85: 1315-1321.
33. O'Doherty, J., Rolls, E. T., Francis, S., Bowtell, R., McGlone, F., Kobal, G., Renner, B. & Ahne, G. (2000) Sensory-specific satiety-related olfactory activation of the human orbitofrontal cortex. Neuroreport 11: 893-897.
34. Finlayson, G., King, N. & Blundell, J. E. (2007) Is it possible to dissociate 'liking' and 'wanting' for foods in humans? A novel experimental procedure. Physiol Behav. 90: 36-42.
35. Rolls, B. J. (1986) Sensory-specific satiety. Nutr. Rev. 44: 93-101.
36. Zald, D. H. (2003) The human amygdala and the emotional evaluation of sensory stimuli. Brain Res. Brain Res. Rev. 41: 88-123.
37. Pelchat, M. L., Johnson, A., Chan, R., Valdez, J. & Ragland, J. D. (2004) Images of desire: food-craving activation during fMRI. Neuroimage. 23: 1486-1493.
38. O'Doherty, J., Dayan, P., Schultz, J., Deichmann, R., Friston, K. & Dolan, R. J. (2004) Dissociable roles of ventral and dorsal striatum in instrumental conditioning. Science 304: 452-454.
39. Cabanac, M. (1971) Physiological role of pleasure. Science 173: 1103-1107.
40. Mela, D. J. (2006) Eating for pleasure or just wanting to eat? Reconsidering sensory hedonic responses as a driver of obesity. Appetite 47: 10-17.
41. Gottfried, J. A., O'Doherty, J. & Dolan, R. J. (2003) Encoding predictive reward value in human amygdala and orbitofrontal cortex. Science 301: 1104-1107.
42. Davidson, T. L. & Jarrard, L. E. (1993) A role for hippocampus in the utilization of hunger signals. Behav. Neural Biol. 59: 167-171.
43. Delparigi, A., Chen, K., Salbe, A. D., Hill, J. O., Wing, R. R., Reiman, E. M. & Tataranni, P. A. (2004) Persistence of abnormal neural responses to a meal in postobese individuals. Int. J. Obes. Relat Metab Disord. 28: 370-377.
44. Gottfried, J. A., O'Doherty, J. & Dolan, R. J. (2002) Appetitive and aversive olfactory learning in humans studied using event-related functional magnetic resonance imaging. J. Neurosci. 22: 10829-10837.
45. Jensen, J., Smith, A. J., Willeit, M., Crawley, A. P., Mikulis, D. J., Vitcu, I. & Kapur, S. (2007) Separate brain regions code for salience vs. valence during reward prediction in humans. Hum. Brain Mapp. 28: 294-302.
46. Jensen, J., McIntosh, A. R., Crawley, A. P., Mikulis, D. J., Remington, G. & Kapur, S. (2003) Direct activation of the ventral striatum in anticipation of aversive stimuli. Neuron 40: 1251-1257.
47. Blundell, J. E., Lawton, C. L., Cotton, J. R. & Macdiarmid, J. I. (1996) Control of human appetite: implications for the intake of dietary fat. Annu. Rev. Nutr. 16: 285-319.
48. Zald, D. H., Lee, J. T., Fluegel, K. W. & Pardo, J. V. (1998) Aversive gustatory stimulation activates limbic circuits in humans. Brain 121 (Pt 6): 1143-1154.
49. Weston, C. S. (2012) Another major function of the anterior cingulate cortex: The representation of requirements. Neurosci. Biobehav. Rev. 36: 90-110.

Supplemental data

Supplemental table 1 Pleasantness and intensity ratings obtained inside the scanner during scan 1 and scan 2 in health, normal weight, young men in both sweet and savoury conditions[1]

	Sweet condition		Savoury condition	
	Scan 1	Scan 2	Scan 1	Scan 2
Pleasantness				
Fruit stimuli	67 ± 14	58 ± 18*	61 ± 16	61 ± 20
Tomato stimuli	48 ± 21	47 ± 21	56 ± 18	48 ± 21*
Control stimuli	51 ± 11	53 ± 14	50 ± 13	52 ± 13
Intensity				
Fruit stimuli	64 ± 11	61 ± 14	67 ± 11	68 ± 12
Tomato stimuli	68 ± 16	70 ± 16	66 ± 13	71 ± 11
Control stimuli	20 ± 16*	20 ± 19*	20 ± 16*	19 ± 13*

[1]Values are means ± SD, n = 15. Rating performed on 100 mm VAS.*Pleasantness of fruit during scan 2 in sweet condition differs from all other pleasantness ratings during the sweet condition. Pleasantness of tomato during scan 2 in savoury condition differs from all other pleasantness ratings during the savoury condition. Intensity rating of the control stimuli differs from all other intensity ratings during all scans and conditions. (Repeated measure ANOVA, post-hoc paired t-test P <0.05 for multiple comparison).

Supplemental table 2 Perceived sweetness and saltiness ratings of the fruit, tomato and control stimuli in health, normal weight, young men in both sweet and savoury conditions[1]

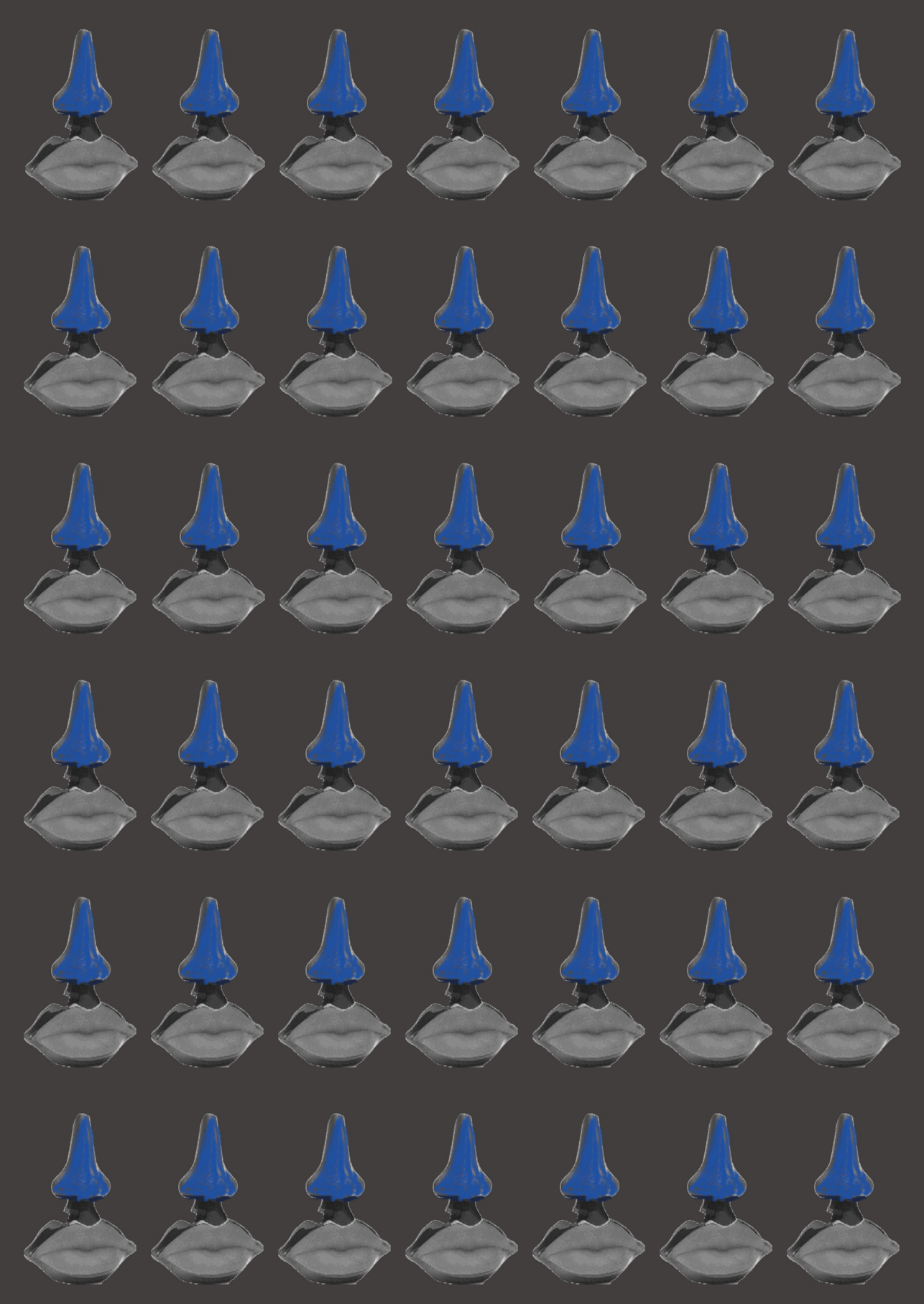
	Sweet condition			Savoury condition		
	Baseline	After preload	After ad libitum	Baseline	After preload	After ad libitum
Sweetness						
Fruit	74 ± 12^{a}	69 ± 17^{a}	72 ± 16^{a}	77 ± 10^{a}	76 ± 10^{a}	74 ± 17^{a}
Tomato	26 ± 18^{b}	18 ± 16^{b}	16 ± 15^{b}	21 ± 14^{b}	12 ± 12^{b}	15 ± 1^{b}
Control	6 ± 6^{b}	9 ± 10^{b}	6 ± 4^{b}	8 ± 12^{b}	13 ± 11^{b}	9 ± 11^{b}
Saltiness						
Fruit	16 ± 22^{b}	16 ± 21^{b}	16 ± 15^{b}	11 ± 8^{b}	7 ± 6^{b}	8 ± 7^{b}
Tomato	50 ± 23^{a}	57 ± 24^{a}	65± 22^{a}	60 ± 22^{a}	69 ± 12^{a}	64 ± 12^{a}
Control	6 ± 5^{b}	7 ± 7^{b}	6 ± 4^{b}	8 ± 6^{b}	11 ± 11^{b}	5 ± 4^{b}

[1]Values are means ± SD, n = 15 Rating performed on 100 mm VAS. a,b Entries within a row having different lower case letter superscripts differ significantly (Repeated measure ANOVA, post-hoc paired t-test P <0.05 for multiple comparison)

Supplemental table 3 Whole brain analyses of effect of consumption on target and non-target taste activation in both sweet and savoury conditions in health, normal weight, young men[1]

Region	Cluster size[2]	Peakvoxel coordinates[3]			Z-score	Region	Cluster size[2]	Peakvoxel coordinates[3]			Z-score
		x	y	z				x	y	z	
Target Taste						**Non Target Taste**					
Cerebelum	193	18	-77	-25	5.20	Midbrain	394	11	-32	-18	4.39
		28	-53	-25	4.88	Cerebelum		7	-49	-18	4.25
Amygdala R	15	25	0	-14	4.93	Cingulate gyrus	52	-7	28	28	3.56
Midbrain	30	7	-35	-21	4.71	Insula R	39	39	11	-14	3.78
Pallidum R	69	-25	-7	-4	3.91			39	0	-7	3.50
Precuneus	32	14	-46	18	4.19						
		14	-49	39	3.78						
Insula R	47	32	-11	21	4.17						

[1]Values are clusters of mean brain activation, n = 15.L: left hemisphere and R: right hemisphere. [2]Reported clusters were thresholded at P < 0.001, uncorrected for multiple comparisons, which corresponds to a Z-score > 3.0. [3]Voxel coordinates are in MNI space (26).



Go to foreign coutries and you will get to know the good things one possesses at home

Chapter 4

Differential effects of satiety on brain response to ortho- vs. retronasally perceived food odours

ABSTRACT

Food aromas sensed orthonasally (via the nose) provide information about food availability, whereas food odours sensed retronasally (via the mouth) provide information about foods and drinks being consumed. Prior work has shown differential brain response to orthonasal vs. retronasal odour perception, especially if the odour is a food odour. We used fMRI to test whether internal state (hungry vs. full) would interact with route of odorant sensation (ortho vs. retro) to influence neural response to food but not non-food odours. In addition we tested whether devaluation of a target food odour, induced by eating the food represented by the odour to satiety, would differ as a function of route, suggesting that different networks encode the value of food cues vs. food receipt. Subjects were scanned while smelling four food and two non-food odours via both routes when they were hungry and immediately following a meal associated with one of the food odours. The hippocampus responded more to food vs. non-food odours regardless of route but this was driven primarily by differential responses in the full state. Preferential response in the insular cortex was observed to orthonasal vs. retronasal odour presentation. However, insular responses to orthonasally presented odour were only sensitive to internal state if the odour represented a food, indicating that route, internal state and odour category interact here. These findings confirm earlier work showing that route impacts neural response to odours and extends knowledge by showing that route impacts the influence of internal state on brain response to odours.

INTRODUCTION

Sensory systems can be categorized in terms of whether they provide information about events and objects distal or proximal to the observer. Olfaction is unique among the senses in that it is a dual sense modality (2). Odours sensed via the nose or "orthonasally" provide information about objects in the external world (i.e. distal), whereas odours sensed from the mouth, or "retronasally" provide information about the foods and drinks in the mouth (i.e. proximal) (2-5). What is remarkable is that although both ortho- and retronasal olfaction depend upon transduction events in the olfactory epithelium, which is located deep within the nasal cavity, retronasally sensed odours are referred to the oral cavity (1, 6); and often confused for tastes (3), whereas orthonasally sensed odours are referred to their source object, or are poorly localized (at least by humans) (7, 8). Accordingly, the same odour sensed retronasally compared to orthonasally preferentially activates the oral somatosensory cortex (9). This is thought to either reflect activation of the central mechanism underlying the referral of the odour to the mouth or to occur as a result of the referral produced by a mechanism elsewhere in the nervous system (9).

There are two important corollaries of the differential localization produced by route. First, since an odorant must be in the mouth to be sensed retronasally, it is highly likely that the odorant represents a food or drink. As such, only food- (or drink) related odours are sensed by both routes. This is important because experience plays a critical role in olfactory neural coding and perception (10-12). Indeed odours can be localized to the mouth by the presence of a taste but only if the taste is congruent (6), indicating that the oral capture illusion is dependent upon experience. Additionally, differential brain response was observed following ortho- versus retronasal perception of chocolate odour, but not of three similarly pleasant and intense non-food odours (9). This indicates that route selectively influences brain response to food odours. A logical explanation for this interaction between odour category (i.e. food versus. non-food) and route (ortho- versus retro-) on brain response relates to the second corollary of differential localization as a function of route; namely, that orthonasal olfaction signals food availability whereas retronasal olfaction signals food consumption. This is important because it is well recognized that there are separable neural circuits representing anticipatory and consumatory food reward (13, 14). Thus, it has been proposed (9, 15, 16) that orthonasally sensed food odours induce responses in regions important for representing incentive, such as the amygdala (17), whereas retronasally sensed odours activate a separate circuit of regions coding the value of the consumed substance, such as the medial orbitofrontal cortex (18). However, this possibility, and indeed the possibility that food odours represent a distinct "category" remains an open question since only one food odour was used in the prior study (9).

The aim of this study was twofold. First, we sought to determine if food odours activate different brain regions compared to similarly intense, pleasant and familiar floral odours. Second, we tested whether the influence of route (ortho- vs. retro-) on food odours generalizes to other types of food odours besides chocolate. Third, we evaluated the influence of internal state (hungry versus full) on brain response to odours as a function of route and odour category (food versus non-food). We undertook this aim because we reasoned that if the effect of route on brain response to chocolate is dependent upon food reward, then the internal state manipulation should produce selective effects for ortho- versus retronasally sensed food, but not floral, odours.

MATERIALS AND METHODS

Subjects

Subjects were recruited from the campus of the University of Dresden Medical School. Sixteen right-handed subjects qualified and participated (mean age 23.7 ± 2.6 y, mean BMI 22.5 ± 1.8 kg/m2). All experimental procedures were approved by the Ethics Committee of the University of the Dresden Medical School. After being informed about the aims and potential risks of the study, all subjects provided informed consent. Only non-smokers, of healthy weight (body mass index 18.5-25), free from psychiatric illness, with unobstructed nasal passages (for insertion of nasal cannulae) and normal olfactory function were included. Normosmia was ascertained by means of a 12-item odour identification test using the Sniffin' Sticks (Burghart Instruments, Wedel, Germany) test kit, which serves as a quick and reliable means of measuring gross olfactory function (19).

Stimuli

Six odours were used, four food and two non-food (floral). The food odours were chocolate (Bell Flavors and Fragrances, Leipzig, Germany; order number 0812498), pineapple (International Flavors & Fragrances. Inc., Code 013834 Allyl Caproate, Lot No. 1502997), tomato (Frey+LauGmbH, Honstadt-Ulzburg, Germany, Order number P0620117) and peach (Frey+LauGmbH, Honstadt-Ulzburg, Germany, Order number P0606040). The non-food odours were rose (2-Phenylethanol; Sigma Aldrich GmbH, Steinheim, Germany, Order number P6134-1L) and lilac (Frey+LauGmbH, Honstadt-Ulzburg, Germany, Order number P0218324). The odorants were presented at 10, 20, 30, 40 or 50% concentrations ortho- and retronasally. In total there where twelve odorant conditions (pineapple ortho = PO, pineapple retro = PR, chocolate ortho = CO, chocolate retro = CR, tomato ortho = TO, tomato retro = TR, peach ortho = PEO, peach retro = PER, lilac ortho = LO, lilac retro = LR, rose ortho = RO, rose retro = RR), each with their own odourless air baseline condition.

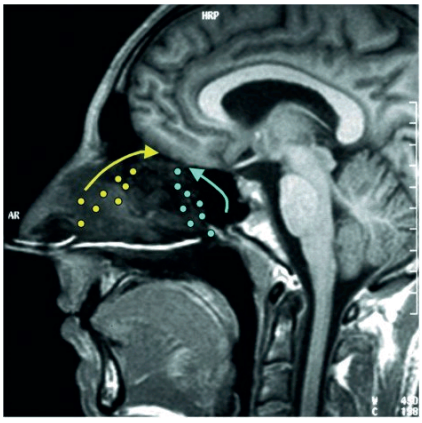


Figure 1 A MRI image showing placement of the nasal cannulae at the external nares, to achieve orthonasal (yellow dots and arrow) and at the retropharynx to achieve retronasal turquoise dots and arrow) odorant delivery. Through the cannulae (white lines in cross-section) odourized air was administrated (1)

Stimuli Delivery

Odours were presented by a dual olfactometer (OM6b; Burghart Instruments, Wedel, Germany). The olfactometer was connected to the inserted nasal canulae, with one connected to the "ortho" tube

and the other connected to the "retro" tube (Figure 1; for more details see (9)). Subjects received a constant stream of odourless air maintained at 80% relative humidity, 36.5ºC and total flow at 7.2 L/min, with odorants embedded in the constant airflow.

Procedure

All subjects first underwent a screening session. After screening, subjects participated in the first behavioural session followed by two scan sessions, one hungry, one full and then a second behavioural session followed by two scan sessions (one hungry, one full). Before each scan subjects were asked to skip two meals (approx 10-15hrs fasted). All behavioural and scan sessions were at least one day apart. During each behavioural and scanning session subjects rated only three odours (orthonasally and retronasally), due to the fact that the olfactometer had a maximum of six odorant channels (three odours, ortho- and retro-). The order was randomized, with the stipulation that two food odours and one floral odour were delivered in each session (Figure 2).

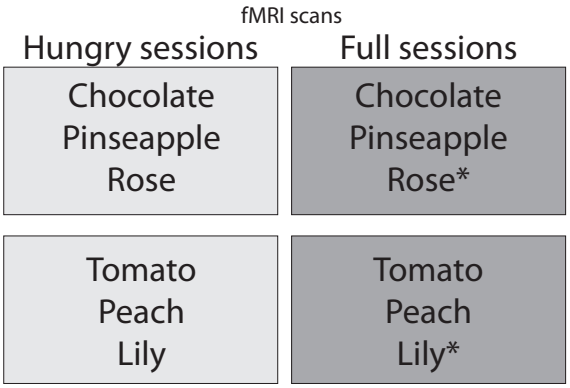


Figure 2 Experimental design. Graphical depiction of design, every rectangle presents one scan session with the orthonasally and retronasally percived odours during the scans. Order of scans was randomized, all subjects underwent all four scans. Left scans were preformed when fasted (10-15h), during the right scans subjects were full.*Subjects comsumed a food associated with one of the odours untill satiety before the full scans.

Screening session

During the screening session subjects completed the Stunkard and Messick's three-factor eating questionnaire to evaluate dietary restraint (20). Weight and height were measured to determine body mass index. Subjects were then trained to perform velophyarngeal closure; using a biofeedback technique, a thermistor was held in front of the nostril so that subjects could see changes in respiratory airflow on an oscilloscope (1, 9). This was done so that changes in respiratory airflow would not impact on the distribution of the odours stimulus inside the nasal cavity. The nasal canulae were inserted into the subjects' wider nose under endoscopic guidance so that the end of one tube extended just beyond the antrum of the nasal cavity and the opening of the other tubing was at the epipharynx. Finally, the perceptual correlates of all odours used in the experiment were evaluated. The odours were rated with visual analog scales (VAS) (0= most negative, 50 = neutral, 100 = most positive) and labelled magnitude scales (LMS) (21) (0 to 100 = logarithmic increases) to evaluated stimulus pleasantness, familiarity, and intensity, and edibility. Subjects were excluded from the study if they found the odours unfamiliar and/or unpleasant (< 50 on the VAS scale). Test odours were required to have sufficient volatility to be delivered using air dilution olfactometry. Subjects who experienced the nasal cannulae as uncomfortable were excluded.

Behavioural sessions

During each behavioural session the odours were adjusted for each individual to be similarly intense and pleasant via both routes of delivery (ortho- and retronasal) so that these perceptual differences could not influence the predicted effects of route of delivery on brain response. Intensity matching was performed during a behavioural session on a separate day prior to the day of the scanning. Ortho- and retronasal concentrations of the odorants were diluted by the experimenter before they were loaded into the olfactometer to grossly match the odorants in intensity. This was also done to best ensure that the odours were sufficiently intense to be identifiable and weak enough to minimize aversion. The olfactometer allowed us to present subjects with 10, 20, 30, 40 and 50% concentrations of the experimental odorants ortho- and retronasally. A 30% retronasal concentration was always the first stimulus presented, followed in close succession by orthonasal delivery at a 30% concentration (approximately ten seconds separating 1st and 2nd stimulus delivery). The order of presentation (retro- to ortho-) was done to minimize adaptation. Most people perceive orthonasal delivery of an odour as being more intense than retronasal (1, 22), starting with a lower concentration was believed to least interfere with intensity perception of future odours. After the odorant pair (retro-ortho), subjects were asked which odour they perceived to be stronger or whether they perceived the odorants to be equally intense. The experimenter would then adjust the concentration of the odorant according to the subject's intensity evaluation. There was approximately a 30 second delay between odorant presentations. The subject had to report the concentration of odorant #1 and odorant #2 as being equal with 3 separate repetitions before the experimenter moved on to the next experimental odour. In one behavioural session pineapple, chocolate and rose odours were matched. Tomato, peach and lilac were matched in the other session. The order was randomized per subject.

fMRI Sessions

In order to obtain sufficient data for inter-trial averaging subjects participated in four scans over four days (Figure 2). Two scans were performed while hungry and two while full (directly after eating a food associated with one of the odours to satiety). The chocolate, pineapple and rose odours were delivered on two sessions (one hungry and one full) and the tomato, peach, and lilac odours on the other two sessions. The eaten food was counterbalanced across subjects. Within-subjects scans were performed at the same time of day. Before and after every scanning session subjects rated hunger as well stimulus intensity pleasantness, familiarity and edibility.

A 30 sec ON" 30 sec "OFF" block design was used (Figure 3). Odorants were delivered as 1 sec air pulses embedded in a constant airflow during the ON period (3 sec inter-stimulus intervals). Odourless air was pulsed in the same fashion during the OFF periods. In half of the blocks, the pulses were delivered retronasally and in the other half of the blocks, the pulses were delivered orthonasally. All of the odorants were delivered once ortho- and retronasally during each experimental run for a total run time of 6 minutes. In total there were six runs per scan session. The sequence of odorants tested and the route of presentation (orthonasal or retronasal) were randomized across subjects.

fMRI data acquisition

fMRI data were acquired using a gradient echo single shot EPI sequence (T2*-weighted, with TE/TR/bandwidth/flip angle = 45 ms/3.0 s/2604 kHz/90°), which was performed to image the Blood Oxygen Level Dependant (BOLD) effect. Twenty-six slices were acquired (3 mm thickness; 0.75 mm gap; field-

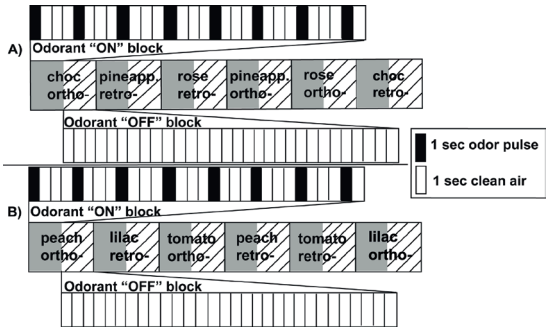


Figure 3 Stimuli design. Graphical depiction of odorant delivering during scan. Figure represents one block of odorant delivery, every scan consisted of 6 blocks. The odorant "ON" trail (grey box) referes to a 30 s odorant delivery: 1 s odour puls (black) followed by 3 s of clean air (white) repeated 8 times. During the 30 s Odorant "OFF" trail a constant stream of odorless air was presented. A represents the two chocolate, pineapple and rose sesions and B; peack, lilac and tomato sessions.

of-view, 192 mm; matrix, 64 × 64) that covered the brain and were oriented parallel to the cribriform plate to minimize bone artefacts. In each functional run, 120 volumes (plus six volumes at the beginning, to equilibrate magnetization) were collected. A complementary T1-weighted, high-resolution structural image set was acquired using a 3D sequence.

Data Analysis

Behavioural data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). To verify that the intensity and pleasantness matching procedure was successful and to analyze the ratings of edibility and familiarity, ratings were assessed with separate 2 x 2 x 2 x 2 repeated-measures multiple analyses of variances (MANOVAs). The variables were: 1) internal state, with two levels, hungry and full; 2) route, with two levels, ortho- and retro-; 3) odour category, with two levels, food and non-food; and 4) time, with two levels, pre- or post scan. Post-hoc testing was performed using t-tests with $p < 0.05$, Bonferroni corrected for multiple comparisons. Hunger ratings from all four scans were compared with a repeated-measure ANOVA, with two factors; internal state (hungry or full) and time of delivery (pre- or post scan). Post-hoc testing was performed using paired t-tests with $p < 0.05$, Bonferroni corrected for multiple comparisons.

fMRI data were preprocessed and analyzed using SPM5 (Welcome Department of Imaging Neuroscience, London, UK,) run with MATLAB 7.5 (The Mathworks Inc, Natick, MA) and the WFU Pickatlas tool (23). First, the functional volumes from every subject were realigned to the first volume of the first run. Next, the anatomical image was co-registered with the mean functional image. Then the images were normalized (retaining 3 × 3 × 3 mm voxels) to Montreal Neurological Institute space (MNI space) (24), and spatially smoothed with a Gaussian kernel of 6mm 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. Data were high-pass filtered with a cut off of 128s.

Eight conditions were modelled in first level analyses: (1) food odour hungry retro; (2) food odour hungry ortho; (3) non-food odour hungry retro; (4) non-food odour hungry retro; (5) food odour full retro; (6) food odour full ortho; (7) non-food odour full retro; (8) non-food odour full ortho. All eight odorant conditions were contrasted with their own odourless baseline. The contrast images of each condition minus its baseline were then entered into a second level 3-way ANOVA (route × internal state × odour category).

Unpredicted peaks were considered significant at $p < 0.05$ corrected across the entire brain. Predicted peaks were considered significant at $p < 0.05$ corrected across voxels within regions of interest defined using peaks from previous studies (9, 17, 25) as the centre of a 10 mm diameter sphere. For display, all maps are thresholded at $p < 0.001$ uncorrected with a cluster criterion of five voxels. Predicted regions were the amygdala, insula, pirifrom, anterior cingulate, orbitofrontal cortex, hippocampus, and the striatum.

RESULTS

Subjective ratings of odorants

No significant main effects were observed for pleasantness or intensity ratings of the odours (Figure 4), but there was an effect of time of delivery on intensity ($F(1,31)= 7.13$ $p < 0.05$), resulting from a significant decrease in intensity ratings post scan for orthonasally delivered food and non-food odours, and retronasally delivered non-food odours, only during the full sessions. No other interactions were present (Figure 4).

There was a significant main effect of odour category for the familiarity ratings ($F(1,31)= 8.78$ $p < 0.05$) (Figure 4), food odours were more familiar then non food odours. An interaction between odour and time of delivery ($F(1,31)= 5.85$ $p < 0.05$) and between internal state and time of delivery ($F(1,31)= 6.17$ $p < 0.05$) was also observed. Post hoc testing showed that food odours were perceived as more familiar before scanning, and post scanning ratings were significant lower when full compared to hungry (Figure 4).

A main effect of odours category ($F(1,31)= 256.99$ $p < 0.05$) was observed for the edibility ratings (Figure 5). Post-hoc testing showed that food odours had higher edibility ratings compared to non-food odours. An interaction was found between odour and time of delivery ($F(1,31)= 6.79$ $p < 0.05$), food odours decreased in edibility post scan, whereas the non food odours increased (Figure 4). We also observed a 3-way interaction between odour, internal state and route ($F(1,31)= 5.714$ $p < 0.05$). For food odours edibility of the retronasally delivered odour were increased during the full scan compared to orthonasal, whereas the orthonasally delivered food odours were decreased during the full scans compared with the hungry. Non food odours showed the opposite, orthonasally delivered odours were perceived as more edible in the hungry scans, whereas retronasally delivered odours in the full scans (Figure 4).

Finally, we tested whether our internal state manipulation changed hunger ratings. There was a main effect of internal state on the hunger rating ($F(1,15)= 54,752$ $p < 0.05$) with hunger ratings higher in the hungry compared to the full scans. A main effect of time of delivery was also observed ($F(1,15)= 4,87$ $p < 0.05$) and an interaction of internal state and time of delivery ($F(1,15)= 9.15$ $p < 0.05$). This effect arose because hunger ratings increased post scan during the full session (Figure 5).

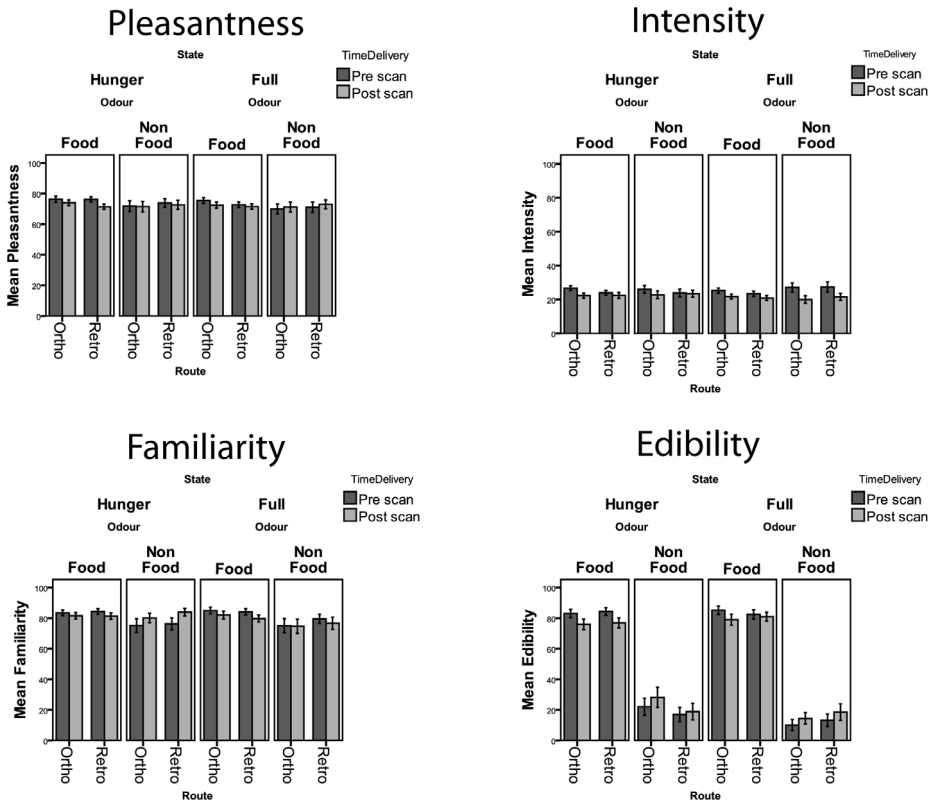


Figure 4 Mean pleasantness, intensity, familiarity and edibility ratings (VAS ± SE) of food and non food odours, rated before (dark grey) and after (light grey) the scan, during two different conditions (hungery (left) or full (right)) and delivered orthonasal or retronasal.

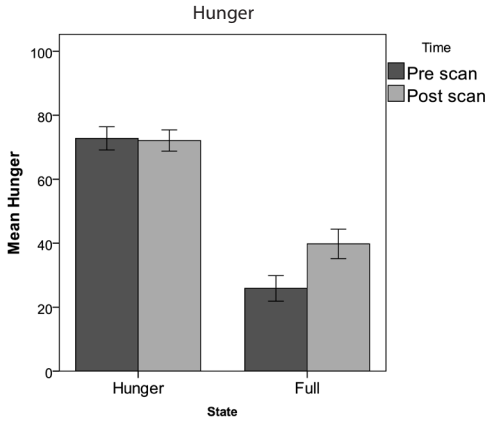


Figure 5 Mean hunger ratings (VAS ± SE) pre (dark grey) and post (light grey) scanning, during the hungry or full scan condition.

Neuroimaging data

Main effects

Main effect of odours

All odours produced activation in the thalamus (-3, -3, 12, z = 5.38), right superior frontal gyrus (3, 51, 42 z = 4.23), triangular part of the inferior frontal gyrus (left) (-54, 30, 9 z = 4.19) and the orbital region of inferior frontal gyrus (L) (-48, 39, -15 z = 4.02) (Figure 6). There was a trend in the piriform primary olfactory cortex (-27, 18, -12; z = 3.18, p = 0.20)

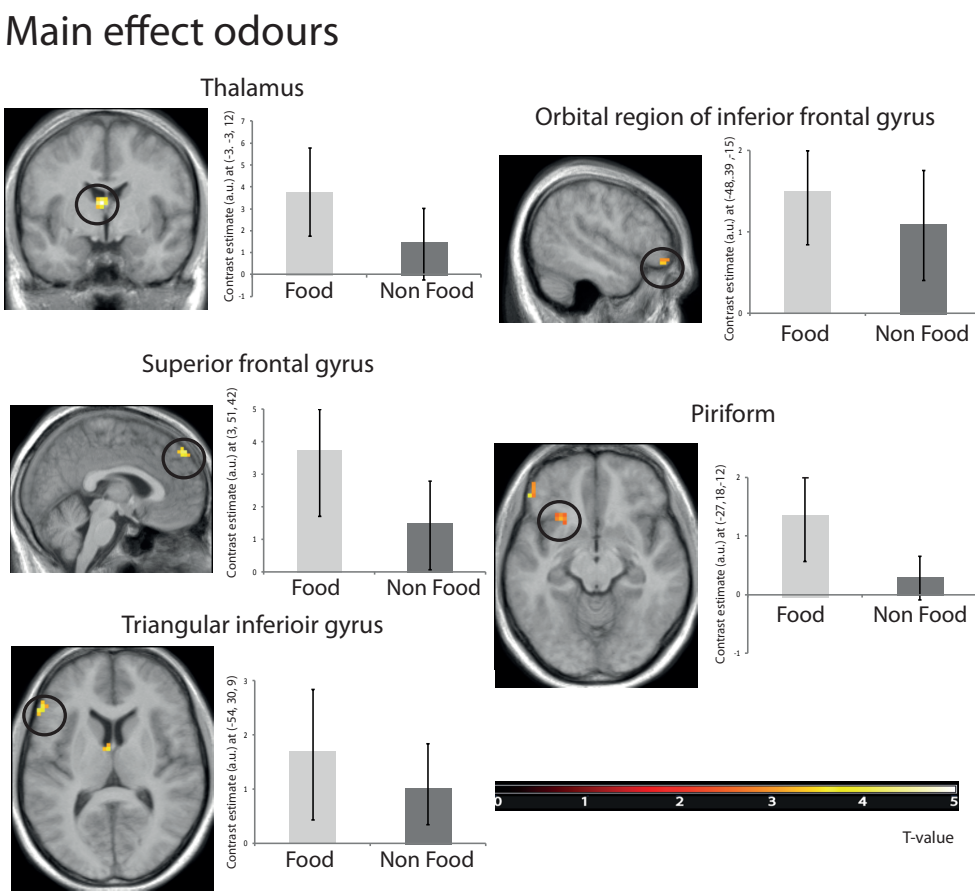


Figure 6 Main effect of all odours versus odourless. Anatomical sections show neural responses with a t-map that os thresholded at $p < 0.001$ uncorrected, with a cluster threshold of $k > 3$. All bar graphs show contrast estimates for teh food and the non-food odorants with their associated baselines substracted.

Main effect of odour category

Food odours evoked a greater activation in the left hippocampus (-33, -30, -6 z = 4.55) than non food odours (Figure 7A), whereas the non food odours did not show any greater activation than the food odours.

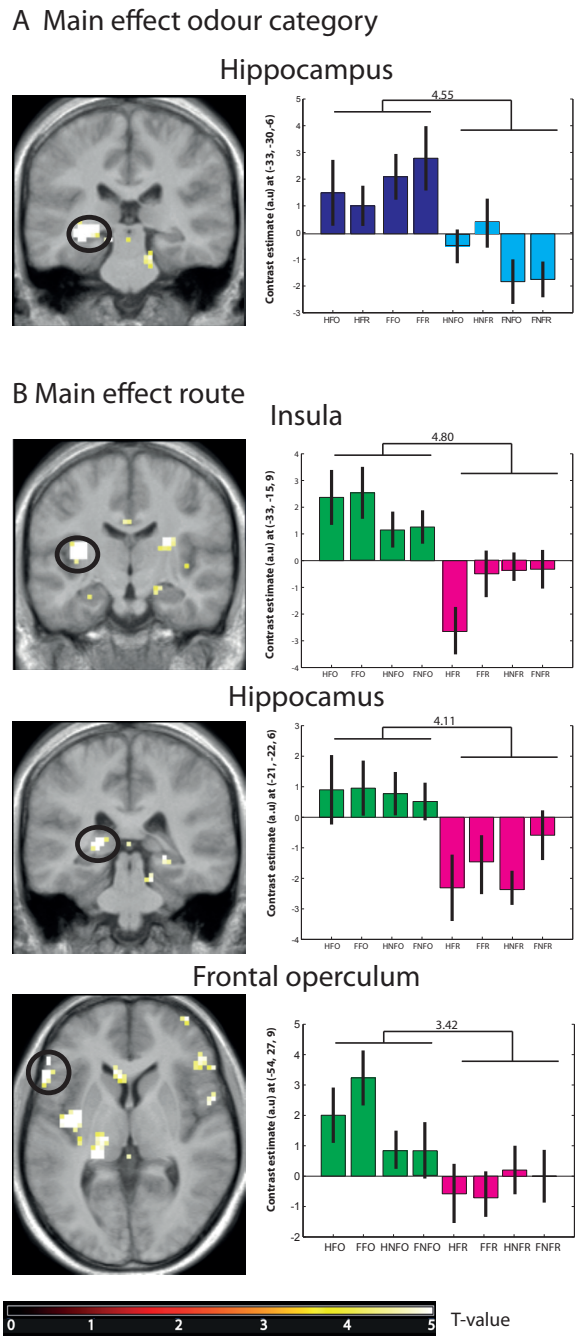


Figure 7 A. Main effect of odour category, food versus non-food odours and **B**. Main effect of route, orthonasal versus retronasal delivery. Anatomical sections show neural responses with a t-map that is thresholded at $p < 0.001$, with a cluster threshold of $k < 3$. All bar graphs show contrast estimates for each of the eight odorants with their associated baseline subtracted. HFO = hunger food orthonasal, FFO = full food retronasal, HNFO = hungry non food orthonasal, FNFO = full non food orthonasal, HFR = hunger food retronasal, FFR = Full food retronasal, FNFR = full non food retronasal.

Main effect of route

Orthonasal odours evoked a greater response in the left insula (-33, -15, 9 z = 4.80), hippocampus (-21, -33, 6 z= 4.11) and left frontal operculum (-57, 21, 3 z = 3.42) and a non significant peak at the right frontal operculum (33, 36, 3 z = 2.53). (Figure 7B) Retronasal odours showed no greater response than the orthonasally perceived odours. No significant differential activation was observed in the somatomotor mouth area, as had been predicted.

Main effect internal state

There was no main effect of hunger.

2-way interactions

Internal state x odour category

During the hungry scans ((hungry food > hungry non-food) > (full food > full non-food)), food odours evoke a greater response in right insula (39, 15, 3; z = 3.90) compared to non-food odours. When subjects were full ((hungry food > hungry non-food) < (full food > full non-food)), food odours evoked a greater response in the amygdala extending to the hippocampus (-27, -6, -24; z = 3.45) compared with non-food odours (Figure 8A).

Odour category x route

Ortho- versus retronasal food versus non-food odours ((food ortho>food retro) > (non-food ortho > non-food retro)) evoked a greater activation in the frontal operculum (-54, 27, 9; z = 4.51). Retro- versus orthonasal food versus non-food odours ((food ortho < food retro) > (non-food ortho < non-food retro)) showed no significant differential activation. Non food odours did not evoke a greater activation as a function of route (Figure 8B).

3-way interactions

Odours x state x route

No 3-way interactions were observed between category (food vs. non-food) or (target vs. non-target), route, and internal state or for category (target vs. non-target food odour), route and internal state.

DISCUSSION

In the current study we investigated the effect of internal state, route of olfactory stimulation and odour category on brain response. Of primary interest was determining whether the effect of route is greater for food compared to non-food odours and whether responses to orthonasal vs. retronasal food aromas are influenced by satiety.

In keeping with our predictions and with prior observations (9), we found that route had a greater impact on food compared to non-food odours. More specifically, replicating our prior study (9) response in the insula and frontal operculum was preferential for orthonasally vs. retronasally presented food but not non-food odours. Extending these findings we observed that the effect in the insula emerged largely as a function of internal state, with response greater to orthonasally vs.

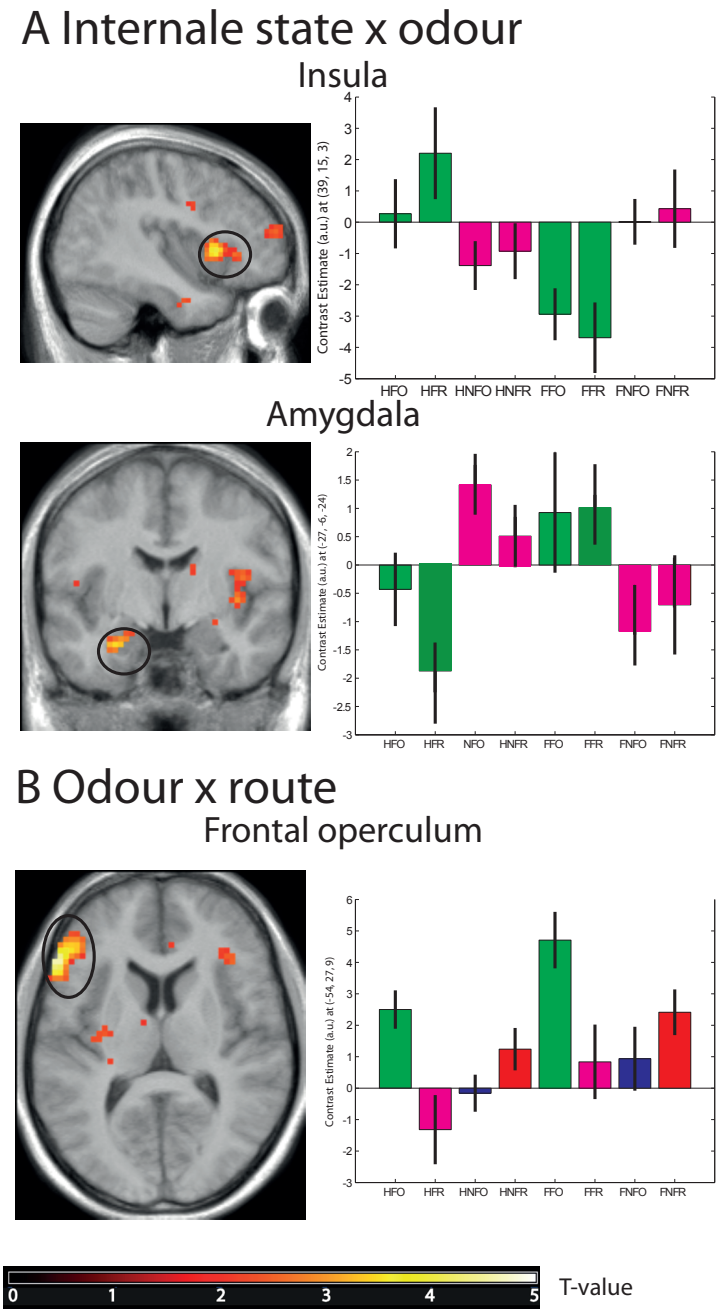


Figure 8 A. Interaction between internal state and odour category. Insula activation is greater for the food vs. non-food odours when hungry, whereas the hippocampus evoked a greater activation for the food vs. non –food odours when full. **B.** Interaction between odour category and route, the frontal operculum evoked a greater activation ortho vs. retro for the food vs. the non-food odours. Anatomical sections show neural responses with a t-map that is thresholded at $p < 0.001$, with a cluster threshold of $k < 3$. All bar graphs show contrast estimates for each of the eight odorants with their associated baseline subtracted. HFO = hunger food orthonasal, FFO = full food retronasal, HNFO = hungry non food orthonasal, FNFO = full non food orthonasal, HFR = hunger food retronasal, FFR = Full food retronasal, FNFR = full non food retronasal.

retronasally presented food odours during hunger. This is consistent with a large literature showing that the insula is sensitive to internal state (26-28), to the value of food cues as a function of internal state (17, 29, 30) and to modulating the incentive value of food outcomes (31).

However, during the hungry state the orthonasally perceived food odours were perceived as more edible compared to the retronasal administrated food odours, this was the opposite in the full sessions. Thus differential response may reflect differences in edibility as a function of route. Future work will be required to resolve this possibility but the current result is consistent with the possibility that the effect of route is dependent upon the relevance of the food odour to feeding behaviours.

The only region where an effect of route was observed independently of hunger was the left frontal operculum. Consistent with the study by Small et al. (9), response was found to be greater for orthonasally vs. retronasally sensed food odours. Future work directed at identifying the reason for this selective response appears warranted. However, given that most effects were dependent upon internal state, our results are consistent with the possibility that route exerts its effect by differentially engaging anticipatory vs. consumatory food reward circuits.

Like the effect of route, the effect of odour category also depended on internal state. A main effect of odour category was observed in the hippocampus with response greater for the food vs. non-food odours. However, inspection of figure 7A illustrates that this was observed primarily during the sated scans. This is consistent with observations that the hippocampus plays an important role in signalling satiety (18, 27, 32). However, we also note that the food odours were perceived as more familiar than the non-food odours. Thus we cannot rule out familiarity in contributing to the observed interaction.

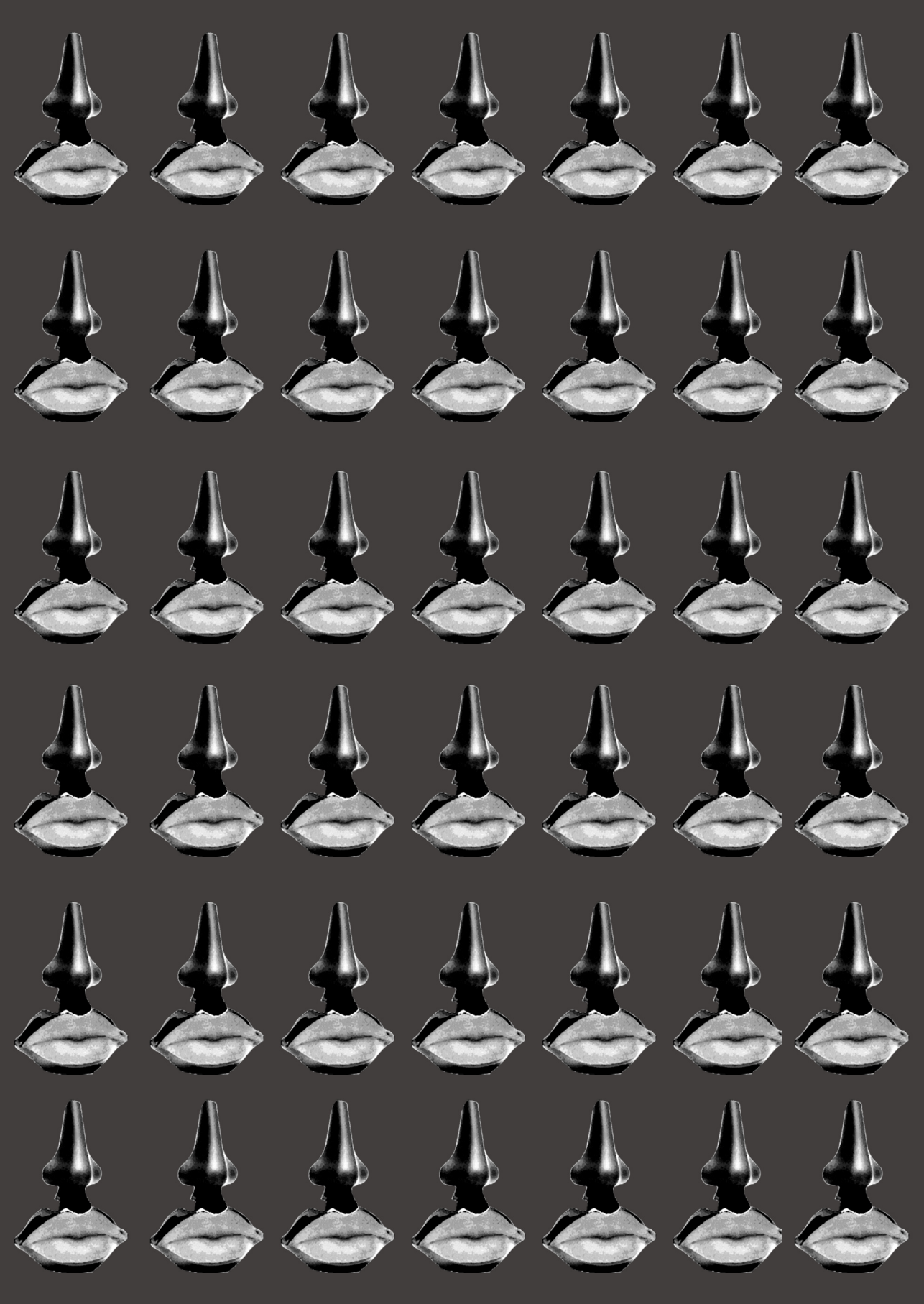
Surprisingly, we did not observe a main effect of route in the somatomotor mouth area. Retronasally sensed odours tend to be referred to the mouth. This effect is greatest when a congruent taste is present (6) but also occurs when no oral stimulation is present (1, 6). In a prior study, using the identical method of odour delivery as was employed in the current study we found greater response in the somatomotor mouth area following retronasal versus orthonasal stimulation (9). This effect did not depend upon odour category and we suggested that it reflected olfactory referral to the mouth. The results of the current study do not support this possibility, as no differential response in the somatomotor mouth area was observed in any of the comparisons of retro- versus orthonasal olfactory stimulation (main effects, 2-way or 3-way interactions). However, in the current study we did not evaluate odour referral. It is therefore conceivable that the odours we used were simply not referred to the mouth when delivered retronasally and thus the somatomotor mouth area not stimulated.

In summary, the results of the current study confirm that route of odour delivery influences brain response to odours, primarily if they are food related. The results also extends knowledge by showing that this effect occurs for many food odours and that many of these selective responses depend upon internal state, which in turn indicates that they are related to the physiological significance or value of the food odour.

REFERENCES

1. Heilmann, S. & Hummel, T. (2004) A new method for comparing orthonasal and retronasal olfaction. Behav. Neurosci. 118: 412-419.
2. Rozin, P. (1982) "Taste-smell confusions" and the duality of the olfactory sense. Percept. Psychophys. 31: 397-401.
3. Murphy, C., Cain, W. S. & Bartoshuk, L. M. (1977) Mutual action of taste and olfaction. Sens. Processes 1: 204-211.
4. Small, D. M. & Prescott, J. (2005) Odor/taste integration and the perception of flavor. Exp. Brain Res. 166: 345-357.
5. Small, D. M. & Green, B. (2011) A Proposed Model of a Flavor Modality. In: The Neural Bases of Multisensory Processes , pp. 705-726.
6. Lim, J. & Johnson, M. B. (2011) Potential mechanisms of retronasal odor referral to the mouth. Chem. Senses 36: 283-289.
7. Kobal, G., Van, T. S. & Hummel, T. (1989) Is there directional smelling? Experientia 45: 130-132.
8. Porter, J., Anand, T., Johnson, B., Khan, R. M. & Sobel, N. (2005) Brain mechanisms for extracting spatial information from smell. Neuron 47: 581-592.
9. Small, D. M., Gerber, J. C., Mak, Y. E. & Hummel, T. (2005) Differential neural responses evoked by orthonasal versus retronasal odorant perception in humans. Neuron 47: 593-605.
10. Wilson, D. A. & Stevenson, R. J. (2006) Learning to smell: olfactory perception from neurobiology to behavior. John Hopkins Univ. Press, Baltimore.
11. Li, W., Howard, J. D., Parrish, T. B. & Gottfried, J. A. (2008) Aversive learning enhances perceptual and cortical discrimination of indiscriminable odor cues. Science 319: 1842-1845.
12. Wilson, D. A., Best, A. R. & Sullivan, R. M. (2004) Plasticity in the olfactory system: lessons for the neurobiology of memory. Neuroscientist. 10: 513-524.
13. Berridge, K. C. (1996) Food reward: brain substrates of wanting and liking. Neurosci. Biobehav. Rev. 20: 1-25.
14. Small, D. M., Veldhuizen, M. G., Felsted, J., Mak, Y. E. & McGlone, F. (2008) Separable substrates for anticipatory and consummatory food chemosensation. Neuron 57: 786-797.
15. Small, D. M. (2008) Flavor and the Formation of Category-Specific Processing in Olfaction. Chemosensory Perception 1: 136-146.
16. Small, D. M. (2009) Individual differences in the neurophysiology of reward and the obesity epidemic. Int. J. Obes. (Lond) 33 Suppl 2: S44-S48.
17. Gottfried, J. A., O'Doherty, J. & Dolan, R. J. (2003) Encoding predictive reward value in human amygdala and orbitofrontal cortex. Science 301: 1104-1107.
18. Small, D. M., Zatorre, R. J., Dagher, A., Evans, A. C. & Jones-Gotman, M. (2001) Changes in brain activity related to eating chocolate: from pleasure to aversion. Brain 124: 1720-1733.
19. Hummel, T., Konnerth, C. G., Rosenheim, K. & Kobal, G. (2001) Screening of olfactory function with a four-minute odor identification test: reliability, normative data, and investigations in patients with olfactory loss. Ann. Otol. Rhinol. Laryngol. 110: 976-981.
20. Peck, R. E. (1959) The SHP test--an aid in the detection and measurement of depression. Arch. Gen. Psychiatry 1: 35-40.
21. Green, B. G., Dalton, P., Cowart, B., Shaffer, G., Rankin, K. & Higgins, J. (1996) Evaluating the 'Labeled Magnitude Scale' for measuring sensations of taste and smell. Chem. Senses 21: 323-334.
22. Voirol, E. & Dagnet, N. (1986) Comparative study of nasal and retronasal olfactory perception. Food Science Technology 19: 316-319.
23. Maldjian, J. A., Laurienti, P. J., Kraft, R. A. & Burdette, J. H. (2003) An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage. 19: 1233-1239.
24. Evans, A. D., Collins, D. L., Millst, S. R., Brown, E. D., Kelly, R. L. & Peters, T. M. (1993) 3D statistical neuroanatomical models from 305 MRI volumes. Proc IEEE Nucl Sci Symp Med Imaging 3: 1813-1817.
25. Zatorre, R. J., Jones-Gotman, M., Evans, A. C. & Meyer, E. (1992) Functional localization and lateralization of human olfactory cortex. Nature 360: 339-340.
26. Haase, L., Cerf-Ducastel, B. & Murphy, C. (2009) Cortical activation in response to pure taste stimuli during the physiological states of hunger and satiety. Neuroimage. 44: 1008-1021.
27. Del Parigi A., Chen, K., Gautier, J. F., Salbe, A. D., Pratley, R. E., Ravussin, E., Reiman, E. M. & Tataranni, P. A. (2002) Sex differences in the human brain's response to hunger and satiation. Am. J. Clin. Nutr. 75: 1017-1022.

28. Tataranni, P. A., Gautier, J. F., Chen, K., Uecker, A., Bandy, D., Salbe, A. D., Pratley, R. E., Lawson, M., Reiman, E. M. & Ravussin, E. (1999) Neuroanatomical correlates of hunger and satiation in humans using positron emission tomography. Proc. Natl. Acad. Sci. U. S. A 96: 4569-4574.
29. LaBar, K. S., Gitelman, D. R., Parrish, T. B., Kim, Y. H., Nobre, A. C. & Mesulam, M. M. (2001) Hunger selectively modulates corticolimbic activation to food stimuli in humans. Behav. Neurosci. 115: 493-500.
30. Malik, S., McGlone, F. & Dagher, A. (2011) State of expectancy modulates the neural response to visual food stimuli in humans. Appetite 56: 302-309.
31. Balleine, B. W. & Dickinson, A. (2000) The effect of lesions of the insular cortex on instrumental conditioning: evidence for a role in incentive memory. J. Neurosci. 20: 8954-8964.
32. Davidson, T. L. & Jarrard, L. E. (1993) A role for hippocampus in the utilization of hunger signals. Behav. Neural Biol. 59: 167-171



In the realm of ideas everything depends on enthusiasm...
in the real world all rests on perseverance

Chapter 5

Neuroimaging satiation: interactions between oral and gastric administration and hormonal responses

ABSTRACT

The complex process of satiation is determined among others, by a food's macronutrient content and sensory properties, gastric distension and hormonal responses. All these factors are integrated in the brain, where the decision to stop eating is made. Here, we investigated the interaction between food administration, hormone and brain responses by administering chocolate milk orally and intragastrically during functional magnetic resonance imaging and measuring peptides in the blood. Naso-gastric infusion of water was used as a control. Oral administration reduced appetite ratings more than both naso-gastric infusions. During gastric chocolate milk infusion insulin and CCK levels increased significantly more than oral administration, whereas the control condition did not evoke and response. Infusion (water and chocolate milk) increased activation in the midbrain, amygdala, hypothalamus, and hippocampus. This suggests that these areas responded to stomach distension and not to nutrient content. After treatment, the thalamus, amygdala, putamen and precuneus were activated more after ingestion compared to infusion of chocolate milk, whereas naso-gastric infusion evoked greater activation in the hippocampus and anterior cingulate cortex. The former is in line with the finding that ingestion increased fullness and decreased desire to eat. Anterior cingulate activation correlated negatively with fullness ratings. Insulin responses correlated positively with putame and negatively with insula activation. In conclusion, we show that food ingestion evokes greater activation in stomach distension and food intake related brain areas than gastric infusion. In line with the idea that oral stimulation is necessary for optimal digestion and absorption, gastric infusion induced greater hormone responses than oral administration. In summary, we provide neural and hormonal evidence for the importance of sensory stimulation for satiation and efficient digestion.

INTRODUCTION

Satiation refers to the process which leads to meal termination. It is a complex process which is determined by many factors, e.g. gastric distension, hormone levels, and a food's macronutrient content and sensory properties (1). Oral (2-5) and gastric (6,7) stimulation contribute separately and together (8) to meal termination. For example, during oral stimulation, the viscosity of a drink influences intake (9), or increased oro-sensory exposure can lower intake of sweet drinks (10). However, gastric processes are equally important for meal termination. This includes stomach distension and hormonal responses related to the macronutrient composition and size of the meal (11,12).

When consuming a food or drink through the mouth the perception of flavour occurs. This includes gustatory, olfactory and somatosensory stimulation (13). The brain responses that are activated through food or liquids on the tongue, travel from the central relay in the brainstem, the nucleus of the solitary tract, via the thalamus to the primary taste cortex in the frontal operculum and insula to reach a secondary cortical taste area in the caudolateral orbitofrontal cortex (14), but also to the amygdala and anterior cingulate cortex (15,16). Odours travel from olfactory bulb to the piriform cortex, from where it travels through the amygdala, frontal operculum and orbitofrontal cortex (17). When food enters the stomach, neural signals from the gastrointestinal tract travel via the vagus nerve to the brainstem and thalamus, which projects to the rest of the brain, in particular the hypothalamus, amygdala and primary sensory cortices (18). The integration of sensory and gastric signals in the brain is difficult to study. In neuroimaging studies in which the stomach was distended with a gastric balloon, activation was observed in the insula, amygdala, posterior insula, left inferior frontal gyrus and anterior cingulate cortex (19,20). To our knowledge, no study has examined the different contributions of oral stimulation and gastric distension by food on brain activation. In addition to neural signals, hormonal signals are important for meal termination. Hormones like insulin, ghrelin and CCK interact with gastric as well as sensory signals during food intake (11,21,22). Peptides secreted from the gastrointestinal tract provide information to the brain which leads to inhibition or stimulation of food intake (12,23). Gut peptides like ghrelin and CCK act on vagal afferents, the brainstem and other brain areas (24-26), in particular the hypothalamus. However, hormonal responses to food administration have rarely been linked to brain responses in humans. Moreover, to date the process of satiation has not been examined in real time in the brain. Therefore, the aim of this study was to assess the effects of oral and gastric food administration on hormone responses and brain responses in time and their interaction. To this end, chocolate milk was administered orally and intra-gastrically during functional magnetic resonance imaging (fMRI) and blood sampling. Water infusion was used as a control treatment to test in how far brain activation is induced by the nutrients or sheer stomach distension.

First we hypothesize that oral administration will activate reward areas, such as the striatum and amygdala compared to infusion of chocolate milk. Second, that distension of the stomach by infusion of nutrients will activate the striatum compared to the control. Third, gastric distension (water or chocolate milk) will evoke activation in the midbrain and hypothalamus, insula and anterior cingulate cortex. We also hypothesize a possible inaction between ghrelin, glucose, insulin and CCK levels and hypothalamus activation.

MATERIALS AND METHODS

Subjects

Subjects were recruited by flyers posted at the University Medical Center Utrecht and Utrecht University. People who responded were ask to fill in the restraint eating section of the Dutch Eating Behaviour Questionnaire (27) and a questionnaire which included questions on demographic variables (gender, age, height, weight), general health, use of medication, dieting/restraint eating, smoking, alcoholic beverage consumption, and contra-indications for MRI (claustrophobia and the presence of metal in the body). The inclusion criteria were: male, healthy BMI (19-25 kg/m2), right handed and aged between 18 and 35 yr. Exclusion criteria for this study included: disliking chocolate milk, smoking, slimming or following a medically prescribed diet, restraint eating (28), having an eating disorder, having a history of or current alcohol consumption > 28 units per week, or any diseases (including neurological, psychiatric diseases and taste and smell disorders) and use of medication. Subjects were informed about their eligibility and the procedure and risks were explained. When subjects met our inclusion criteria they were invited for a training session. After the training sessions, subjects could decide to participate or to withdraw for the following fMRI sessions. 16 men participated in the training session. Of these 16 subjects 14 (mean age 24.6 ± 3.8 yr, mean BMI 22.3 ± 1.6 kg/m2) participated in the fMRI sessions. All experimental procedures were approved by the Medical Ethical Committee of the University Medical Center Utrecht. Before the experiments written informed consent was obtained from all subjects.

Stimuli

Two stimuli were used. During the training session, and the two fMRI sessions with a caloric load , chocolate milk (Chocomel®, FrieslandCampina, Ede, the Netherlands. Per 100mL; energy content of 354 kJ, 84 kcal, 3.5 g proteins, 12 g carbohydrates of which 12 g sugars, 2.5 fat g of which 2.0 g were saturated, 0.5 g fibers, 0.04 g sodium and 110 mg calcium) was used. For the control session water (Dutch tap water) was used. To adjust for viscosity differences, guargum (a starch-based thickening agent) was added. Guargum (E number E412) is allowed in the EU. For the 500 mL control load, 5 gram (1%) guargum was added to 500mL water.

Stimulus delivery

Loads were administered with the use of a computer-controlled peristaltic pump (323 DU, Watson-Marlow Ltd, Falmouth, Cornwall, UK), so as to stimulate a normal drinking pattern (sips rather than continuous infusion). The pump was used with a silicon tube (inner diameter 4.8 mm). In the oral session, this tube was in the mouth of the subject. In the gastric sessions this tube was connected to the naso-gastric tube (length 110 mm with an inner diameter of 2.67mm). The pump was programmed to deliver 100mL/min with a sip size of 12 mL (delivered in 3 s) followed by a 4 s delay (for swallowing). Sip and swallow onset were cued on a screen. Subjects were acquainted with the drinking procedure in a training session and could stop the pump any time by pressing a button. First, 250 mL was administered in 2.5 min followed by a pause of 30 seconds. In this pause subjects rated their anxiety, desire to eat and fullness with a button box. Subsequently, the other load of 250 mL was administered, after which subjects again gave their ratings. All instructions were displayed on a screen through a computer interface, run by the computer program PRESENTATION (Neurobehavioral Systems Inc).

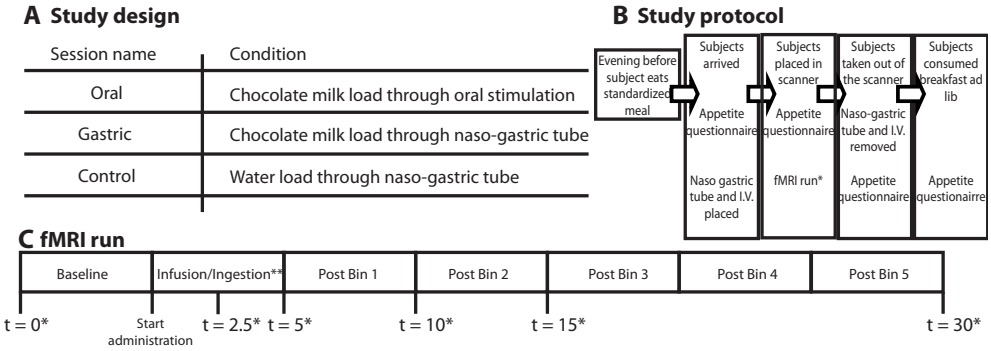


Figure 1 Experimental design. A, Summary of study design. B, Study protocol: timeline of events during one session. C, Timeline of events during fMRI run, *at all time points (shown in minutes) blood was drawn and fullness, desire to eat and anxiety were rated.

Experimental procedures

The study had a randomized cross over design with three conditions (Figure 1A). The first condition was the oral condition (1), which consisted of the ingestion of a caloric load through a tube in the mouth. The second condition (2) was the administration of a caloric intra-gastric load through a naso-gastric tube. The third condition (3) was the control condition, which consisted of administration of a non-caloric intra-gastric load through a naso-gastric tube. Before enrolling in the fMRI sessions, all subjects participated in a training session. The three conditions during the fMRI sessions were randomly allocated to a selected treatment order, based on enrolment in the study.

Trainings session

During the training session subjects were asked to come in fasted for at least 2 hours. A qualified nurse inserted a naso-gastric tube. After insertion of the tube, subjects rested to allow the water used to assist insertion of the tube to leave the stomach and to become comfortable with the tube. During tube insertion the nurse and the subject could see how well the naso-gastric tube was tolerated. To simulate the position in the MR scanner, subjects were asked to lie down on a doctor's table. A tube was placed between the teeth of the subject (like a straw) after which an oral load of 500 ml was administered through this tube with the use of computer-controlled pump (as described in the stimulus delivery part). During this session, subjects experienced the insertion of a naso-gastric tube and became familiar with the drinking procedure (drinking supine in a controlled manner). When everything went well subjects could participate in the fMRI study.

fMRI sessions

After an overnight fast, subjects came into the lab in the morning between 8am and 11am. The evening before all subjects consumed a standardized meal (provided by the researchers), and were instructed not to eat anything after 10pm. Subjects randomly received three conditions on three separated scan days. Upon arrival an appetite questionnaire was filled in (hunger, fullness, thirst, desire to eat, quantity to eat, desire to eat something sweet or savoury, nauseous, and anxiety rated on a Visual Analog Scale (VAS))(29). A qualified nurse placed a naso-gatric tube and an i.v canula, after which the

appetite questionnaire was filled in again. Subsequently, subjects were placed into the scanner and the baseline blood sample was obtained. A towel was placed underneath the subject's left side, such so that the stomach position was more resembles in the position when standing/sitting, in order to approximate normal gastric filling (Figure 1B).

First, a five min anatomical scan was obtained, after which the 35 min fMRI run started (Figure 1C). The first five minutes of the functional scan were the baseline measure. After baseline, stimulus administration started: either an oral load through a tube in the mouth, or an intra gastric load through the naso-gastric tube. The load was administered with the use of a peristaltic pump. After administration, subjects continued to be scanned for 30 min. The start of administration of the load was defined as t = 0. At t = 2.5, 5, 10, 15 and 30 min another blood sample were drawn. At these same time points, subjects rated their anxiety, desire to eat and fullness on a VAS by use of a button box. After the functional scan the subject was taken out of the scanner and the naso-gastric tube and i.v. was removed. Subsequently the appetite questionnaire was filled in again, after which subjects were provided with an ad libitum breakfast buffet consisting of bread, butter, milk, cheese, ham, jam, and chocolate sprinkles. Subjects could were placed in a separate room with a table with all the food items on top, and were instructed to eat as much as they would like. Food weight and macronutrient intake were recorded. After breakfast the appetite questionnaire was filled in once more.

Blood sampling and biochemical analysis

Blood samples were collected at baseline, before ingestion/infusion (t = 0), and at 2.5 and at 5, 10, 15 and 30 min after ingestion/infusion (t = 5, 10, 15 and 30) in tubes containing EDTA to prevent clotting. Blood samples for ghrelin and CCK-8 were collected in EDTA tubes which also contained Aprotinin and were kept on ice. After each blood drawn, 2 mL of phyciological salt solution (NaCl 0.9%) was injected into the caula to prevent it from clotting; before each blood draw an extra tube was drawn to remove the physiological salt solution. Plasma was obtained by centrifugation (1000×g/3000 rpm for 10 min at 4 ˚C).

Glucose levels were analyzed with the hexokinase method (Glucose HK 125 kit, Abbott). The inter- and intra-assay CV was < 1.1%. Insulin samples were analyzed using the architect insulin assay (Architect insulin reagent kit 8K41, Abbott), with an inter- and intra-assay CV of < 2.6 %.

Active and total ghrelin were measured using human ELISA kits (Millipore RIA GHRT-88HK, Billerica, MA, USA). The lowest level of active ghrelin that could be detected with the kit was 3.9 pg/mL. The intra-assay CV was 10% at mean concentrations of 1000 pg/mL and 4.4% at 3000 pg/ml. The inter-assay CV was 14.7% at 1000 pg/ml and 16.7% at 3000 pg/mL.

The lowest level of total ghrelin that could be detected was 82 pg/mL. The intra-assay CV was 9.5% at 235.76 pg/ml and 6.7& at 138.56 pg/ml. The inter-assay CV at the same concentrations was 13.7% and 9.6%.

Plasma CCK-8 concentrations were measured using a commercial RIA kit (Eurodiagnostica RIA RB302, Malmö, Sweden). Sensitivity of this RIA assay was 0.1 pmol/L. The inter-assay CV was 13.7% at mean concentrations of 4.2 pmol/L, and 2.0% at 20.6 pmol/L. The intra-assay CV for the same mean concentrations was 5.5% and 2.0%.

All analyses within one subject were done in one run. For all biochemical analyses concentrations below the detection limit were set at the lower detection limit. When concentrations were far above the highest calibration value, measurements were added as missing value in analyses. This only occurred for two subjects in one condition for CCK and insulin.

fMRI data acquisition

The scans were performed on a 3-Tesla Philips Achieva at the University Medical Center Utrecht. First a T1-weighted anatomical scan was acquired (TR/TE = 61/8.4 ms, flip angle = 30°, FOV = 288 × 175 mm, 175 axial slices, voxelsize = 1 × 1 × 1 mm). Second, a functional MRI scan was made (2D gradient echo EPI sequence, TR/TE= 1400/23 ms, flip angle =70°, FOV = 208 × 120 × 256 mm, 43 interleaved axial slices, voxelsize = 4 × 4 × 4 mm). The duration of each functional scan was 35 min, during which 1490 volumes were obtained.

Statistical analyses: Subjective and hormonal data

Subjective ratings were analyzed using SPSS 16.0 (SPSS Inc, Chicago, USA). Data are presented as means ± SD. Mean fullness, desire to eat, hunger, thirst and anxiety ratings were tested by means of a mixed model ANOVA with a fixed treatment effect, time effect and treatment × time interaction. Subjects were added as random variables. To control for the differences at baseline, the baseline values were added as a covariate in all models. Post-hoc, paired t-tests were performed ($p < 0.05$ LSD-corrected for multiple comparisons).

The same mixed model procedure was performed for the blood parameters insulin, CCK, ghrelin active and total, and glucose. CCK, insulin and ghrelin active were not normally distributed and were log-transformed with a natural logarithm before testing.

Total areas under the curves (AUC) were calculated for appetite and anxiety ratings, and all blood parameters. Comparison of the AUC between the three conditions was performed by means of one-way ANOVA, adjusted for subjects and baseline values. Post-hoc, paired t-tests were performed ($p < 0.05$ LSD corrected for multiple comparisons).

Ad libitum breakfast intake after the scan was analyzed by means of a one-way ANOVA, adjusted for subjects. Post-hoc, paired t-tests were performed $p < 0.05$ LSD corrected for multiple comparisons).

fMRI data processing and analysis

fMRI data were pre-processed and analyzed using SPM8 (Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) run with MATLAB 7.5 (The Mathworks Inc, Natick, MA) and the WFU Pickatlas-tool (30). First the functional volumes of every subject were realigned to the first volume of the first run. Second, the anatomical image was co-registered with the additional functional volume with a flip angle of 27°, after which this was co-registered with mean functional image. Third the images were normalized (retaining 4 × 4 × 4 mm voxels) to Montreal Neurological Institute space (MNI space) (31), and spatially smoothed with a Gaussian kernel of 8mm 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.

Within-subject analyses

First level analyses were performed by splitting every functional run into time bins: one pre-treatment bin (baseline bin T0), one treatment bin (T1) and five post treatment bins (T2-6). Every bin was 4.5 min (30 second was subtracted per bin due to the rating procedure). A regressor was created to be able to exclude instructions and ratings from the other bins; this was neglected in future analyses. For each subject and condition the BOLD signal averages of the baseline bin was subtracted for every treatment and post treatment bins, using regression, analysis resulting in 6 contrast images per scan session (32, 33).

Group analyses

Contrasts images from the first level analyses were entered into a 6 time (T1-T6) × 3 conditions repeated measure ANOVA. All peaks were considered significant at $P < 0.05$ FWE-corrected across the entire brain.

Regions of interest (ROI's) were gastric distension and reward areas reported in previous studies, which include the amygdala, insula, inferior frontal gyrus, anterior cingulate cortex, hypothalamus and striatum (19). ROI masks were made using the WFU Pickatlas tool (30).

In subsequent analyses, changes in hormone levels and VAS ratings were added as covariates. Significant correlations ($p < 0.001$, uncorrected for multiple comparisons, $k > 5$)(34,35) between brain activation and changes in hormonal levels or VAS ratings were tested for in SPM8.

For all significant clusters mean parameter estimates were obtained with the use of the MarsBaR toolbox.

RESULTS

Subjective ratings

Fullness ratings increased after administration (time effect $p < 0.0001$) and differed per treatment (treatment effect $p < 0.0001$). When the chocolate milk was administered orally, fullness ratings increased significantly more than the other two conditions ($p < 0.0001$). Desire to eat ratings decreased over time ($p < 0.0001$) and differed between treatments ($p < 0.0001$). There was an interaction between time and treatment (time × treatment effect $p < 0.0001$). During the oral condition at $t = 5$ the desire to eat ratings were significantly lower than the two gastric conditions ($p < 0.0001$), the same occurred at $t = 10$ and $t = 15$ ($p < 0.005$). After 30 minutes the desire to eat ratings after the oral ingestion were lower compared to the control condition ($p < 0.05$). Time to peak for fullness and desire to ratings was at 5 minutes after administration started, so immediately after consumption. The area under the curve for desire to eat ($p < 0.005$) and fullness ($p < 0.05$) was significantly different when comparing the oral with the control and gastric conditions.

Anxiety ratings were significantly increased in the oral condition (effect of treatment $p < 0.05$), with a time to peak at 2.5 and 5 minutes. Anxiety ratings decreased over time (effect of time $p < 0.0001$). There was no significant difference in AUC (Figure 2).

When comparing hunger ratings before and after the scan, the ratings were decreased after the scan in the oral condition ($p < 0.0001$) but not in the other two conditions. After the scan, thirst ratings were significantly decreased after the oral chocolate milk administrating ($p < 0.05$), but not after the gastric and control condition.

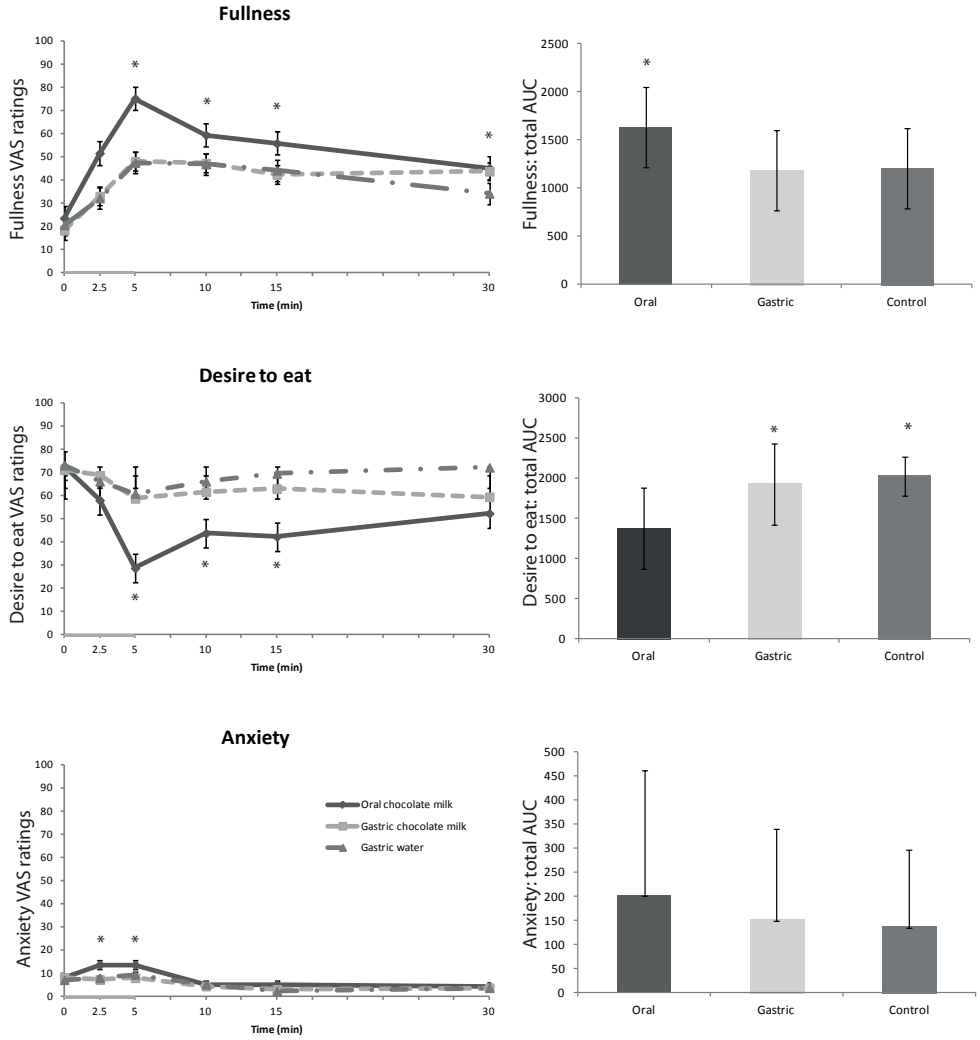


Figure 2 Left pane: mean (± SD) subjective ratings obtained in the scanner during the three conditions (◆ = oral chocolate milk, ■ = gastric chocolate milk, ▲ = control gastric water) *represents significant difference between condition at that time point. Right pane: the mean areas under the curve (± SD) for the measures plotted on the left for all three conditions.

Ad libitum intake

For ad libitum intake a main effect of treatment was observed ($p < 0.05$). After the control condition (618 kcal ± 48) subjects consumed significantly more than after the oral condition (484 kcal ± 56) ($p < 0.05$), and almost significantly more than after the gastric condition (515 kcal ± 60) ($p = 0.05$).

Blood parameters

Glucose and insulin levels showed an effect of time ($p < 0.0001$) and treatment ($p < 0.0001$). Also an interaction effect was observed (time × treatment $p < 0.0001$). Glucose concentrations at 15 and 30 minutes after starting the administration were significantly higher in the two chocolate milk conditions compared to the control condition ($p < 0.0001$). Insulin blood levels were higher at 10, 15 and 30 min after administration started when comparing oral versus gastric ($p < 0.0001$), oral versus control ($p < 0.05$), and gastric versus control ($p < 0.0001$). Time to peak for both blood parameters was at 30 minutes after the start of the administration. The areas under the curve were significantly lower for the control condition compared to the oral and gastric condition. Insulin AUC also differed between the oral and gastric condition.

Total ghrelin and CCK concentrations both showed an effect of time ($p < 0.0001$), treatment ($p < 0.0001$), and an interaction effect (time × treatment $p < 0.0001$). CCK levels were increased at 2.5 and 5 min after the onset of administration in the gastric condition compared to the oral and control condition ($p < 0.05$). At t =10 in all three conditions the CCK levels were significantly different ($p < 0.0001$), with the highest concentration in the gastric condition, then the oral, and the lowest for the control condition. After 15 and 30 min the CCK concentrations in the control condition were significantly lower than in the oral and gastric conditions ($p < 0.0001$). CCK AUC was significantly higher for the gastric condition compared to control. Total ghrelin levels were increased at t = 10 and 15 for the oral compared to the gastric condition ($p < 0.05$), after 15 min the blood parameter levels were also increased compared to the control condition ($p < 0.05$). At t = 30 the concentrations during the control condition were increased compared to the other two ($p < 0.005$). Total ghrelin AUC was significantly higher for the oral condition compared to the gastric condition. Active ghrelin levels only showed a significant effect of time ($p < 0.0001$), the concentration decreased after administration. No difference between AUC's was observed (Figure 3).

Neuroimaging data

Effect of gastric infusion

When comparing the gastric infusion time bin of chocolate milk and water versus baseline, an increased BOLD signal in the hypothalamus, amygdala, hippocampus and midbrain was observed (Table 1 and Figure 4). When comparing chocolate milk versus water infusion, or the other way around, no difference were observed.

Effects after gastric infusion with chocolate milk or water

Gastric infusion with water increased activity in the putamen (MNI 22, 12, -10 z = 5.07) compared to gastric infusion with chocolate milk (Figure 5).

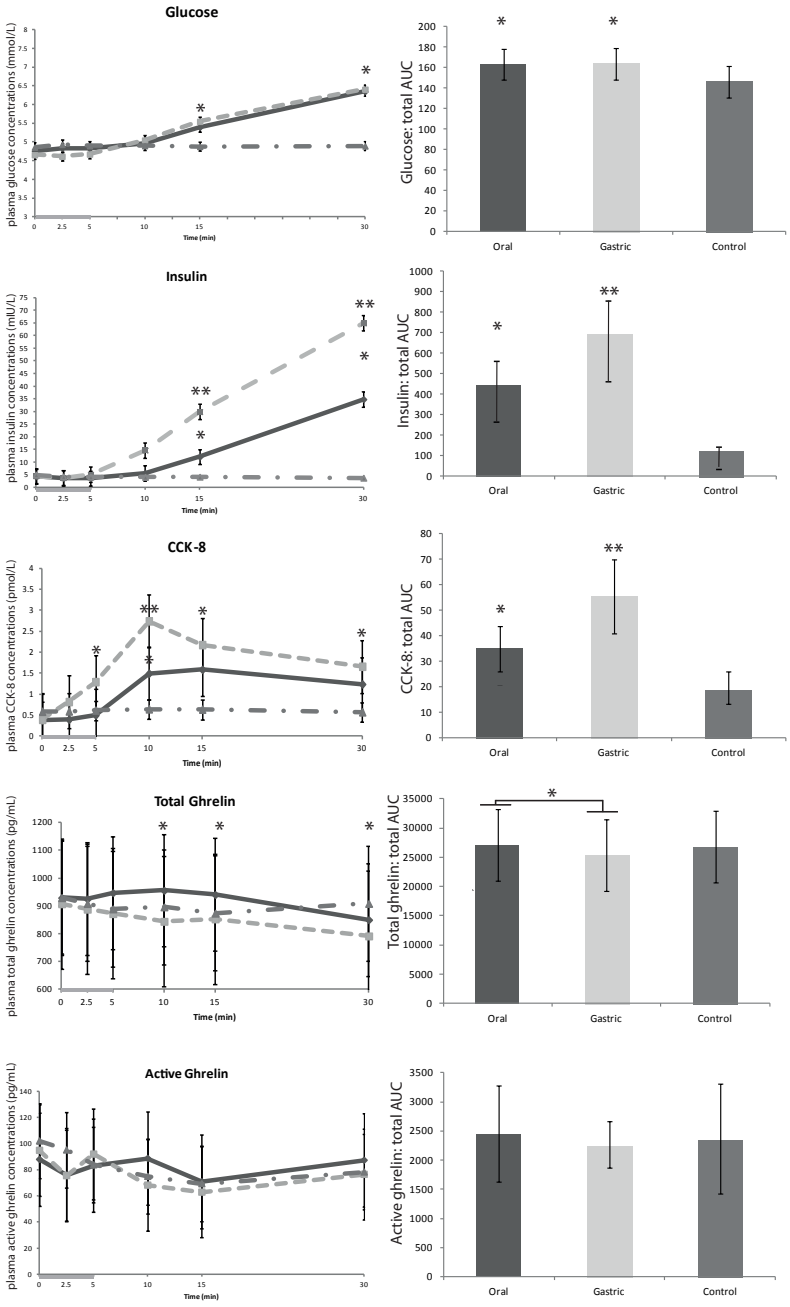


Figure 3 Left pane: mean (± SD) values of blood parameters for all three conditions (◆ = oral chocolate milk, ■ = gastric chocolate milk, ★ = control gastric water), * represents significant difference between condition at that time point. Right pane: the mean areas under the curve (± SD) for the blood measures plotted on the left for all three conditions.

Table 1 Brain activation by gastric infusion in healthy, normal wieght, youngh men[1]

Regions	Peakvoxel coordinates[3]			Z-score
	x	y	z	
Midbrain	6	-28	-18	Inf
	14	-15	-2	6.05
Hippocampus L	34	-8	-22	5.76
	25	-24	-10	4.54
Amygdala ROI R	26	-4	-26	5.32
Amygdala ROI L	-26	0	-26	3.83
	-22	-8	-22	3.32
Hypothalamus ROI R	12	2	-8	4.66
	2	-8	2	4.09

[1]Values are clusters of mean brain activation, n = 14. L: left hemisphere and R: right hemisphere. Clusters are differences in BOLD response between infusion bin and baseline bin. Reposted cluster were thresholded at P < 0.05 FWE-corrected, with a cluster extent threshold k > 10 voxels. Voxel coordinates are in MNI space (31).

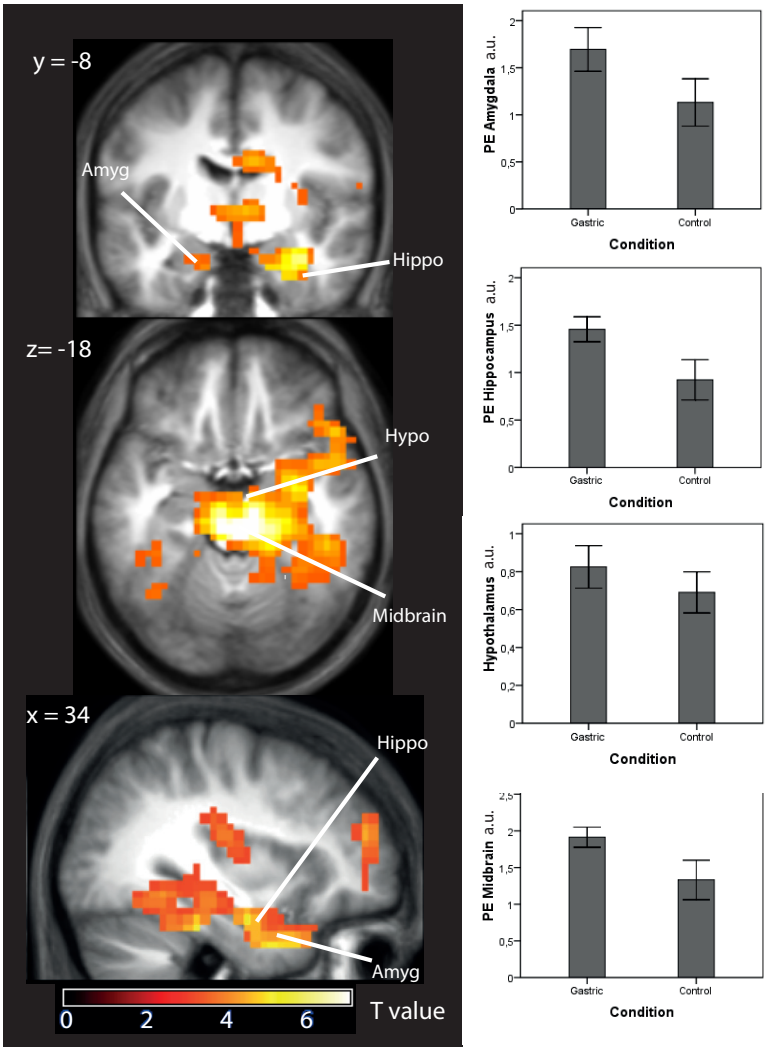


Figure 4 Effect of gastric infusion on brain activation. Left plane: T-map of the response to chocolate milk and water infusion versus baseline overlaid onto the mean anatomical scan. Right plane: Mean parameter estimates (a.u. ± SEM) for the gastric chocolate milk and control infusion within the significant cluster.

Oral versus gastric chocolate milk administration

After chocolate milk administration activity in the amygdala, thalamus, left precuneus and putamen was increased for the oral conditions compared to the gastric chocolate milk load, whereas the ACC and hippocampus were more activated in the gastric condition (Table 2 and Figure 5).

Table 2 Effect of treatment with chocolate milk on brain activity in healthy, normal weight, young men. Left table: increased activity in the oral condition. Right table: increased activity during the gastric chocolate milk condition[1]

Region	Peakvoxel coordinates[3]			Z- score	Region	Peakvoxel coordinates[3]			Z- score
	x	y	z			x	y	z	
oral > gastric chocolate milk					**oral < gastric chocolate milk**				
Thalamus L	-2	-16	6	5.24	Hippocampus ROI R	34	-8	-26	5.65
Thalamus R	18	-24	-10	4.74	Hippocampus ROI L	-30	-32	-2	4.61
Precuneus L	-2	-68	50	4.98	ACC ROI	6	28	14	5.15
Precuneus R	10	-54	52	4.65					
	10	-72	46	4.12					
Amygdala ROI R	34	0	-26	4.67					
	26	-4	-26	4.47					
	30	-4	-14	4.47					
Putamen ROI R	26	16	-6	4.57					
	30	0	-10	3.74					

[1]Values are clusters of mean brain activation, n = 15. Contrasts were calculated using t-tests on the contrast images of before and after preload of the sweet and savory target taste. L: left hemisphere and R: right hemisphere. [2]Reported clusters were thresholded at P < 0.001, uncorrected for multiple comparisons, which corresponds to a Z-score > 3.0. [3]Voxel coordinates are in MNI space (26).

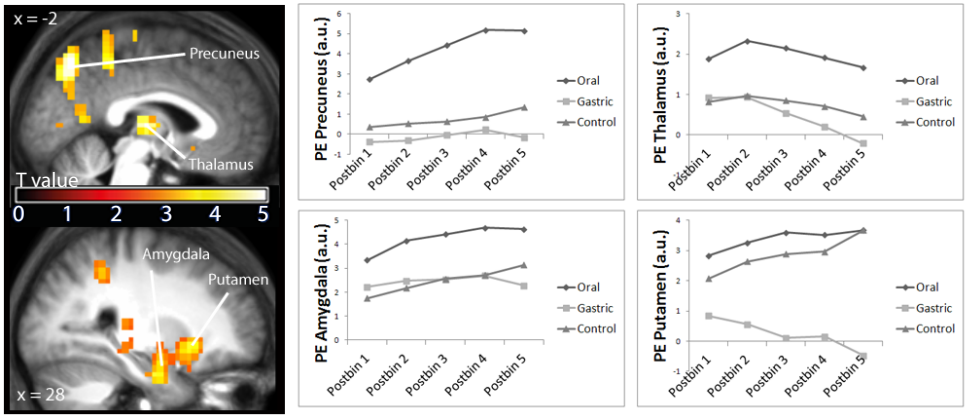


Figure 5 Effect of treatment on brain activation (P < 0.05, FWE-corrected). Right pane: Mean parameter estimates (a.u.) over time from selected areas. Area under the curve was greater for the oral condition in all brain areas, and for the control condition in the putamen.

Brain activation and subjective ratings

Changes in fullness ratings in the gastric condition were correlated (r = 0.47) with activation in the ACC (MNI (-6, -16, 30), z = 3.72, FWE-corrected p = 0.022) (Figure 6). The oral and control condition did not show any correlations. Also desire to eat ratings did not correlate with brain activation.

Brain activation and hormone levels

During the gastric condition insulin changes were correlated positively with putamen activation (MNI (34, 0, 6), z = 3.81, FWE corrected p = 0.011, r = 0.56), and negatively with middle and posterior insula activation (MNI (-38, 0, 2), z = 3.81, FWE-corrected = 0.047, r = - 0.67) (Figure 6). Brain activation during the control and oral condition was not correlated with insulin changes. CCK, glucose, total and active ghrelin responses did not correlate with any brain activation in (Figure 6).

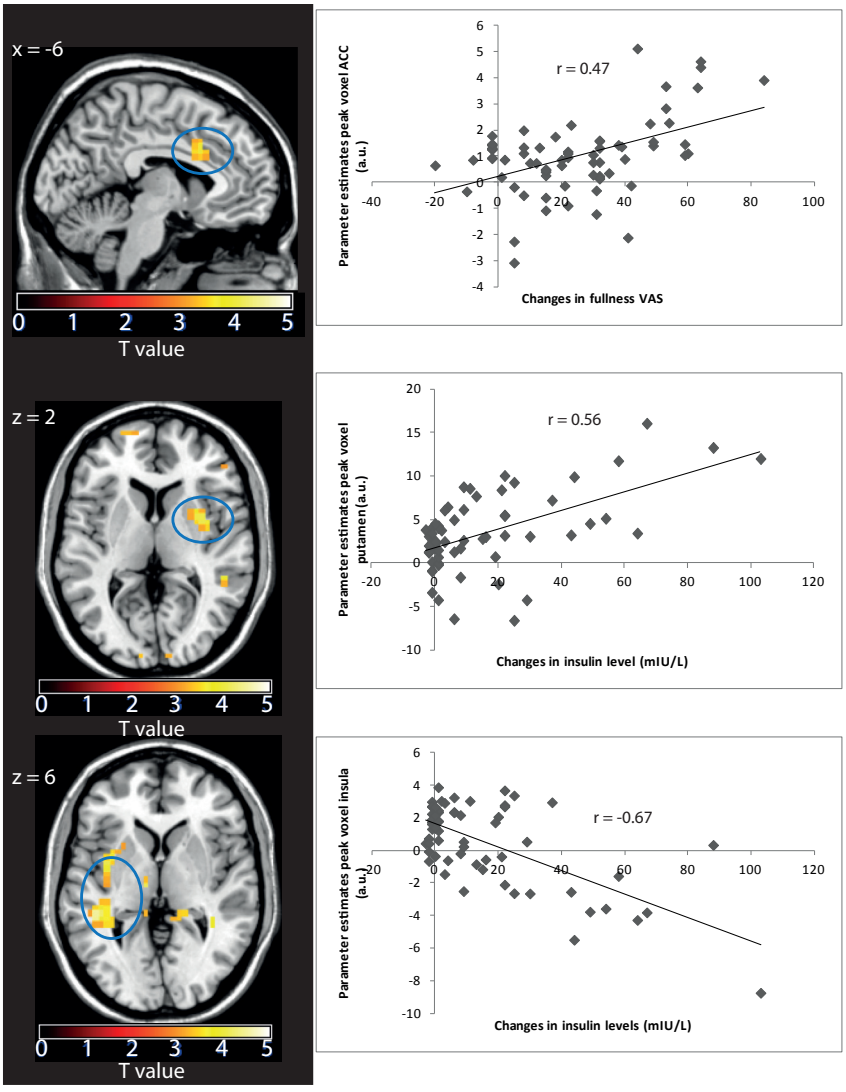


Figure 6 Correlation between fullness and insulin changes and brain activity (P < 0.001 uncorrected for multiple comparisons). Left pane: T-map of the brain activation in ACC, putamen and insula overlaid onto the mean anatomical scan. Right plane: scatter plot of the parameter estimates of the brain areas of the left pane and changes in fullness and insulin in the gastric condition.

DISCUSSION

We investigated the effect of gastric infusion and food ingestion on hormone levels and brain activation. CCK concentrations increased significantly more after gastric chocolate milk infusion in the first 10 min after administration, whereas insulin levels increased in the last 15 minutes. Total ghrelin levels where increased more in the oral condition. The effect of infusion on amygdala, midbrain, hippocampus, and hypothalamus activation was independent of the caloric content of the load. We also observed differential brain responses between oral and gastric administration of chocolate milk and between chocolate milk and water infusion (control condition).

Subjective responses

Oral administration decreased appetite more than both the gastric infusions. Moreover, after oral administration fullness ratings increased more than in the gastric condition, even though the same load was administrated in a similar fashion. The same was observed for the desire to eat ratings, which decreased significantly after ingestion, but not after infusion. Cecile already observed in 1999 that hunger and fullness are not only related to gastric distension but are, to some extent, independently influenced by orosensory stimulation (8). In previous behavioural studies the energy content of the load or meal affected the appetite ratings 60 min later (36). In our study, gastric infusion of chocolate milk also evoked an increased fullness ratings compared to the control condition after 30 min. This is in line with the finding that after 30 minutes the nutrient content of a load is the most important factor affecting appetite ratings whereas shortly after intake the oral stimulation is most important (37-40). Thirst ratings were decreased after the oral condition, due to the oral stimulation, which made subjects more aware of the liquid intake.

Glucose and Insulin

Glucose concentrations increased in a similar same pattern in both chocolate milk conditions, whereas insulin responses differed. Insulin responses were significantly greater at 15 and 30 min in the gastric condition. This suggests that during the gastric condition more insulin was needed to keep the glucose concentrations at a normal level, even though the same caloric load was administered. In the oral condition we did not observe a cephalic phase insulin response (41), however in liquids meals this does not always occur, presumably because these are less appetizing or not perceived as a real ‘meal’ (42).

CCK

CCK levels increased more during gastric infusion, even though the chocolate milk loads had the same nutrient content. CCK is released from the small intestine (43) in the gastrointestinal tract (44), following consumption, especially in response to fat, and inhibits gastric emptying (45) and suppresses energy intake (46,47). Gastric infusion may have caused the early CCK peak observed in this study, although the load entered the stomach distension only a few seconds later in the oral condition. The changes in CCK response during the oral condition started 10 min after onset, although still a decreased response compared to the gastric conditions, and at 15 and 30 min the oral and gastric condition did not differ from each other in CCK response, but did from the control condition. To our knowledge, such differences in the CCK response between gastric infusion and oral stimulation

have never been observed. It suggests that the process of nutrient uptake is more efficient after oral stimulation, since a higher CCK secretion, as observed after gastric infusion requires more energy. This is in line with the observed insulin effects and the idea that oral stimulation is necessary for optimal absorption and digestion (42).

Ghrelin

Ghrelin is mainly released by the stomach and has an appetite stimulating effect; plasma levels are higher during fasting and decrease after consumption (48). It normally plays an important role in meal initiation (49), it increases when a subject is intent to eat and decrease during the meal. In this study chocolate milk was administered, which is a caloric load but not a meal. This may explain why the decline in ghrelin after administration was relatively modest. Active ghrelin was thought to be biologically active part of ghrelin (50), but recently it has been suggested that the inactive ghrelin also exerts some biological effects (51,52). Therefore, we measured both total and active ghrelin. In line with other studies we found that total ghrelin levels decreased after food administration over time. However, there are some differences between the conditions, in the gastric and control conditions the total ghrelin concentrations were lower than the oral condition, but the response was higher at 30 min for the control conditions. However, all the changes in the total ghrelin levels were small compared to the ghrelin concentration decrease after a meal intake (53). Active ghrelin levels decreased after administration but did not differ between conditions.

Overall, oral administration evoked less insulin and CCK secretion and did not lower total ghrelin as much as gastric infusion of chocolate milk. Even though subjects felt fuller during the oral condition, the above hormone responses were greater after gastric detention. This underpins the importance of cephalic stimulation for efficient digestion and absorption.

Ad libitum intake

After oral administration, subjects consumed fewer calories. This was expected since during the control condition no energy was administered. In previous studies, when providing a preload with less calories, more was consumed during a meal 60min after the load, then after a more caloric load (36).

Brain responses

We observed the effects of stomach filling, which evoked an increased activity in the midbrain, hypothalamus, amygdala and hippocampus. Considering no difference was found between the control and the gastric condition, this increased activity was a consequence of stomach filling independent of the macronutrient content. In the process of satiation, when the stomach is filled, the neural signals from the gastrointestinal tract travel via the vagus nerve to the brainstem. The gut-hindbrain communication is of importance in this process; the higher cognitive centres interact with the hindbrain, but are not required for the process of satiation (11). When tasting, the cephalic neural signals travel from the brainstem to the thalamus, which projects to the rest of the brain, in particular the hypothalamus, amygdala and primary sensory cortices (18,54). Here, we observed that during gastric infusion many of these areas responded to gastric filling. Wang et al. (19) also observed amygdala activation during stomach distension with a balloon filled with water. Our results confirm that stomach distension (40) is an important factor for meal termination (19,20). Unfortunately,

brain responses during oral food administration could not be assessed, due to movement artefacts associated with swallowing.

The water infusion and oral ingestion caused a greater activation than the chocolate milk infusion in the putamen after treatment. The putamen is involved in the expectation of reward and stimulus-action reward (55,56). Gastric administration of chocolate milk and subsequent detection of calories in the gastrointestinal tract may constitute an unexpected reward, due to the lack of predicting oral stimulation. The putamen response after water infusion was an unexpected finding and would need replication.

Oral administration of chocolate milk evoked greater activation in the thalamus, precuneus, amygdala and putamen than gastric infusion after treatment. This may be due to the stronger stimulation present in the oral condition and associated heightened attention, which is also reflected in increased fullness ratings. Wang et al. Found that the amygdala, thalamus and left precuneus were activated when the stomach was distended with a volume of 500ml (19). All these areas play a role in feeding behaviour. The amygdala is involved in processing aversive and rewarding stimuli (57), but has also been found to be activated by the sensory properties of a food in primates (58) and by visual food cues by humans (59). The thalamus is a sensory relay area and has been shown to be involved in the preparatory (the food seeking aspects) of food behaviour(60). The brain could have reacted more to perception of fullness and not to the actual gastric distension as soon in previous studies (19,20).

In the gastric condition we found a negative correlation between ACC activation and fullness ratings. ACC activation has been observed in a several studies in which the internal state was altered (61-63). E.g., recently we reported a negative correlation between taste activation of the anterior part of the ACC and subsequent ad libitum intake, suggesting that the ACC reflects the degree of satiety (fullness). Here, we found that increased middle ACC activation compared to baseline reflects greater changes in fullness. The absence of this correlation in the oral condition may be due to the fact that subject s drank on command, which may have yielded different fullness ratings than normal self-paced ingestion.

In line with other studies, insulin responses correlated positively with putamen activation and negatively with insula activation over 30 min in the gastric condition. Insula activity (64,65)and activation (62,66) is greater when subjects are hungry. The putamen is a reward area (55,56). Thus, we demonstrate that the insulin response, which is indicative of the amount of carbohydrate being absorbed, affects activity of these limbic areas in proportion to the degree of nutrient repletion. These correlations were not found in the oral condition. This may be due to the smaller magnitude of hormone responses in the oral condition.

In conclusion, CCK and insulin increased more after gastric infusion of chocolate milk. This is in line with the idea that oral stimulation is necessary for optimal digestion and absorption; gastric infusion induced greater hormone responses than oral administration. The midbrain, amygdala, hippocampus, and hypothalamus, all areas that play an important role in the regulation of food intake were activated by gastric filling, independent of nutrient content. Oral administration evoked greater activation after food administration in several brain areas involved in gustatory and reward processes, and was associated with increased fullness and decreased desire to eat. Thus, we

provided neural and hormonal evidence for the importance of oral sensory stimulation for satiation and optimal digestion. Future research should further investigate the complex interplay between oral sensory stimulation, gastric filling, hormones and brain responses.

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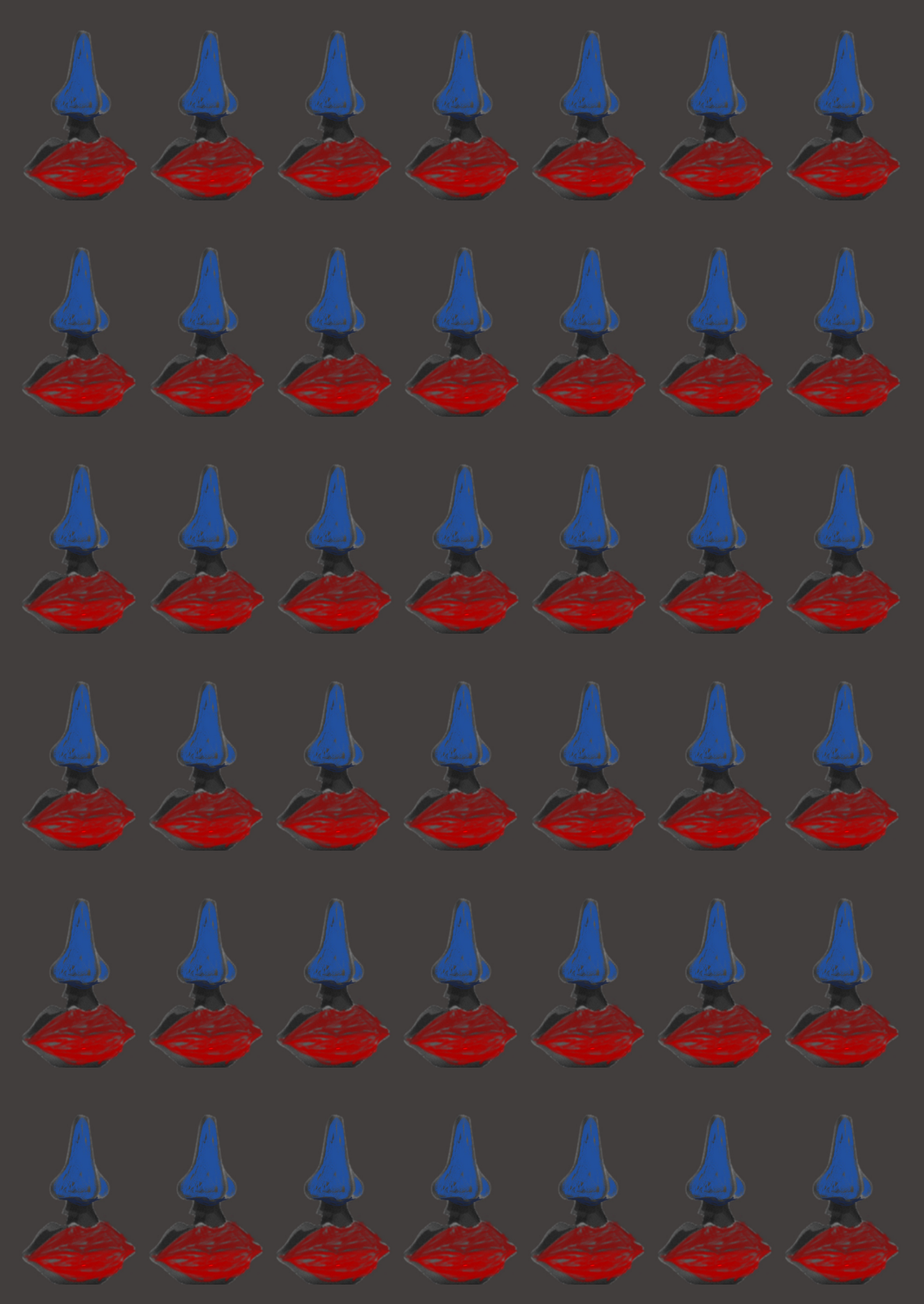
REFERENCES

1. Blundell, J. E. & Halford, J. C. (1994) Regulation of nutrient supply: the brain and appetite control. Proc. Nutr. Soc. 53: 407-418.
2. Rolls, B. J., Rowe, E. A. & Rolls, E. T. (1982) How sensory properties of foods affect human feeding behavior. Physiol Behav. 29: 409-417.
3. Sorensen, L. B., Moller, P., Flint, A., Martens, M. & Raben, A. (2003) Effect of sensory perception of foods on appetite and food intake: a review of studies on humans. Int. J. Obes. Relat Metab Disord. 27: 1152-1166.
4. Rolls, B. J., Rolls, E. T., Rowe, E. A. & Sweeney, K. (1981) Sensory specific satiety in man. Physiol Behav. 27: 137-142.
5. Smeets, A. J., Lejeune, M. P. & Westerterp-Plantenga, M. S. (2009) Effects of oral fat perception by modified sham feeding on energy expenditure, hormones and appetite profile in the postprandial state. Br. J. Nutr. 101: 1360-1368.
6. Stratton, R. J., Stubbs, R. J. & Elia, M. (2008) Bolus tube feeding suppresses food intake and circulating ghrelin concentrations in healthy subjects in a short-term placebo-controlled trial. Am. J. Clin. Nutr. 88: 77-83.
7. Shide, D. J., Caballero, B., Reidelberger, R. & Rolls, B. J. (1995) Accurate energy compensation for intragastric and oral nutrients in lean males. Am. J. Clin. Nutr. 61: 754-764.
8. Cecil, J. E., Francis, J. & Read, N. W. (1998) Relative contributions of intestinal, gastric, oro-sensory influences and information to changes in appetite induced by the same liquid meal. Appetite 31: 377-390.
9. Zijlstra, N., Mars, M., de Wijk, R. A., Westerterp-Plantenga, M. S. & de, G. C. (2008) The effect of viscosity on ad libitum food intake. Int. J. Obes. (Lond) 32: 676-683.
10. Weijzen, P. L., Smeets, P. A. & de Graaf C. (2009) Sip size of orangeade: effects on intake and sensory-specific satiation. Br. J. Nutr. 102: 1091-1097.
11. Cummings, D. E. & Overduin, J. (2007) Gastrointestinal regulation of food intake. J. Clin. Invest 117: 13-23.
12. Delzenne, N., Blundell, J., Brouns, F., Cunningham, K., De, G. K., Erkner, A., Lluch, A., Mars, M., Peters, H. P. & Westerterp-Plantenga, M. (2010) Gastrointestinal targets of appetite regulation in humans. Obes. Rev. 11: 234-250.
13. Lundstrom, J. N., Boesveldt, S. & Albrecht, J. (2011) Central Processing of the Chemical Senses: an Overview. ACS Chem. Neurosci. 2: 5-16.
14. Rolls, E. T. (1989) Information processing in the taste system of primates. J. Exp. Biol. 146: 141-164.
15. Kringelbach, M. L., de Araujo, I. & Rolls, E. T. (2004) Taste-related activity in the human dorsolateral prefrontal cortex. Neuroimage. 21: 781-788.
16. Small, D. M. (2006) Central gustatory processing in humans. Adv. Otorhinolaryngol. 63: 191-220.
17. Zatorre, R. J., Jones-Gotman, M., Evans, A. C. & Meyer, E. (1992) Functional localization and lateralization of human olfactory cortex. Nature 360: 339-340.
18. Hellstrom, P. M., Geliebter, A., Naslund, E., Schmidt, P. T., Yahav, E. K., Hashim, S. A. & Yeomans, M. R. (2004) Peripheral and central signals in the control of eating in normal, obese and binge-eating human subjects. Br. J. Nutr. 92 Suppl 1: S47-S57.
19. Wang, G. J., Tomasi, D., Backus, W., Wang, R., Telang, F., Geliebter, A., Korner, J., Bauman, A., Fowler, J. S. et al. (2008) Gastric distention activates satiety circuitry in the human brain. Neuroimage. 39: 1824-1831.
20. Stephan, E., Pardo, J. V., Faris, P. L., Hartman, B. K., Kim, S. W., Ivanov, E. H., Daughters, R. S., Costello, P. A. & Goodale, R. L. (2003) Functional neuroimaging of gastric distention. J. Gastrointest. Surg. 7: 740-749.
21. Mars, M., Stafleu, A. & de, G. C. (2012) Use of satiety peptides in assessing the satiating capacity of foods. Physiol Behav. 105: 483-488.
22. de, G. C., Blom, W. A., Smeets, P. A., Stafleu, A. & Hendriks, H. F. (2004) Biomarkers of satiation and satiety. Am. J. Clin. Nutr. 79: 946-961.
23. Blundell, J. E. & Gillett, A. (2001) Control of food intake in the obese. Obes. Res. 9 Suppl 4: 263S-270S.
24. Batterham, R. L., ffytche, D. H., Rosenthal, J. M., Zelaya, F. O., Barker, G. J., Withers, D. J. & Williams, S. C. (2007) PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans. Nature 450: 106-109.
25. Naslund, E. & Hellstrom, P. M. (2007) Appetite signaling: from gut peptides and enteric nerves to brain. Physiol Behav. 92: 256-262.

26. Hillebrand, J. J., de, W. D. & Adan, R. A. (2002) Neuropeptides, food intake and body weight regulation: a hypothalamic focus. Peptides 23: 2283-2306.
27. Van Strien, T., Frijters JER, Bergers GPA & Defares PB (1986) The Dutch Eating Behaviour Questionnaire (DEBQ) for assessment of restrained, emotional and external eating behaviour. Int J Eat Disord 295-315.
28. Van Strien, T. (1997) The concurrent validity of a classification of dieters with low versus high susceptibility toward failure of restraint. Addict. Behav. 22: 587-597.
29. Blundell, J., de, G. C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., Mela, D., Salah, S., Schuring, E. et al. (2010) Appetite control: methodological aspects of the evaluation of foods. Obes. Rev. 11: 251-270.
30. Maldjian, J. A., Laurienti, P. J., Kraft, R. A. & Burdette, J. H. (2003) An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage. 19: 1233-1239.
31. Evans, A. D., Collins, D. L., Millst, S. R., Brown, E. D., Kelly, R. L. & Peters, T. M. (1993) 3D statistical neuroanatomical models from 305 MRI volumes. Proc IEEE Nucl Sci Symp Med Imaging 3: 1813-1817.
32. McKie, S., Del-Ben, C., Elliott, R., Williams, S., del, V. N., Anderson, I. & Deakin, J. F. (2005) Neuronal effects of acute citalopram detected by pharmacoMRI. Psychopharmacology (Berl) 180: 680-686.
33. Lassman, D. J., McKie, S., Gregory, L. J., Lal, S., D'Amato, M., Steele, I., Varro, A., Dockray, G. J., Williams, S. C. & Thompson, D. G. (2010) Defining the role of cholecystokinin in the lipid-induced human brain activation matrix. Gastroenterology 138: 1514-1524.
34. Cox, R. W. (1996) AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput. Biomed. Res. 29: 162-173.
35. Forman, S. D., Cohen, J. D., Fitzgerald, M., Eddy, W. F., Mintun, M. A. & Noll, D. C. (1995) Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. Magn Reson. Med. 33: 636-647.
36. Cecil, J. E., Castiglione, K., French, S., Francis, J. & Read, N. W. (1998) Effects of intragastric infusions of fat and carbohydrate on appetite ratings and food intake from a test meal. Appetite 30: 65-77.
37. Rolls, B. J., Hetherington, M. & Burley, V. J. (1988) Sensory stimulation and energy density in the development of satiety. Physiol Behav. 44: 727-733.
38. Rolls, B. J., Fedoroff, I. C., Guthrie, J. F. & Laster, L. J. (1990) Foods with different satiating effects in humans. Appetite 15: 115-126.
39. Kissileff, H. R., Gruss, L. P., Thornton, J. & Jordan, H. A. (1984) The satiating efficiency of foods. Physiol Behav. 32: 319-332.
40. Jones, K. L., Doran, S. M., Hveem, K., Bartholomeusz, F. D., Morley, J. E., Sun, W. M., Chatterton, B. E. & Horowitz, M. (1997) Relation between postprandial satiation and antral area in normal subjects. Am. J. Clin. Nutr. 66: 127-132.
41. Bellisle, F., Louis-Sylvestre, J., Demozay, F., Blazy, D. & Le, M. J. (1983) Reflex insulin response associated to food intake in human subjects. Physiol Behav. 31: 515-521.
42. Smeets, P. A., Erkner, A. & de Graaf C. (2010) Cephalic phase responses and appetite. Nutr. Rev. 68: 643-655.
43. Liddle, R. A., Goldfine, I. D., Rosen, M. S., Taplitz, R. A. & Williams, J. A. (1985) Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction. J. Clin. Invest 75: 1144-1152.
44. Liddle, R. A. (1997) Cholecystokinin cells. Annu. Rev. Physiol 59: 221-242.
45. Liddle, R. A., Morita, E. T., Conrad, C. K. & Williams, J. A. (1986) Regulation of gastric emptying in humans by cholecystokinin. J. Clin. Invest 77: 992-996.
46. Beglinger, C., Degen, L., Matzinger, D., D'Amato, M. & Drewe, J. (2001) Loxiglumide, a CCK-A receptor antagonist, stimulates calorie intake and hunger feelings in humans. Am. J. Physiol Regul. Integr. Comp Physiol 280: R1149-R1154.
47. Kissileff, H. R., Pi-Sunyer, F. X., Thornton, J. & Smith, G. P. (1981) C-terminal octapeptide of cholecystokinin decreases food intake in man. Am. J. Clin. Nutr. 34: 154-160.
48. Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E. & Weigle, D. S. (2001) A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50: 1714-1719.
49. Cummings, D. E. (2006) Ghrelin and the short- and long-term regulation of appetite and body weight. Physiol Behav. 89: 71-84.
50. Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. & Kangawa, K. (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402: 656-660.

51. Asakawa, A., Inui, A., Fujimiya, M., Sakamaki, R., Shinfuku, N., Ueta, Y., Meguid, M. M. & Kasuga, M. (2005) Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. Gut 54: 18-24.
52. Gauna, C., Meyler, F. M., Janssen, J. A., Delhanty, P. J., Abribat, T., van, K. P., Hofland, L. J., Broglio, F., Ghigo, E. & van der Lely, A. J. (2004) Administration of acylated ghrelin reduces insulin sensitivity, whereas the combination of acylated plus unacylated ghrelin strongly improves insulin sensitivity. J. Clin. Endocrinol. Metab 89: 5035-5042.
53. Williams, D. L. & Cummings, D. E. (2005) Regulation of ghrelin in physiologic and pathophysiologic states. J. Nutr. 135: 1320-1325.
54. Berthoud, H. R. (2002) Multiple neural systems controlling food intake and body weight. Neurosci. Biobehav. Rev. 26: 393-428.
55. Haruno, M. & Kawato, M. (2006) Different neural correlates of reward expectation and reward expectation error in the putamen and caudate nucleus during stimulus-action-reward association learning. J. Neurophysiol. 95: 948-959.
56. Berns, G. S., McClure, S. M., Pagnoni, G. & Montague, P. R. (2001) Predictability modulates human brain response to reward. J. Neurosci. 21: 2793-2798.
57. O'Doherty, J., Rolls, E. T., Francis, S., Bowtell, R. & McGlone, F. (2001) Representation of pleasant and aversive taste in the human brain. J. Neurophysiol. 85: 1315-1321.
58. Kadohisa, M., Verhagen, J. V. & Rolls, E. T. (2005) The primate amygdala: Neuronal representations of the viscosity, fat texture, temperature, grittiness and taste of foods. Neuroscience 132: 33-48.
59. van der Laan, L. N., de Ridder, D. T., Viergever, M. A. & Smeets, P. A. (2011) The first taste is always with the eyes: a meta-analysis on the neural correlates of processing visual food cues. Neuroimage. 55: 296-303.
60. Reilly, S. (1998) The role of the gustatory thalamus in taste-guided behavior. Neurosci. Biobehav. Rev. 22: 883-901.
61. Small, D. M., Zatorre, R. J., Dagher, A., Evans, A. C. & Jones-Gotman, M. (2001) Changes in brain activity related to eating chocolate: from pleasure to aversion. Brain 124: 1720-1733.
62. Haase, L., Cerf-Ducastel, B. & Murphy, C. (2009) Cortical activation in response to pure taste stimuli during the physiological states of hunger and satiety. Neuroimage. 44: 1008-1021.
63. Spetter, M. S., de Graaf, C., Viergever, M. A. & Smeets, P. A. M. (2012) Anterior cingulate taste activation predicts ad libitum intake of sweet and savoury drinks in healthy normal-weight men. Journal of Nutrition in press.
64. Tataranni, P. A., Gautier, J. F., Chen, K., Uecker, A., Bandy, D., Salbe, A. D., Pratley, R. E., Lawson, M., Reiman, E. M. & Ravussin, E. (1999) Neuroanatomical correlates of hunger and satiation in humans using positron emission tomography. Proc. Natl. Acad. Sci. U. S. A 96: 4569-4574.
65. Del Parigi A., Chen, K., Gautier, J. F., Salbe, A. D., Pratley, R. E., Ravussin, E., Reiman, E. M. & Tataranni, P. A. (2002) Sex differences in the human brain's response to hunger and satiation. Am. J. Clin. Nutr. 75: 1017-1022.
66. Cornier, M. A., Von Kaenel, S. S., Bessesen, D. H. & Tregellas, J. R. (2007) Effects of overfeeding on the neuronal response to visual food cues. Am. J. Clin. Nutr. 86: 965-971.



Doubt grows with knowledge

Chapter 6

General Discussion

GENERAL DISCUSSION

The main aim of this research project was to understand the effect of internal state on brain activity associated with different taste and odour properties. To this end, the brain activation in response to differential taste and odour stimuli when either being hungry or satiated, and additionally, the process of satiation was studied. This chapter starts with a brief overview of the main findings. Next, we discuss the new insights in the effects of food and odour properties on brain activation and changes in the brain activation due to satiation/satiety. Finally, recommendations for future research are made.

OVERVIEW OF MAIN FINDINGS

Satiation is affected by sensory properties of a food, such as intensity, stimulus quality and stomach distension.

- Intensity of a taste can influence the satiation process. When increasing the concentration of a NaCl and sucrose solution, we observed that the middle insula activation increased. Amygdala and anterior insula activation were only associated with an increased concentration of NaCl, probably due to the unpleasantness of that stimulus (Chapter 2).

- Consumption of sweet and savoury foods may alter the satiation and satiety process differently. When consuming a sweet or savoury beverage, striatal activation decreased. Taste activation of brain reward areas changed following food consumption. There were no differences in brain activation changes between sweet and savoury juice. The anterior cingulate response predicted subsequent ad libitum juice intake, independent of the quality of the taste (Chapter 3).

- If an odour is associated with food or non-food, the route of delivery and internal state affect the brain differentially. Insula response was increased for food odours when hungry dependent on the route, this does not occur when full (Chapter 4).

- In the process of satiation, amygdala, hypothalamus and hippocampus activation are associated with gastric infusion, independent of the nutrient content of the load (Chapter 5). In line with the idea that oral stimulation is necessary for optimal absorption and digestion, gastric infusion induced greater hormone responses than oral administration (Chapter 5).

CENTRAL INTEGRATION OF SENSORY INFORMATION AND INTERNALE STATE

As noted in the introduction of this thesis, to fully understand the neural mechanisms behind food intake regulation, one should focus on pleasure and reward, satiety and satiation, and its neural and hormonal afferent pathways.

The role of the different food and odour properties

To gain insights in what the effect was of internal state on brain activation associated with different food/odour properties, we investigated the effect of different sensory qualities on brain activation in

Chapters 2-4. We reported that intensity influences insula and striatum activation (Chapter 2), but changes in amygdala and hippocampus activation due to intensity differences could not be totally separated from the valence of the stimuli. When changing the valence of a stimulus, we observed a decrease in striatal activation (Chapter 3). When comparing odour categories (food vs. non-food) and the route of delivery and internal state, while keeping the pleasantness and intensity equal, a different effect on hippocampus and insula activation was observed (Chapter 4). These results suggest that the valence or reward value, affected by internal state changes or the physiological function of a stimulus, contribute to differences in brain activation.

The importance of pleasantness when varying intensity was also observed in a behavioural study (1). Ad libitum soup intake did not differ when keeping the palatability similar, and changing the saltiness (intensity) of a soup. The pleasantness of a taste is more important for determining meal size than the taste intensity. When varying intensity in a meal, pleasantness changes are closely related. In the intensity brain response study of Small et al. (2), only two stimuli per taste quality were used. The same was done in the soup intake study; two salt intensities were consumed (low and high). By using only two stimuli, the researchers were able to select two stimuli which were rated similarly in palatability. Instead we looked at the dose-response curve of NaCl and sucrose intensity, and here it was unfortunately impossible to keep the pleasantness equal. When observing the differential effects of intensity and pleasantness on brain responses by using the same stimuli (3), attention appears to be a important factor. When subjects were asked to focus on the taste intensity, insula activation increased. Tasting the same stimuli but focusing on its pleasantness evoked a greater response in the medial orbitofrontal cortex. This implies that when studying the changes in brain responses to different sensory properties, subjects should be instructed thoroughly about the property they should focus on when tasting. In our study we explicit asked the subjects to rate the intensity (Chapter 2) or reward value (Chapter 3) of the stimuli when it was presented. If stimuli are totally matched, e.g. macro-nutrients, pleasantness etc. expect for one sensory property, different brain responses between the products as a result of their different properties could perhaps be observed. However, people often already have expectations about a taste, for example, savoury tasting products are associated with proteins and fat, and sweet products with sugar, even if the products are totally matched in macro-nutrients. The question therefore remains whether the content of the product is more important than its taste quality and its expectations.

In the process of food intake the smell of a food is highly underestimated. Already before ingestion the smell of a food prepares the body for food intake, by means of the cephalic phase response. Olfaction is a dual sense modality. Odours sensed orthonasally provide information about objects in the external world, such as the availability of bread, whereas odours sensed retronasally provide information about foods and drinks being consumed (4). Prior work has demonstrated differential brain response to ortho- versus retronasal perception of chocolate, but not for non-food odours (5). This category-specific (food vs. non-food) effect of route on brain response was suggested to reflect engagement of anticipatory (ortho) vs. consummatory (retro) food reward circuits (6). Our results confirm that route of odorant delivery influences brain response to odours, primarily if they are food-related (Chapter 4). The results show that this effect occurs for many food odours and that many of these selective responses depend upon internal state, which in turn indicates that they are related to the physiological significance or value of the food odour.

The role of internal state

We observed that anterior cingulate response predicted subsequent ad libitum juice intake, irrespective of quality of taste. These findings extended our understanding of the representation of food-specific appetite in the human brain and suggest that neuroimaging may provide objective and more accurate measure of food motivation than self-report measures (Chapter 3). In odours, route difference only seem to play a important role when hungry (Chapter 4) or neither hungry nor full (5). When full, the human body does not anticipate as strongly on food availability or possible consumption. Cabanac (7) already argued in the 70's that internal state influences the perception of a stimulus, especially the pleasantness. When full, the decreased energy need of the human body affects the neural activation while smelling odours and tasting a juice (Chapter 3-4).

So, when full, neural activation associated with a stimulus is altered compared to a hungry state. In Chapter 5 we studied the effect of the actual stomach filling and oral stimulation, due to consumption, on brain activation. Brain areas associated with the process of satiation, e.g. the midbrain, amygdala, hippocampus, and hypothalamus, increased in activation during stomach filling, independent of the nutrient content of the load. This raises the question if it is actual the size of the meal, or its macronutrient content which influences the process of satiation. After oral stimulation greater activation was observed in several brain areas, and an increased fullness and decreased desire to eat. This reflects the effect of sensory stimulation on the brain and the awareness of the consumption. This suggests that the food properties are of importance for meal termination when being orally exposed. Also hormone levels responded differently to gastric infusion (Chapter 5). Hormone levels that influence the digestion changed less after oral consumption compared to infusion of chocolate milk. This suggested the importance of sensory stimulation for satiation and optimal digestion. The combination of oral and gastric factors is necessary to cause the optimal effect on satiety and suppression of hunger. The interplay between stomach filling, oral exposure and food properties define the satiation process.

METHODOLOGICAL CONSIDERATIONS

The fMRI environment and stimuli

When studying brain activation, subjects lie on their back in a scanner, a small space, and hear a horrible loud noise; until now, this is the optimal way to study brain activation related to food regulation in humans. Perhaps, in the future, when scanned, a more 'real' environment could be developed, by using a computer program were subjects actually see someone or themselves tasting or smelling the food in a nice, restaurant-like environment, or even hear the sound of chewing or drinking. At this moment, only liquid stimuli can be provided in the scanner, due to the movements during chewing when food is solid, which limits our generalisation to normal solid foods. Therefore, a lot of challenges are still out there, such as creating a virtual reality or optimizing liquid stimuli by the industry, to optimize the research of brain responses within the limitations of the scanner environment.

Study population

In all four fMRI studies we only used young healthy adults; in three out of four we only used male volunteers; which was done to limit variation in our data. All subjects had a healthy BMI (between 19

and 25); considering that when a person is overweight (BMI above 25) their perception of for example a sweet solution can be altered (8) and therefore evoke a differential brain response compared to healthy adults. The same holds for reward value of a food, overweight subjects may experience reward at a different level, which influences the brain activation (9). These are interesting differences, and may lead to more research on brain responses in lean and obese subjects, and understanding the underlying neural networks of overeating. All subjects were right-handed; it has been suggested that when people are left-handed there brain networks are differently built. Therefore, we did not want to risk any effects and excluded all left-handed people. In a previous study by Smeets et al. (10), differences have been observed in brain responses between the sexes. Future studies that include differences between females and males may give some interesting information regarding food intake. The optimal research with a combination of female, male, lean and overweight participants, all at different age groups is very time-consuming and expensive, so choices need to be made. By data sharing or cooperation this kind of studies are more feasible, and the differences and similarities between groups can be studied.

FUTURE DICRECTIONS

In the studies presented in this thesis, brain responses were altered by varying food properties and internal state. Optimizing the experimental stimuli is an important issue that should be addressed in the future. This is unfortunately really hard to realize, especially, the production of liquids that are equal in all properties or macronutrient composition, except for one factor. Liquids are mainly associated with sweet sugary drinks. Savoury drinks are rare and mostly related to a meal (soup), and bitter tasting drinks contain caffeine or alcohol. Studies that investigate these factors are needed to confirm or refine the general and food-specific effects reported here. When optimizing the stimuli for brain related research, one should not forget the expectations and learned associations subjects will have with a stimuli. Most of the flavour preferences and dietary behaviours are learned (11). This field of research has been studied in behavioural studies in the last decade, and making the link to underlying brain mechanisms would be of interest.

The effect of direct infusion on the brain was mainly caused by stomach distension, considering no differences between the water and chocolate milk infusion were found. Future studies should assess the effect of oral stimulation on brain activation in relation to food intake. Also relating feeding-induced hormone responses to human brain activation is rarely observed; it seems difficult to correlate hormone responses to brain responses. Hormone concentrations and their impact on neuronal substrates may help to understand obesity and even influence treatments to regulate feeding behaviour. Future studies should further address the gut-brain axis and its hormonal interaction.

Overall, the use of neuroimaging in food research can help us to understand the underlying mechanism behind food intake, and people's preference and choice. It makes it possible to asses more information than by only doing behavioural studies.

CONCLUSIONS

The internal state affects the physiological need of a stimulus, and thereby influences the brain responses. First, we observed that the striatum, insula, hippocampus, and amygdala decreased or increased in activation when the valence of a stimulus changed. These limbic areas, which are known to be involved in the regulation of food intake, also reflect changes in valence. The changes in reward value and internal state and their effects on brain activity are closely linked. The valence of a stimulus contributes extremely to its neural response. Second, sensory stimulation is important for satiation and optimal digestion. This is reflected in both the neural and hormonal responses to food administration. To better understand the regulation of food intake, future studies should address the interaction between cephalic phase response to different foods, internal state, and food preferences.

REFERENCES

1. Bolhuis, D. P., Lakemond, C. M., de Wijk, R. A., Luning, P. A. & de, G. C. (2010) Effect of salt intensity on ad libitum intake of tomato soup similar in palatability and on salt preference after consumption. Chem. Senses 35: 789-799.
2. Small, D. M., Gregory, M. D., Mak, Y. E., Gitelman, D., Mesulam, M. M. & Parrish, T. (2003) Dissociation of neural representation of intensity and affective valuation in human gustation. Neuron 39: 701-711.
3. Grabenhorst, F. & Rolls, E. T. (2008) Selective attention to affective value alters how the brain processes taste stimuli. Eur. J. Neurosci. 27: 723-729.
4. Rozin, P. (1982) "Taste-smell confusions" and the duality of the olfactory sense. Percept. Psychophys. 31: 397-401.
5. Small, D. M., Gerber, J. C., Mak, Y. E. & Hummel, T. (2005) Differential neural responses evoked by orthonasal versus retronasal odorant perception in humans. Neuron 47: 593-605.
6. Berridge, K. C. (1996) Food reward: brain substrates of wanting and liking. Neurosci. Biobehav. Rev. 20: 1-25.
7. Cabanac, M. & Fantino, M. (1977) Origin of olfacto-gustatory alliesthesia: intestinal sensitivity to carbohydrate concentration? Physiol Behav. 18: 1039-1045.
8. Sartor, F., Donaldson, L. F., Markland, D. A., Loveday, H., Jackson, M. J. & Kubis, H. P. (2011) Taste perception and implicit attitude toward sweet related to body mass index and soft drink supplementation. Appetite 57: 237-246.
9. Frankort, A., Roefs, A., Siep, N., Roebroeck, A., Havermans, R. & Jansen, A. (2011) Reward activity in satiated overweight women is decreased during unbiased viewing but increased when imagining taste: an event-related fMRI study. Int. J. Obes. (Lond).
10. Smeets, P. A., de Graaf C., Stafleu, A., van Osch, M. J., Nievelstein, R. A. & van der Grond J. (2006) Effect of satiety on brain activation during chocolate tasting in men and women. Am. J. Clin. Nutr. 83: 1297-1305.
11. Brunstrom, J. M. (2005) Dietary learning in humans: directions for future research. Physiol Behav. 85: 57-65.

Summary/Samenvatting

SUMMARY

The main aim of this research project was to understand the effect of internal state on brain activity associated with different food and odour properties. To this end, the brain activation in response to differential taste and odour stimuli when either being hungry or satiated, and additionally, the process of satiation was studied. In Chapter 1 the gustatory and olfactory pathways and associated brain activation and networks were described. Also what is known so far about the process of satiation and satiety and the effect on brain activation is described in this chapter. Intensity of a taste can influence the satiation process. In chapter 2 we investigated the effect of intensity differences on brain activation. We used two basic tastes, sweet (sucrose) and salt (NaCl) as taste stimuli and varied the molarity of the solutions (0M, 0.25M, 0.5M en 1M) to investigate the dose-effect responses. Both stimuli were rated at the same intensity per concentration. However, the pleasantness of the highest concentrations differed between the two stimuli; the salt stimulus was perceived as aversive. When increasing the concentration of a NaCl and sucrose solution, we observed that the middle insula activation increased. Amygdala and anterior insula activation were only associated with an increased concentration of NaCl, probably due to the unpleasantness of the stimulus and the physiological function of NaCl.

In chapter 3 the effect of consumption on taste activation was investigated. When consuming a juice the pleasantness of that juice decreased compared to juices not consumed independent of the taste quality. Using these differences in pleasantness changes as a covariant, we investigated the food specific wanting effect on brain responses. When consuming a sweet or savoury beverage, striatal activation decreased. Taste activation of brain reward areas changed following food consumption. There were no differences in brain activation changes between sweet and savoury juice. When tasting a juice the anterior cingulate response predicted subsequent ad libitum juice intake, independent of the quality of the taste, when the anterior cingulate response was increased, subject consumed less of the associated juice.

Chapter 4 describes the effect of consumption on brain responses to food and non-food odours when perceived retro- or orthonasal. Odours sensed orthonasally provide information about objects in the external world, such as the availability of bread, whereas odours sensed retronasally provide information about foods and drinks being consumed. We observed that when an odour is associated with food or non-food, the route of delivery and internal state affect the brain differentially. Insula response is increased for food odours when hungry dependent on the route, this did not occur when full.

Last, we investigated the effect of stomach filling on brain activation and the effect on hormone changes in chapter 5. We varied the administration of chocolate milk, by intra-gastrically administrating the chocolate milk or ingestion true the mouth with the use of a tube. As a control we used intra-gastric infusion of water. In the process of satiation, amygdala, hypothalamus and hippocampus activation were associated with gastric infusion, independent of the nutrient content of the load. Oral stimulation evoked greater activation in several food intake related brain areas then when it was directly infused into the stomach, whereas, CCK and insulin concentrations increased more after gastric infusion of chocolate milk. In line with the idea that oral stimulation is necessary for optimal absorption and digestion, gastric infusion induced greater hormone responses than oral administration. In conclusion, the internal state affects the physiological need of a stimulus, and thereby influences the brain

responses. Sensory stimulation is important for satiation and optimal digestion.

To better understand the regulation of food intake, future studies should address the interaction between cephalic phase response to different foods, internal state and food preferences.

SAMENVATTING

Het doel van het onderzoek zoals beschreven in dit proefschrift is het beter begrijpen van de effecten van honger en verzadiging op hersenactiviteit die geassocieerd is met verschillende smaak- en geureigenschappen, maar ook het proces van verzadiging zelf.

In hoofdstuk 1 wordt een introductie gegeven van wat er tot nu toe bekend is over geur, smaak, de geassocieerde hersenactiviteit en netwerken. Ook wordt er beschreven hoe het proces van verzadiging tijdens of na voedselinname verloopt. Eveneens worden de betrokken hersengebieden toegelicht.

In hoofdstuk 2 wordt het effect van intensiteitveranderingen van de basissmaken zoet (sucrose) en zout (NaCl) op hersenactiviteit onderzocht. Er is gekeken welke hersengebieden reageerden als de intensiteit van de smaken versterkt werd. De vier zoete en zoute (0M, 0.25M, 0.5M en 1M) oplossingen werden per molaire (M) als even intens ervaren door de proefpersonen. Ze vonden de 1M NaCl echter wel minder aangenaam dan de 1M sucrose. Als de intensiteit van zowel de zoete als zoute stimuli toenam, verhoogde de activiteit van het middelste gedeelte van de insula. Echter bij het verhogen van de intensiteit van de zoute oplossingen verhoogde de activiteit van de anterior insula en de amygdala ook. De verschillen in hersenactiviteit zijn waarschijnlijk te verklaren door de verschillen in aangenaamheid en de waarschuwingsfunctie van het menselijk lichaam op een te hoge zoutinname.

In hoofdstuk 3 wordt onderzocht wat het effect van consumptie van een sap is op smaakactiviteit in de hersenen. Na het consumeren van een zoete vruchtensap of hartige tomatensap was de aangenaamheid van de geconsumeerde sap gedaald. Een niet geconsumeerde sap was echter net zo lekker gebleven als voor consumptie. Als de verschillen in aangenaamheid als covariant in de berekening werden meegenomen bleek de activiteit in het striatum te zijn afgenomen na consumptie. Dit gold alleen als men het geconsumeerde sap proefde, niet voor het niet geconsumeerde sap. Deze de-activatie is te verklaren door afname van het specifieke verlangen naar voedselproducten met de geassocieerde smaak. Er was geen verschil in smaakactiviteit tussen de zoete en hartige drank. Wanneer beide sappen werden geproefd correleerde de activiteit van de anterior cingulate cortex met de inname naderhand. Hoe actiever de anterior cingulate, hoe minder de proefpersonen consumeerden.

Hoofdstuk 4 beschrijft het effect van consumptie op hersenactiviteit wanneer een voedsel- of niet-voedselgeur retro- dan wel orthonasal wordt waargenomen. Geuren die orthonasal worden waargenomen geven informatie over de buitenwereld, zoals de aanwezigheid van brood. Retronasale geuren worden geassocieerd met voedsel of drank wanneer het wordt geconsumeerd, dus via de mond. We observeerden dat de insula-activiteit was verhoogd wanneer een voedselgeur werd waargenomen als men honger had en dit afhankelijk was van de route (ortho vergeleken met retro). Dit zien we niet wanneer men vol was.

Als laatste hebben we in hoofdstuk 5 onderzocht wat het effect van maagvulling was op hersenactiviteit en hormoonveranderingen. Proefpersonen kregen tijdens een hersenscan chocolademelk door een dik rietje via de mond te drinken of direct in de maag gespoten via een maagsonde. Als controleconditie werd water via een maagsonde in de maag gebracht. Om de hormoonconcentraties te bepalen werd tijdens de scans ook bloed afgenomen. Tijdens de directe maagvulling van de chocolademelk en het water verhoogde de activiteit van de amygdala, hypothalamus en hippocampus vergeleken met de baseline. Deze is dus onafhankelijk van de inhoud van de drank. Deze hersengebieden zijn in eerdere studies geassocieerd met maagvulling en regulatie van voedselinname. Wanneer de chocolademelk via de mond werd ingenomen was de hersenactiviteit in de 25 minuten na de inname groter in verschillende gebieden die gerelateerd zijn aan voedselinname vergeleken met de directe maagvulling. De concentraties van CCK (een hormoon wat reageert op vet in de maag) en insuline waren na directe maagvulling verhoogd vergeleken met de orale inname van chocolademelk. Deze bevindingen zijn in lijn met het idee dat orale stimulatie nodig is voor een optimale inname en vertering van voedsel. Het lichaam werkt effectiever als het voedsel eerst in de mond is waargenomen. Zowel op hormonaal niveau, als in de hersenen.

In de toekomst zullen de stimuli die tijdens hersenscans gebruikt worden verder moeten worden geoptimaliseerd. Zoals het geheel gelijk maken van de eigenschappen van de stimuli, met uitzondering van die ene eigenschap die je wilt onderzoeken. Daarbij moet niet vergeten worden wat het effect is van verwachtingen en geleerde associaties van een stimulus. Hierdoor kunnen de resultaten nog meer gegeneraliseerd worden. Hersenonderzoek in het veld van voeding kan ons helpen om de mechanismes van voedselinname en de voorkeuren en keuzes van mensen beter te begrijpen. Het maakt het mogelijk om meer te weten te komen dan met alleen gedragstudies.

Conclusie: of men honger heeft of vol zit heeft invloed op de fysiologische waarde van de stimulus en hierdoor beïnvloedt het de hersenactiviteit. Sensorische stimulatie is van groot belang tijdens het beëindigen van een maaltijd en voor een optimale vertering. Om de regulatie van voedselinname beter te begrijpen zal er in de toekomst onderzoek gedaan moet worden naar het effect van verzadiging van verschillende soorten voedsel en de effecten van voedsel voorkeuren op hersenactiviteit.

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Maartje

LIST OF PUBLICATIONS

Journal articles

Spetter M.S., Smeets P.A.M., de Graaf C., Viergever M.A., Representation of sweet and salty taste intensity in the brain. Chemical Senses. 2010 Nov;35(9):831-40.

Spetter M.S., de Graaf C., Viergever M.A., Smeets P.A.M., Anterior cingulate taste activation predicts ad libitum intake of sweet and savory drinks. Journal of Nutrion. 2012 Apr;142(4):795-802.

Spetter M.S., Negoias S., Hummel T., Bender G., Veldhuizen M.G., Small D.M., Differential effects of satiety on brain response to ortho- vs. retronasally perceived food odors. In preparation for submission.

Spetter M.S., Mars. M., de Graaf C., Viergever M.A., Smeets P.A.M., The effects of oral and gastric food administration on hormonal responses. In preparation for submission.

Spetter M.S., de Graaf C., Viergever M.A., Mars. M., Smeets P.A.M., Neuroimaging of satiation; the interactions between oral and gastric stimulation, and the related hormonal responses. In preparation.

Smeets P.A.M., Charonnier L., van Meer F., van der Laan L.N., **Spetter M.S.,** Food-induced brain responses and eating behaviour. Nutrition Research Reviews. Submitted

Conference proceedings

Spetter M.S., Smeets P.A.M., de Graaf C., Viergever M.A., Representation of sweet and salty taste intensity in the brain (NeuroImage suppl. Human Brain Mapping 2009, San Fransisco, USA, poster presentation).

Spetter M.S., Smeets P.A.M., de Graaf C., Viergever M.A., Representation of sweet and salty taste intensity in the brain, a fMRI study (Appetite suppl. Pangborn 2009, Florence, Italy, poster presentation).

Spetter M.S., Smeets P.A.M., de Graaf C., Viergever M.A., Representation of sweet and salty taste intensity in the brain (Proceedings of the International Society for Magnetic Resonance in Medicine 2010, Stockholm, Sweden, e-poster presentation).

Spetter M.S., de Graaf C., Viergever M.A., Smeets P.A.M., Sweet and savoury sensory-specific satiation in the brain (34th British feeding and Drinking Group meeting 2010, Maastricht, the Netherlands, oral presentation).

Spetter M.S., Bender G., Hummel T., Negoias S., Veldhuizen M.G., Small D.M., Differential effects of satiety on brain response to ortho- vs. retronasally perceived food (Chemical Senses suppl. Association for Chemoreception Sciences 2011, Clearwater, FL, USA, poster presentation).

Spetter M.S., de Graaf C., Viergever M.A., Smeets P.A.M., Effect of consumption on sweet and savory taste activation and correlation with ad libitum intake in the human brain (9th Pangborn Sensory Science Symposium 2011, Toronto, Canada, oral presentation).

Awards

Student Bursary Award, 9th Pangborn Sensory Science Symposium, September 2011, Toronto, Canada

CURRICULM VITAE

Maartje Spetter was born on May 25th 1984 in 's-Gravenhage, the Netherlands. In 2002 she graduated from secondary school (v.w.o. I.S.W., 's-Gravenzande). In de same year she started her bachelor education Public Health Sciences at the University of Maastricht. During her bachelor she specialized in physical activity (bewegingswetenschappen) and bioregulation (biologische gezondheidskunde). In the beginning of 2005 she enrolled in the Erasmus program, and followed several public health courses at the Kuopio University, Finland. She graduated in 2005 and during her bachelor thesis and research project at the department of Human Biology she focused on sensory effects on fat metabolism and satiety. Maartje enrolled in the master program E-MeNu (European master – Metabolism and Nutrition) in September 2005, which was a part of the Physical Activity master of Public Health Sciences at the University of Maastricht. During her final research and thesis in 2006 she had a closer look on the effects of the glycemic index of foods in subjects with the metabolic syndrome. After her thesis she worked at the department of Human Biology (University Maastricht) as a research assistant on a research project aimed on the effects of resistant starch, protein and capsaicin on hormones and energy use. To get more research experience Maartje went to Pamplona, Spain to do an internship at the Universidad Navarra at the Physiology & Nutrition department. In October 2007 Maartje started her PhD at the Images Sciences Institute, University Medical Center Utrecht (Utrecht University). The project was in co-operation with Wageningen University and part of a large project focusing on sensory satiation. In the summer and autumn of 2010, as part of her PhD, Maartje moved to New Haven, USA to work in the laboratory of Affective Sensory Neuroscience at JB Pierce, Yale University. The results of the research conducted at the UMCU and JB Pierce are described in this thesis.

Der Mensch muß bei dem Glauben verharren, daß das Unbegreifliche begreiflich sei; er würde sonst nicht forschen.

— Johann Wolfang von Goethe